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Long-term prediction of major coronary or ischemic stroke event in a low-incidence European population: model development and evaluation of clinical utility

Short Title: Clinical utility of long-term CVD risk prediction in primary prevention

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ARTICLE SUMMARY

Article focus

- Primary prevention of cardiovascular disease (CVD) has been recently moved towards the concepts of "lifetime" and "long-term" risk, especially in young subjects and women.
- There is no long-term risk prediction model available for European populations; in addition, the evaluation of the clinical benefit of long-term prediction has not been provided so far.
- We aim to develop a 20-year risk score equation in a northern Italian population of men and women considered at low incidence of major cardiovascular events; and to evaluate the clinical utility of the model for risk stratification in primary CVD prevention program.

Key Messages

- In our population, the 20-year risk model had satisfactory discrimination ability as compared to short-term risk prediction. The importance of long-term prediction for early identification of young subjects and women at increased likelihood of event during their remaining lifespan is confirmed.
- Risk stratification based on the predicted 20-year risk had a better clinical Net Benefit with respect to a stratification based on the number of risk factors, in men and women.
- In both genders, the optimal treatment allocation based on 20-year risk can be determined according to different public health strategies, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment.

Strengths and limitations of this study

- Our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. We also mention the high-quality of follow-up

procedures, including case ascertainment for non-fatal events and a consistent event validation according to MONICA criteria over the whole follow-up period.

- Our 20-year risk prediction model is the first attempt to characterize long-term risk of first coronary or ischemic stroke event in a low-incidence European population. To allow applying our equation to different populations, as Supplementary Material we provide the baseline survival term as well as the calibration slope. However, an external validation study might be desirable. We also remind that our underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. Caution is therefore required before generalizing our findings to different contexts.

ABSTRACT

Objective. To develop a long-term prediction model of first major cardiovascular event and to assess its clinical utility in a low-incidence European population.

Setting. Four independent population-based cohorts enrolled between 1986 and 1993 in Northern Italy.

Participants and methods. N=5,247 35-69 years old men and women free of cardiovascular disease at baseline. Absolute 20-year risk of first fatal or non-fatal coronary or ischemic stroke event (MONICA validated) was estimated from gender-specific Cox models.

Main outcome measures. Model discrimination (Area Under the ROC-Curve, AUC). "High-risk" subjects were defined based on several threshold values for the 20-year predicted risk. Clinical utility was defined in terms of fraction of missed events (events among those

considered at low-risk) and unnecessary treatment (false:true positives ratio). A Net Benefit curve was also provided.

Results. Kaplan-Meier 20-year risk was 16.1% in men (315 events) and 6.1% in women (123 events). Model discrimination (AUC=0.737 in men, 0.801 in women) did not change significantly as compared to 10-year prediction time interval. In men, with respect to risk stratification based on the number of risk factors, a 20% predicted risk cut-off would miss less events (36% vs. 50%) and reduce unnecessary treatment (false:true positive ratio: 2.2 vs. 3.0); the Net Benefit was higher over the whole range of threshold values. Similar considerations hold for women.

Conclusions. Long-term prediction has good discrimination ability and is clinically useful for risk stratification in primary prevention. A clinical utility analysis is recommended to identify the optimal stratification according to different public health goals.

INTRODUCTION

Current European and American guidelines for primary prevention of major coronary and stroke events recommend the use of a multivariable risk prediction method to identify high risk subjects [1, 2]. Several risk scores are available in different US [3, 4] and European [5] populations of middle-aged adults, including the Italian one [6], to estimate the risk of first fatal and non-fatal cardiovascular event over a 10 year time interval. Primary prevention however has been recently moved towards the concepts of "lifetime" [7] and "long-term" risks [8], motivated also by the increasing life expectancy in western Countries. To this extent, 10-year risk prediction models are inadequate to distinguish between those at both low short-term and long-term risks, and those at low short-term but at elevated long-term risk due to the presence of non-optimal risk factors levels [9-11]. In the Framingham Study population, an unfavorable risk factor profile led to an increased 30-year risk of first cardiovascular event, independently on the age at the risk factors assessment [10]. In a representative sample of the Italian population, about 80% of individuals classified at low 10-year risk had increased lifetime risk according to US definition (>=40%), potentially leading to a consistent number of un-prevented events that might have been prevented if lifetime risk had been considered [11]. This group was largely composed of women and young subjects, suggesting that long-term prediction models for risk stratification may be even more beneficial in populations at low incidence of cardiovascular disease [12]. To this extent, the development of a specific long-term risk prediction should be preferred with respect to re-calibration of risk models derived in high-incidence countries [13]. However, extending the range of risk prediction is not a straightforward operation. Although several studies have shown that a single measurement of risk factor is predictive of future events after 30 plus years [10, 14], behavioral changes and risk factors modification may affect model

discrimination. High-quality follow-up data, with a consistent event definition and validation over-time, are also required. Finally, subjects' stratification in risk categories is often based on arbitrary cut-points of absolute risk [15] which may show no benefit in clinical practice [16]. The evaluation of the clinical benefit of long-term prediction by means of some standard measure [17] has not been provided so far and is therefore required [8].

The aim of the present paper is to develop a 20-year risk score equation in a European population of men and women considered at low incidence of major cardiovascular events. In addition to standard model calibration and discrimination tools, we evaluate the clinical utility of the model for risk stratification.

METHODS

Study population

The Brianza population comprises residents in 173 municipalities in the area between Milan and the Swiss border, Northern Italy. The CAMUNI study includes four independent population surveys carried out between 1986 and 1994 as part of either the WHO-MONICA Project[3 surveys; 18] or the PAMELA study [19]. Participation rates were 70.1%, 67.2%, and 70.8% for the three MONICA surveys, respectively, and 64% for the PAMELA Study, with no differences between men and women. Both the baseline screening and the follow-up for all the surveys were approved by the ethical committee of the Monza Hospital.

Baseline assessment of risk factors

Cardiovascular risk factors were collected at baseline strictly adhering to the standardized procedures and quality standards of the WHO-MONICA Project [20]. Height and weight were measured on subjects without shoes and wearing light clothing. Trained technicians collected blood pressure at right arm on subjects in sitting position and at rest, using a standard mercury sphygmomanometer equipped with two side cuff bladders, for normal and

obese subjects. Systolic and diastolic blood pressure were assessed twice, at 5 minutes apart, recording the first and fifth phase of the Korotkoff sounds. The study variable for systolic blood pressure is the average of the two measurements. Venous blood specimens were taken from the ante-cubital vein on fasting subjects (12 hours or more). Serum total cholesterol, HDL-cholesterol and blood glucose were determined using the enzymatic methods; HDL-cholesterol fraction was separated using the Phosphotungstate-Mg^{++} method [20]. A standardized interview was administered to participants by trained interviewers. Information on the use of anti-hypertensive treatment in the last two weeks was dichotomised as yes/no; similarly, cigarette smoking habit was dichotomised as current versus past/never smokers. Diabetes mellitus was defined using self-reported diagnoses, information on insulin and oral hypoglycaemic treatments and fasting blood glucose exceeding 7 mmol/L (126 mg/dl). The presence at baseline of a previous history of MI, unstable angina pectoris, cardiac revascularization or stroke was defined based on self-reported information.

Study endpoint and follow-up procedures

The study endpoint is defined as the occurrence of first major coronary event (myocardial infarction, acute coronary syndrome and coronary revascularization) as well as for first ischemic stroke or carotid endarterectomy, fatal and non-fatal [13]. Data completeness for fatal events was assured through a systematic collection of death certificates provided by local health units; vital status and death certificates were available for 99% of the subjects. Suspected out-of-hospital deaths were investigated through interview of relatives. Suspected hospitalized coronary (discharge code ICD-IX 410 or 411 and ICD-IX CM 36.0-9 for coronary revascularization) and stroke events (ICD-IX 430-432, 434, 436; ICD-IX CM 38.01-39.22 or 39.50-39.52 with at least one 430-438 as discharge code, for carotid endarterectomy) were identified through deterministic and probabilistic record linkages with

regional hospital discharge databases, obtaining a satisfactory performance in case finding, as reported [18, 21]. All acute events were investigated and validated according to the MONICA diagnostic criteria [20]; the ischemic subtype for stroke was attributed after review of the available clinical information.

Statistical analysis

The CUORE Project 10-year risk equation for the Italian population [6, 18] constituted the base for the development of the 20-year risk prediction model. We considered gender-specific Cox regression models with age, total cholesterol, HDL-cholesterol, systolic blood pressure, anti-hypertensive treatment, cigarette smoking and diabetes. After a preliminary check on linearity, total- and HDL-cholesterol were included in the model as categorical variables in four standard classes [4, 22]. The interaction between systolic blood pressure and anti-hypertensive treatment was not statistically significant (p-value 0.84 in men and 0.12 in women, respectively). Finally, no violations in the proportional hazard assumption were observed using a standard test for time-dependent variables.

Model calibration was assessed through the Grønnesby-Bogan goodness-of-fit test [23]. The Area Under the ROC-curve (AUC), as well as sensitivity and specificity in the top and bottom predicted risk quintiles, were computed taking censorship into account [24]. Correction for over-optimism and confidence intervals for the AUC were obtained through 1000 bootstrapped samples [25]. To assess the hypothesis of a loss in discrimination ability due to a longer prediction period, we estimated the 10-year predicted probability of event in our database, using the same set of risk factors but with shorter follow-up period, i.e. up to the end of 2002 for all the subjects. We then compared the AUC of both models, by considering bootstrapped confidence intervals for the difference in the betas.

To assess the clinical utility of the long-term model for risk stratification, we considered two different public health goals. One is to decrease the number of events occurring among those considered at "low-risk". If we assume that a subject classified at "high risk" will be targeted for prevention (either lifestyle intervention or treatment), any event occurring outside this category is "not-identified" or "missed" by the prevention strategy. The second strategy aims instead to reduce un-necessary treatment, by decreasing the number of non-events among those considered at "high-risk". Under the two scenarios, "high-risk "subjects are defined as those with predicted risk above a certain cut-off value. Clinical utility is defined in terms of *i)* fraction of "missed" events; *ii)* probability of event among those classified at high risk; and *iii)* false positive/true positive ratio, for several threshold values in the 20-year predicted risk. We also provide a decision curve analysis based on the net benefit: Net Benefit = (true positives - w*false positives)/n, where n is the sample size and the weight w represents the ratio between the harm of un-necessary treatment and the harm of missing a case at that given value of predicted risk [17]. All the analyses were conducted using the SAS software 9.2.

RESULTS

N=5,426 (2,703 men) subjects were enrolled in the age range 35-69 years. N=205 subjects (3.8%; n=14 events) had at least one missing data; we considered data imputation (R *transcan* function, [26]) and excluded only those with missing values in more than 4 covariates of interest (n=6 men and n=3 women). Finally n=120 men and n=45 women with a positive history of CVD at baseline were also excluded, reducing the sample size to 2,574 men and 2,673 women.

Baseline characteristics of the study population, by gender, are shown in **Table 1**. During a median follow-up time of 15 years (interquartile range: 12-20), we observed 315 first CVD

events in men (233 coronary events) and 123 in women (n=85 coronary events). The Kaplan-Meier estimate for 20-year risk was 16.1% and 6.1% in men and women, respectively.

Model development

The beta-coefficients for the 20-year risk prediction model, as well as the baseline survival term and the calibration slope [25], are provided in the Supplementary Material (**Table S1)**. All the risk factors were statistically significant, except for anti-hypertensive treatment, though its point estimate reflected a 30% increase in hazard in both men and women; the variable was retained in the model for comparability with the short-term CUORE model [6]. There were no significant differences in the set of beta estimates for the 20-year model as compared to those from the 10-year risk model for the risk factors in the model (data not shown). The model calibration was satisfactory, in men (Grønnesby-Bogan goodness-of-fit chi-square 6.7, p-value 0.67) and in women (chi-square 9.6, p-value 0.38).

We found no statistically significant difference in the overall discrimination ability between long- and short-term prediction models, in men (0.736 vs. 0.731) and in women (0.801 vs. 0.816; **Table 2)**. Only 5% of 20-year events in men occurred among subjects with a predicted risk below the 20th percentile (bottom quintile); the corresponding figure in women is 2%. The relative risk of event for being above the 80th percentile vs. below the 20th percentile of 20-year risk was 9.5 (i.e. 35.1/3.7) in men and 22.4 (i.e. 20.2/0.9) in women. Finally, the value of the 80th percentile for 20-year risk was more than twice as high than the similar percentile for 10-year risk in men (26.8 vs. 10.8) and more than three times as high in women (10.1 vs. 3.0). A similar consideration holds for the 20th percentile of risk or the median value.

Clinical utility

Table 3a and **Table 3b** describe strategies for the identification of high-risk subjects, based on predicted 20-year risk, in men and women respectively. A cut-off value of 10% twenty year risk in men would result in a 9% of "missed" events (i.e. events among those with predicted risk below the cut-point), with a probability of event of 23% and one true positive for every 3.4 false positive subjects (**Table 3a**). In the second scenario, by choosing the 20% twenty year risk threshold value, the fraction of missed events was 36%. Note that about 30% of events occurred for a predicted 20-year risk between 20% and 30%. Finally, using the number of risk factors to define high risk subjects would result in a higher fraction of missed events, with no changes in specificity or in the prevalence of subjects considered at high risk. Among women, a cut-off value of 2% would result in a 5% of missed events, with a probability of event of 9% and a true positive for every 10.1 false positive women (**Table 3b**). In the second scenario, the probability of event among those with absolute risk greater than 10% was 20.4%, with a true positive for every 3.9 false positive subjects. However, the fraction of missed events would be 32%; this number can be reduced by lowering the cut-off value to 8%. By considering at high risk those with 2 or more risk factor would result in a higher fraction of missed events, with no gain in specificity or in the probability of event in the group. **Figure 1** illustrates the decision curve analysis based on the Net Benefit [17], for men (left) and women (right). The figure suggests a greater net benefit for the predicted risk with respect to the number of risk factors over the whole range of values, thus generalizing the findings from Table 3a and Table 3b.

DISCUSSION

In this paper we present the 20-year prediction model of first major coronary or ischemic stroke event in a Northern Italian population of men and women aged 35 to 69 years at baseline. To our knowledge, this is the first long-term prediction model in a low-incidence,

European population. The discrimination ability of the long-term model did not significantly drop with respect to a 10-year risk prediction model derived on the same population. Risk stratification based on the predicted 20-year risk can be modulated according to different prevention aims, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment. Under both scenarios, the predicted 20-year risk showed an overall better Net Benefit with respect to a risk stratification based on the number of risk factors.

Our data confirmed previous findings on predictiveness of a single measurement of risk factors on long-term CVD risk, in the Italian [27] as well as in other populations [10, 14]. Event discrimination for the 20-year risk prediction model did not change significantly from 10-year's, although in women it decreased from 0.814 to 0.801. In the Framingham Offspring Study updating the baseline measurement of blood pressure and lipids with a later assessment poorly affected model discrimination and reclassification [28] and cardiometabolic risk factors clustering has been found to be quite stable over time [29].

As in the Framingham population, in our study the long-term predicted risk was more than simply *n*-times the short-term risk prediction [10]. In addition in the age range 35 to 49 years, the long-term predicted risk in subjects with 1 or more non-optimal or elevated risk factors (defined as in [7]) was 3-times the short-term risk in men, and 4-times in women (see **Figure S1** in the Supplementary Material). This conveys the importance of long-term prediction for early identification of young subjects and women at increased likelihood of event during their remaining lifespan. We observed in our data a modest net reclassification improvement (computed as in [24]) for the 20-year risk prediction model over the re-calibrated 10-year risk, in men (1.8%) and in women (4.5%). The net reclassification increased when we considered subjects with a low 10-year predicted risk but a cluster of 2 or

more risk factors (5.4% and 7.6% in men and women, respectively; data not shown). Subjects' stratification is often based on arbitrarily-chosen thresholds of predicted risk [15], which may limit the clinical utility of risk prediction models [16]. We considered two strategies for the identification of "high-risk" subjects with contrasting goals, either to decrease the fraction of missed events or to decrease un-necessary treatment. These can be implemented by choosing threshold values for the predicted risk driven by either sensitivity or by specificity, respectively. Despite the lowering costs of statin treatment with respect to the costs of one un-prevented event, the high sensitivity scenario might not be cost-effective [30]. These two scenarios might be combined to adopt a more complex risk stratification, as often present in clinical practice [1-2, 12]. For instance, if we consider at "low-risk" the 36% of men with 20-year absolute risk less than 10%, the fraction of missed events would be 9%, i.e. 31 first events in 20 years. About 31% of men with absolute risk between 10% and 20% could be addressed for lifestyle modification or treatment according to the presence of specific risk factors; this category accounts for about 20% of cases. Finally, the 33% of men with predicted risk above the 20% could be targeted with treatment intervention; they account for 68% of events, and out of 3.2 treated men, one is a case. A similar stratification can be provided for women, with different threshold values reflecting gender-specific underlying risk as for the cardiovascular age assessment [15].

Among the study strengths and limitations, our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. The underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. As Supplementary Material we provide the baseline survival term as well as a calibration slope [25] to allow applying our equation to different contexts. However, a validation study in a different population might be desirable to

investigate the generalizability of our findings. We also mention a high-quality of follow-up procedures, including case ascertainment for non-fatal events [21] and a consistent event validation according to MONICA criteria, resulting in a Standardized Incidence Rate for the study cohorts above 1 over the whole follow-up period [18]. Finally, the study endpoint reflects the clinical need to treat the "global" ischemic risk of a given patient, and not its separate components [3].

In conclusions, we provide a model to predict long-term risk of first major ischemic cardiovascular event in a low-incidence population. Risk stratification based on long-term risk can be clinically useful, especially for young subjects and women. A clinical utility analysis is required to identify the optimal stratification, according to different public health goals.

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Competing Interests

None.

Contributors

Conception and design: MMF, GM, GC and GV. All authors interpreted the data and critically reviewed the paper. Statistical analyses: GV, WC. GV drafted the manuscript and is the guarantor. All authors have read and approved the final version of the manuscript.

Data sharing

There is no additional data available

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Tables and figures

Table 1. Baseline characteristics (mean (SD) or %) of the study population and number of incident events, by gender. Men and women, 35-69 years old, CVD-free at baseline.

	Men (n=2574)	Women (n=2673)
Age (years)	50.8 (9.1)	50.3 (9)
Years of schooling	8.5 (4.2)	7.3 (3.4)
Total Cholesterol (mmol/L)	5.8 (1.1)	5.8 (1.1)
HDL-Cholesterol (mmol/L)	1.3 (0.3)	1.6 (0.4)
Body Mass Index (Kg/m^2)	26.2 (3.5)	25.6 (4.7)
Systolic Blood Pressure (mmHg)	134.8 (19.3)	131.6 (20.2)
Diastolic Blood Pressure (mmHg)	85.9 (10.6)	82.8 (10.8)
Anti-hypertensive treatment (%)	11.8	16.0
Fasting plasma glucose (mmol/L)	5.4 (1.3)	5.1 (1.2)
Diabetes (%)	6.7	4.0
Current smoker (%)	37.1	19.6
Incident coronary event (n)	233	85
Incident ischemic strokes (n)	99	43
Incident CVD event (n)	315	123
20-year absolute risk of CVD^	16.1	6.1

^: Kaplan-Meier estimate.

Table 2. Discrimination ability for the 10-year and the 20-year risk prediction models.
Men and women, 35-69 years old, CVD-free at baseline

	Men		Women	
	10-year risk	**20-year risk**	**10-year risk**	**20-year risk**
AUC (95% CI)	0.731 (0.702; 0.761)	0.737 (0.713; 0.764)	0.814 (0.779; 0.853)	0.801 (0.771; 0.833)
Subjects with predicted risk below the 20th percentile				
20th percentile of risk	2.3	6.3	0.3	1.1
Fraction of events* (%)	4.4	5.1	1.4	2.0
Probability of event in the group^ (%)	0.8	3.7	0.2	0.9
Subjects with predicted risk above the 80th percentile				
80th percentile of risk	10.8	26.8	3.0	10.1
Sensitivity* (%)	49.9	45.6	68.7	62.0
Specificity (%)	82.4	85.5	81.1	83.1
Probability of event in the group^ (%)	19.4	35.1	7.5	20.2

The Area Under the ROC-curve (AUC) was estimated taking censorship into account, and adjusting for over-optimism (n=1000 bootstrap).
*: Probability of belonging to the group, given that the subject is a case. ^: Kaplan-Meier estimate of the probability of event in the group.

Table 3a. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment. Men, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2574	100.0	0.0	-	16.1	5.2
1+ Major Risk Factor[#]	1842	71.6	13.7	32.5	19.5	4.1
20-year absolute risk > 10%	1645	63.9	9.1	41.2	22.9	3.4
20-year absolute risk > 15%	1169	45.4	22.1	60.9	27.7	2.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors[#]	828	32.2	50.4	73.6	24.9	3.0
20-year absolute risk > 20%	841	32.7	35.7	73.7	31.7	2.2
20-year absolute risk > 30%	415	16.1	62.6	88.9	37.4	1.7

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Table 3b. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment.
Women, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2673	100.0	0.0	-	6.1	15.3
1+ Major Risk Factor#	1654	61.9	17.7	40.1	8.2	11.3
20-year absolute risk > 2%	1733	64.8	4.5	37.4	9.0	10.1
20-year absolute risk > 5%	1067	39.9	14.7	63.2	13.1	6.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors#	640	23.9	42.3	79.5	14.8	5.8
20-year absolute risk > 8%	698	26.1	22.7	77.1	18.2	4.5
20-year absolute risk > 10%	545	20.4	32.1	82.7	20.4	3.9

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Figure 1: Decision curve for the 20-year risk prediction model in the CAMUNI population, Northern Italy.
Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

Net Benefit: (TP-w*FP)/n, where TP = True Positive; FP = False Positive; w = (Absolute risk threshold)/(1- (Absolute risk threshold)); n=sample size
Number of risk factors: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

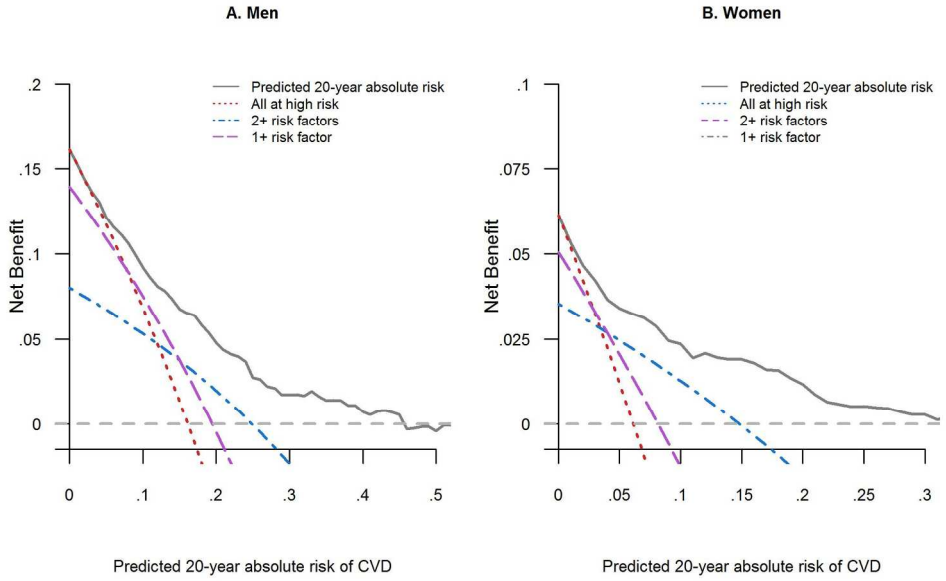

Figure 1: Decision curve for the 20-year risk prediction model in the CAMUNI population, Northern Italy. Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

Net Benefit: (TP-w*FP)/n, where TP = True Positive; FP = False Positive; w = (Absolute risk threshold)/(1-(Absolute risk threshold)); n=sample size
Number of risk factors: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

180x119mm (300 x 300 DPI)

Supplementary material

Table S1: Beta-coefficients, standard errors and baseline survival for the 20-year risk prediction model in Northern Italy. Men and women, 35 to 69 years old, free of CVD at baseline.

	Men			Women		
	Beta	**SE**	**p-value**	**Beta**	**SE**	**p-value**
Age (years)	0.058	0.008	<.0001	0.084	0.014	<.0001
Total Cholesterol^						
200-240 mg/dl	0.388	0.161		0.553	0.287	
240-280 mg/dl	0.690	0.167	<.0001	0.607	0.310	0.027
> 280 mg/dl	0.923	0.198		0.996	0.328	
HDL-Cholesterol°						
<45 mg/dl	0.403	0.160		0.804	0.250	
45-50 mg/dl	0.367	0.186	0.013	0.364	0.309	0.015
50-60 mg/dl	0.024	0.177		0.261	0.225	
Systolic Blood Pressure (mmHg)	0.011	0.003	0.0003	0.015	0.005	0.001
Anti-hypertensive treatment (yes/no)	0.247	0.154	0.11	0.267	0.209	0.20
Smoking (yes/no)	0.521	0.117	<.0001	0.994	0.216	<.0001
Diabetes (yes/no)	0.744	0.163	<.0001	1.020	0.249	<.0001
Baseline 20-year survival ($S_0(20)$)*		0.94168			0.98502	
G(μ)		4.35638			6.20915	
Calibration Slope		0.948			0.937	

SE = Standard Error. ^: reference group: total cholesterol<=200 mg/dl. °: reference group: HDL-cholesterol >60 mg/dl. *: at the mean value for continuous RFs, and at the reference class for categorical variables. 20-year risk: 1- $S_0(20)^{\wedge}[\exp(\sum\beta X - G(\mu)]$.
Calibration slope: correction term that could be used in different population to shrink the beta-coefficients. See reference[25] for more details.

Figure S1: Distribution of predicted 10-year and 20-year risk of first major CVD event, according to the number of risk factors. Men (left) and women (right), 35 to 49 years old, free of CVD at baseline.

Risk factors stratification derived from Lloyd-Jones [7].
All optimal: total cholesterol <180 mg/dl, HDL-Cholesterol >= 40 mg/dl [men] or >= 50 mg/dl [women], blood pressure <120/80 mmHg, non smoker, non diabetic;
1+ non-optimal: total cholesterol 180 to 199 mg/dl, systolic blood pressure 120 to 139 mmHg, diastolic blood pressure 80 to 89 mmHg, non smoker, non diabetic
1+ elevated: total cholesterol 200 to 239 mg/dl, systolic blood pressure 140 to 159 mmHg, diastolic blood pressure 90 to 99 mmHg, non smoker, non diabetic
Major risk factor: total cholesterol >=240 mg/dl, HDL-Cholesterol <40 mg/dl [men] or <50 mg/dl [women], systolic blood pressure>=160 mmHg or treatment, diastolic blood pressure >=100 mmHg, smoker, or diabetic

Supplementary material for the paper:

Long-term prediction of major coronary or ischemic stroke event in a low-incidence European population: model development and evaluation of clinical utility.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Actions
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	The study setting is clearly stated in the abstract.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Done
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	See the introduction section at pages 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	See page 4, end of introduction section
Methods			
Study design	4	Present key elements of study design early in the paper	See the Methods section (pages 4-7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Relevant information on cohorts setting, location and periods of recruitment are provided in the paragraphs "Study population" (page 4), "Baseline assessment of risk factors" (page 4) and "Study endpoint and follow-up procedures" (page 5).
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	See the paragraphs "Study population" (page 4) and "Study endpoint and follow-up procedures" (page 5).
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	See the paragraph "Statistical analysis", page 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	See paragraphs "Baseline assessment of risk factors" (page 4), and "Statistical analysis" (page 6-7). Exposure group: not applicable for this analysis.
Bias	9	Describe any efforts to address potential sources of bias	See the Methods section.
Study size	10	Explain how the study size was arrived at	See the first period in the "Results" section (page 7)
Quantitative	11	Explain how quantitative variables were	See the "Statistical Analysis" paragraph

variables		handled in the analyses. If applicable, describe which groupings were chosen and why	(page 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	See the "Statistical Analysis" paragraph (page 6)
		(*b*) Describe any methods used to examine subgroups and interactions	See the "Statistical Analysis" paragraph (page 6)
		(*c*) Explain how missing data were addressed	See the first line in the "Results" section (page 7)
		(*d*) If applicable, explain how loss to follow-up was addressed	See the "Statistical Analysis" paragraph (page 6) for details on the survival analysis techniques
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Participation rates are reported in the paragraph "Study population" (page 4). Exposure group: not applicable for this analysis.
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	See Table 1. Exposure group: not applicable for this analysis.
		(b) Indicate number of participants with missing data for each variable of interest	See the "Results" section, first period (page 7). Exposure group: not applicable for this analysis.
		(c) Summarise follow-up time (eg, average and total amount)	"Results" section, second period (page 7). Exposure group: not applicable for this analysis..
Outcome data	15*	Report numbers of outcome events or summary measures over time	Number of events, by type, are reported in Table 1. Exposure group: not applicable for this analysis.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	The study model is reported in Table S1, supplementary material; the analysis is multivariable by nature.
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable

Discussion			
Key results	18	Summarise key results with reference to study objectives	See the first part of the Discussion section, page 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Study limitations are reported and discussed at pages 11-12.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Done
Generalisability	21	Discuss the generalisability (external validity) of the study results	See pages 11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Source of funding is reported at page 12.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

Long-term prediction of major coronary or ischemic stroke event in a low-incidence Southern European population: model development and evaluation of clinical utility

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Long-term prediction of major coronary or ischemic stroke event in a low-incidence Southern European population: model development and evaluation of clinical utility

Short Title: Clinical utility of long-term CVD risk prediction in primary prevention

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ABSTRACT

Objective. To develop a long-term prediction model of first major cardiovascular event and to assess its clinical utility in a low-incidence European population.

Setting. Four independent population-based cohorts enrolled between 1986 and 1993 in Northern Italy.

Participants and methods. N=5,247 35-69 years old men and women free of cardiovascular disease at baseline. Absolute 20-year risk of first fatal or non-fatal coronary or ischemic stroke event (MONICA validated) was estimated from gender-specific Cox models.

Main outcome measures. Model discrimination (Area Under the ROC-Curve, AUC). "High-risk" subjects were defined based on several threshold values for the 20-year predicted risk. Clinical utility was defined in terms of fraction of missed events (events among those considered at low-risk) and unnecessary treatment (false:true positives ratio). A Net Benefit curve was also provided.

Results. Kaplan-Meier 20-year risk was 16.1% in men (315 events) and 6.1% in women (123 events). Model discrimination (AUC=0.737 in men, 0.801 in women) did not change significantly as compared to 10-year prediction time interval. In men, with respect to risk stratification based on the number of risk factors, a 20% predicted risk cut-off would miss less events (36% vs. 50%) and reduce unnecessary treatment (false:true positive ratio: 2.2 vs. 3.0); the Net Benefit was higher over the whole range of threshold values. Similar considerations hold for women.

Conclusions. Long-term prediction has good discrimination ability and is clinically useful for risk stratification in primary prevention. A clinical utility analysis is recommended to identify the optimal stratification according to different public health goals.

ARTICLE SUMMARY

Article focus

- Primary prevention of cardiovascular disease (CVD) has been recently moved towards the concepts of "lifetime" and "long-term" risk, especially in young subjects and women.
- There is no long-term risk prediction model available for low-incidence Southern European populations; in addition, the evaluation of the clinical benefit of long-term prediction has not been provided so far.
- We aim to develop a 20-year risk score equation in a northern Italian population of men and women considered at low incidence of major cardiovascular events; and to evaluate the clinical utility of the model for risk stratification in primary CVD prevention program.

Key Messages

- In our population, the 20-year risk model had satisfactory discrimination ability as compared to short-term risk prediction. The importance of long-term prediction for early identification of young subjects and women at increased likelihood of event during their remaining lifespan is confirmed.
- Risk stratification based on the predicted 20-year risk had a better clinical Net Benefit with respect to a stratification based on the number of risk factors, in men and women.
- In both genders, the optimal treatment allocation based on 20-year risk can be determined according to different public health strategies, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment.

Strengths and limitations of this study

- Our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. We also mention the high-quality of follow-up

procedures, including case ascertainment for non-fatal events and a consistent event validation according to MONICA criteria over the whole follow-up period.

- Our 20-year risk prediction model is the first attempt to characterize long-term risk of first coronary or ischemic stroke event in a low-incidence European population. A limitation of our study is the lack of a formal external validation, although we provide a cross-validation analysis. We also remind that our underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. Caution is therefore required before generalizing our findings to different contexts.

INTRODUCTION

Current European and American guidelines for primary prevention of major coronary and stroke events recommend the use of a multivariable risk prediction method to identify high risk subjects [1, 2]. Several risk scores are available in different US [3, 4] and European [5] populations of middle-aged adults, including the Italian one [6], to estimate the risk of first fatal and non-fatal cardiovascular event over a 10 year time interval. Primary prevention however has been recently moved towards the concepts of "lifetime" [7] and "long-term" risks [8], motivated also by the increasing life expectancy in western Countries. To this extent, 10-year risk prediction models are inadequate to distinguish between those at both low short-term and long-term risks, and those at low short-term but at elevated long-term risk due to the presence of non-optimal risk factors levels [9-11]. In the Framingham Study population, an unfavorable risk factor profile led to an increased 30-year risk of first cardiovascular event, independently on the age at the risk factors assessment [10]. In a

representative sample of the Italian population, about 80% of individuals classified at low 10-year risk had increased lifetime risk according to US definition (>=40%), potentially leading to a consistent number of un-prevented events that might have been prevented if lifetime risk had been considered [11]. This group was largely composed of women and young subjects, suggesting that long-term prediction models for risk stratification may be even more beneficial in populations at low incidence of cardiovascular disease [12]. To this extent, the development of a specific long-term risk prediction should be preferred with respect to re-calibration of risk models derived in high-incidence countries [13]. However, extending the range of risk prediction is not a straightforward operation. Although several studies have shown that a single measurement of risk factor is predictive of future events after 30 plus years [10, 14], behavioral changes and risk factors modification may affect model discrimination. High-quality follow-up data, with a consistent event definition and validation over-time, are also required. Finally, subjects' stratification in risk categories is often based on arbitrary cut-points of absolute risk [15] which may show no benefit in clinical practice [16]. The evaluation of the clinical benefit of long-term prediction by means of some standard measure [17] has not been provided so far and is therefore required [8].

The aim of the present paper is to develop a 20-year risk score equation in a European population of men and women considered at low incidence of major cardiovascular events. In addition to standard model calibration and discrimination tools, we evaluate the clinical utility of the model for risk stratification.

METHODS

Study population

The Brianza population comprises residents in 173 municipalities in the area between Milan and the Swiss border, Northern Italy. The CAMUNI study includes four independent

population surveys carried out between 1986 and 1994 as part of either the WHO-MONICA Project [3 surveys; 18] or the PAMELA study [19]. Participation rates were 70.1%, 67.2%, and 70.8% for the three MONICA surveys, respectively, and 64% for the PAMELA Study, with no differences between men and women. Both the baseline screening and the follow-up for all the surveys were approved by the ethical committee of the Monza Hospital.

Baseline assessment of risk factors

Cardiovascular risk factors were collected at baseline strictly adhering to the standardized procedures and quality standards of the WHO-MONICA Project [20]. Height and weight were measured on subjects without shoes and wearing light clothing. Trained technicians collected blood pressure at right arm on subjects in sitting position and at rest, using a standard mercury sphygmomanometer equipped with two side cuff bladders, for normal and obese subjects. Systolic and diastolic blood pressure were assessed twice, at 5 minutes apart, recording the first and fifth phase of the Korotkoff sounds. The study variable for systolic blood pressure is the average of the two measurements. Venous blood specimens were taken from the ante-cubital vein on fasting subjects (12 hours or more). Serum total cholesterol, HDL-cholesterol and blood glucose were determined using the enzymatic methods; HDL-cholesterol fraction was separated using the Phosphotungstate-Mg^{++} method [20]. A standardized interview was administered to participants by trained interviewers. Information on the use of anti-hypertensive treatment in the last two weeks was dichotomised as yes/no; similarly, cigarette smoking habit was dichotomised as current versus past/never smokers. Diabetes mellitus was defined using self-reported diagnoses, information on insulin and oral hypoglycaemic treatments and fasting blood glucose exceeding 7 mmol/L (126 mg/dl). The presence at baseline of a previous history of MI, unstable angina pectoris, cardiac revascularization or stroke was defined based on self-reported information.

Study endpoint and follow-up procedures

The study endpoint is defined as the occurrence of first major coronary event (myocardial infarction, acute coronary syndrome and coronary revascularization) as well as for first ischemic stroke or carotid endarterectomy, fatal and non-fatal [13]. Data completeness for fatal events was assured through a systematic collection of death certificates provided by local health units; vital status and death certificates were available for 99% of the subjects. Suspected out-of-hospital deaths were investigated through interview of relatives. Suspected hospitalized coronary (discharge code ICD-IX 410 or 411 and ICD-IX CM 36.0-9 for coronary revascularization) and stroke events (ICD-IX 430-432, 434, 436; ICD-IX CM 38.01-39.22 or 39.50-39.52 with at least one 430-438 as discharge code, for carotid endarterectomy) were identified through deterministic and probabilistic record linkages with regional hospital discharge databases, obtaining a satisfactory performance in case finding, as reported [18, 21]. All acute events were investigated and validated according to the MONICA diagnostic criteria [20]; the ischemic subtype for stroke was attributed after review of the available clinical information.

Statistical analysis

Our 20-year risk prediction model is based on gender-specific Cox regression models with age, total cholesterol, HDL-cholesterol, systolic blood pressure, anti-hypertensive treatment, cigarette smoking and diabetes. These predictors are core risk factors included in the CUORE Project [6, 13] as well as in other 10-year risk equations [3, 4]. After a preliminary check on linearity, total- and HDL-cholesterol were included in the model as categorical variables in four standard classes [4, 22]. The interaction between systolic blood pressure and anti-hypertensive treatment was not statistically significant (p-value 0.84 in men and 0.12 in women, respectively). There was no evidence of any cohort effect in the full model, in men

(3 df test p-value: 0.2) nor in women (p-value: 0.5). Finally, no violations in the proportional hazard assumption were observed using a standard test for time-dependent variables.

Model calibration was assessed through the Grønnesby-Bogan goodness-of-fit test [23]. The Area Under the ROC-curve (AUC), as well as sensitivity and specificity in the top and bottom predicted risk quintiles, were computed taking censorship into account [24]. Correction for over-optimism and confidence intervals for the AUC were obtained through 1000 bootstrapped samples [25]. To assess the hypothesis of a loss in discrimination ability due to a longer prediction period, we estimated the 10-year predicted probability of event in our database, using the same set of risk factors but with shorter follow-up period, i.e. up to the end of 2002 for all the subjects (number of events: 234 in men, 79 in women). We then compared the AUC of the two models by looking at their respective bootstrapped confidence intervals. To assess the clinical utility of the long-term model for risk stratification, we considered two different public health goals. One is to decrease the number of events occurring among those considered at "low-risk". If we assume that a subject classified at "high risk" will be targeted for prevention (either lifestyle intervention or treatment), any event occurring outside this category is "not-identified" or "missed" by the prevention strategy. The second strategy aims instead to reduce un-necessary treatment, by decreasing the number of non-events among those considered at "high-risk". Under the two scenarios, "high-risk "subjects are defined as those with predicted risk above a certain cut-off value. Clinical utility is defined in terms of *i)* fraction of "missed" events; *ii)* probability of event among those classified at high risk; and *iii)* false positive/true positive ratio, for several threshold values in the 20-year predicted risk. We also provide a decision curve analysis based on the net benefit: Net Benefit = (true positives - w*false positives)/n, where n is the sample size and the weight w represents the ratio between the harm of un-necessary treatment

and the harm of missing a case at that given value of predicted risk [17]. All the analyses were conducted using the SAS software 9.2.

RESULTS

N=5,426 (2,703 men) subjects were enrolled in the age range 35-69 years. N=205 subjects (3.8%; n=14 events) had at least one missing data; we considered data imputation (R *transcan* function, [26]) and excluded only those with missing values in more than 4 covariates of interest (n=6 men and n=3 women). Finally n=120 men and n=45 women with a positive history of CVD at baseline were also excluded, reducing the sample size to 2,574 men and 2,673 women.

Baseline characteristics of the study population, by gender, are shown in **Table 1**. During a median follow-up time of 15 years (interquartile range: 12-20), we observed 315 first CVD events in men (233 coronary events) and 123 in women (n=85 coronary events). The Kaplan-Meier estimate for 20-year risk was 16.1% and 6.1% in men and women, respectively.

Model development

The beta-coefficients for the 20-year risk prediction model, as well as the baseline survival term and the calibration slope [25], are provided in the Supplementary Material (**Table S1)**. All the risk factors were statistically significant, except for anti-hypertensive treatment, though its point estimate reflected a 30% increase in hazard in both men and women; the variable was retained in the model for comparability with the short-term CUORE model [6]. There were no significant differences in the set of beta estimates for the 20-year model as compared to those from the 10-year risk model for the risk factors in the model (data not shown). The model calibration was satisfactory, in men (Grønnesby-Bogan goodness-of-fit chi-square 6.7, p-value 0.67) and in women (chi-square 9.6, p-value 0.38); calibration plots are available as supplementary material (**Figure S1**)

We found no statistically significant difference in the overall discrimination ability between long- and short-term prediction models, in men (0.736 vs. 0.731) and in women (0.801 vs. 0.816; **Table 2)**. Only 5% of 20-year events in men occurred among subjects with a predicted risk below the 20th percentile (bottom fifth); the corresponding figure in women is 2%. The relative risk of event for being above the 80th percentile vs. below the 20th percentile of 20-year risk was 9.5 (i.e. 35.1/3.7) in men and 22.4 (i.e. 20.2/0.9) in women. Finally, the value of the 80th percentile for 20-year risk was more than twice as high than the similar percentile for 10-year risk in men (26.8 vs. 10.8) and more than three times as high in women (10.1 vs. 3.0). A similar consideration holds for the 20th percentile of risk or the median value.

Clinical utility

Table 3a and **Table 3b** describe strategies for the identification of high-risk subjects, based on predicted 20-year risk, in men and women respectively. A cut-off value of 10% twenty year risk in men would result in a 9% of "missed" events (i.e. events among those with predicted risk below the cut-point), with a probability of event of 23% and one true positive for every 3.4 false positive subjects (**Table 3a**). In the second scenario, by choosing the 20% twenty year risk threshold value, the fraction of missed events was 36%. Note that about 30% of events occurred for a predicted 20-year risk between 20% and 30%. Finally, using the number of risk factors to define high risk subjects would result in a higher fraction of missed events, with no changes in specificity or in the prevalence of subjects considered at high risk. Among women, a cut-off value of 2% would result in a 5% of missed events, with a probability of event of 9% and a true positive for every 10.1 false positive women (**Table 3b**). In the second scenario, the probability of event among those with absolute risk greater than 10% was 20.4%, with a true positive for every 3.9 false positive subjects. However, the fraction of missed events would be 32%; this number can be reduced by lowering the cut-off

value to 8%. By considering at high risk those with 2 or more risk factor would result in a higher fraction of missed events, with no gain in specificity or in the probability of event in the group. **Figure 1** illustrates the decision curve analysis based on the Net Benefit [17], for men (left) and women (right). The figure suggests a greater net benefit for the predicted risk with respect to the number of risk factors over the whole range of values, thus generalizing the findings from Table 3a and Table 3b.

DISCUSSION

In this paper we present the 20-year prediction model of first major coronary or ischemic stroke event in a Northern Italian population of men and women aged 35 to 69 years at baseline. To our knowledge, this is the first long-term prediction model in a low-incidence, European population. The discrimination ability of the long-term model did not significantly drop with respect to a 10-year risk prediction model derived on the same population. Risk stratification based on the predicted 20-year risk can be modulated according to different prevention aims, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment. Under both scenarios, the predicted 20-year risk showed an overall better Net Benefit with respect to a risk stratification based on the number of risk factors.

Our data confirmed previous findings on predictiveness of a single measurement of risk factors on long-term CVD risk, in the Italian [27] as well as in other populations [10, 14]. Event discrimination for the 20-year risk prediction model did not change significantly from 10-year's, although in women it decreased from 0.814 to 0.801. In the Framingham Offspring Study updating the baseline measurement of blood pressure and lipids with a later assessment poorly affected model discrimination and reclassification [28] and cardiometabolic risk factors clustering has been found to be quite stable over time [29].

As in the Framingham population, in our study the long-term predicted risk was more than simply *n*-times the short-term risk prediction [10]. In addition in the age range 35 to 49 years, the long-term predicted risk in subjects with 1 or more non-optimal or elevated risk factors (defined as in [7]) was 3-times the short-term risk in men, and 4-times in women (see **Figure S2** in the Supplementary Material). This conveys the importance of long-term prediction for early identification of young subjects and women at increased likelihood of event during their remaining lifespan. We observed in our data a modest net reclassification improvement (computed as in [24]) for the 20-year risk prediction model over the re-calibrated 10-year risk, in men (1.8%) and in women (4.5%). The net reclassification increased when we considered subjects with a low 10-year predicted risk but a cluster of 2 or more risk factors (5.4% and 7.6% in men and women, respectively; data not shown). Subjects' stratification is often based on arbitrarily-chosen thresholds of predicted risk [15], which may limit the clinical utility of risk prediction models [16]. We considered two strategies for the identification of "high-risk" subjects with contrasting goals, either to decrease the fraction of missed events or to decrease un-necessary treatment. These can be implemented by choosing threshold values for the predicted risk driven by either sensitivity or by specificity, respectively. Despite the lowering costs of statin treatment with respect to the costs of one un-prevented event, the high sensitivity scenario was not cost-effective over a 10-year period [30]. These two scenarios might be combined to adopt a more complex risk stratification, as often present in clinical practice [1-2, 12]. For instance, if we consider at "low-risk" the 36% of men with 20-year absolute risk less than 10%, the fraction of missed events would be 9%, i.e. 31 first events in 20 years. About 31% of men with absolute risk between 10% and 20% could be addressed for lifestyle modification or treatment according to the presence of specific risk factors; this category accounts for about 20% of cases. Finally,

the 33% of men with predicted risk above the 20% could be targeted with treatment intervention; they account for 68% of events, and out of 3.2 treated men, one is a case. A similar stratification can be provided for women, with different threshold values reflecting gender-specific underlying risk as for the cardiovascular age assessment [15].

Among the study strengths and limitations, our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. The underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. A major limitation is the lack of an external validation. External validation for long-term prediction models is in general an issue [10]; we provide the over-optimism adjusted AUC as well as the calibration slope [25] to allow applying our equation to different contexts (see Supplementary Material). We also mention a high-quality of follow-up procedures, including case ascertainment for non-fatal events [21] and a consistent event validation according to MONICA criteria, resulting in a Standardized Incidence Rate for the study cohorts above 1 over the whole follow-up period [18]. Finally, the study endpoint reflects the clinical need to treat the "global" ischemic risk of a given patient, and not its separate components [3].

In conclusions, we provide a model to predict long-term risk of first major ischemic cardiovascular event in a low-incidence population. Risk stratification based on long-term risk can be clinically useful, especially for young subjects and women. A clinical utility analysis is required to identify the optimal stratification, according to different public health goals.

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Competing Interests

None.

Contributors

Conception and design: MMF, GM, GC and GV. All authors interpreted the data and critically reviewed the paper. Statistical analyses: GV, WC. GV drafted the manuscript and is the guarantor. All authors have read and approved the final version of the manuscript.

Data sharing

There is no additional data available.

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Tables and figures

Table 1. Baseline characteristics (mean (SD) or %) of the study population and number of incident events, by gender. Men and women, 35-69 years old, CVD-free at baseline.

	Men (n=2574)	Women (n=2673)
Age (years)	50.8 (9.1)	50.3 (9)
Years of schooling	8.5 (4.2)	7.3 (3.4)
Total Cholesterol (mmol/L)	5.8 (1.1)	5.8 (1.1)
HDL-Cholesterol (mmol/L)	1.3 (0.3)	1.6 (0.4)
Body Mass Index (Kg/m^2)	26.2 (3.5)	25.6 (4.7)
Systolic Blood Pressure (mmHg)	134.8 (19.3)	131.6 (20.2)
Diastolic Blood Pressure (mmHg)	85.9 (10.6)	82.8 (10.8)
Anti-hypertensive treatment (%)	11.8	16.0
Fasting plasma glucose (mmol/L)	5.4 (1.3)	5.1 (1.2)
Diabetes (%)	6.7	4.0
Current smoker (%)	37.1	19.6
Incident coronary event (n)	233	85
Incident ischemic strokes (n)	99	43
Incident CVD event (n)	315	123
20-year absolute risk of CVD^	16.1	6.1

^: Kaplan-Meier estimate.

Table 2. Discrimination ability for the 10-year and the 20-year risk prediction models.
Men and women, 35-69 years old, CVD-free at baseline

	Men		Women	
	10-year risk	**20-year risk**	**10-year risk**	**20-year risk**
AUC (95% CI)	0.731 (0.702; 0.761)	0.737 (0.713; 0.764)	0.814 (0.779; 0.853)	0.801 (0.771; 0.833)
Subjects with predicted risk below the 20th percentile				
20th percentile of risk	2.3	6.3	0.3	1.1
Fraction of events* (%)	4.4	5.1	1.4	2.0
Probability of event in the group^ (%)	0.8	3.7	0.2	0.9
Subjects with predicted risk above the 80th percentile				
80th percentile of risk	10.8	26.8	3.0	10.1
Sensitivity* (%)	49.9	45.6	68.7	62.0
Specificity (%)	82.4	85.5	81.1	83.1
Probability of event in the group^ (%)	19.4	35.1	7.5	20.2

The Area Under the ROC-curve (AUC) was estimated taking censorship into account, and adjusting for over-optimism (n=1000 bootstrap).
*: Probability of belonging to the group, given that the subject is a case. ^: Kaplan-Meier estimate of the probability of event in the group.

Table 3a. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment. Men, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2574	100.0	0.0	-	16.1	5.2
1+ Major Risk Factor[#]	1842	71.6	13.7	32.5	19.5	4.1
20-year absolute risk > 10%	1645	63.9	9.1	41.2	22.9	3.4
20-year absolute risk > 15%	1169	45.4	22.1	60.9	27.7	2.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors[#]	828	32.2	50.4	73.6	24.9	3.0
20-year absolute risk > 20%	841	32.7	35.7	73.7	31.7	2.2
20-year absolute risk > 30%	415	16.1	62.6	88.9	37.4	1.7

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Table 3b. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment.
Women, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2673	100.0	0.0	-	6.1	15.3
1+ Major Risk Factor#	1654	61.9	17.7	40.1	8.2	11.3
20-year absolute risk > 2%	1733	64.8	4.5	37.4	9.0	10.1
20-year absolute risk > 5%	1067	39.9	14.7	63.2	13.1	6.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors#	640	23.9	42.3	79.5	14.8	5.8
20-year absolute risk > 8%	698	26.1	22.7	77.1	18.2	4.5
20-year absolute risk > 10%	545	20.4	32.1	82.7	20.4	3.9

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Figure 1: Decision curve for the 20-year risk prediction model in the CAMUNI population, Northern Italy. Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

Net Benefit: (TP-w*FP)/n, where TP = True Positive; FP = False Positive; w = (Absolute risk threshold)/(1- (Absolute risk threshold)); n=sample size
Number of risk factors: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Long-term prediction of major coronary or ischemic stroke event in a low-incidence Southern European population: model development and evaluation of clinical utility

Comment [g1]: Added. See reviewer 1, comment #1

Short Title: Clinical utility of long-term CVD risk prediction in primary prevention

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ABSTRACT

Objective. To develop a long-term prediction model of first major cardiovascular event and to assess its clinical utility in a low-incidence European population.

Setting. Four independent population-based cohorts enrolled between 1986 and 1993 in Northern Italy.

Participants and methods. N=5,247 35-69 years old men and women free of cardiovascular disease at baseline. Absolute 20-year risk of first fatal or non-fatal coronary or ischemic stroke event (MONICA validated) was estimated from gender-specific Cox models.

Main outcome measures. Model discrimination (Area Under the ROC-Curve, AUC). "High-risk" subjects were defined based on several threshold values for the 20-year predicted risk. Clinical utility was defined in terms of fraction of missed events (events among those considered at low-risk) and unnecessary treatment (false:true positives ratio). A Net Benefit curve was also provided.

Results. Kaplan-Meier 20-year risk was 16.1% in men (315 events) and 6.1% in women (123 events). Model discrimination (AUC=0.737 in men, 0.801 in women) did not change significantly as compared to 10-year prediction time interval. In men, with respect to risk stratification based on the number of risk factors, a 20% predicted risk cut-off would miss less events (36% vs. 50%) and reduce unnecessary treatment (false:true positive ratio: 2.2 vs. 3.0); the Net Benefit was higher over the whole range of threshold values. Similar considerations hold for women.

Conclusions. Long-term prediction has good discrimination ability and is clinically useful for risk stratification in primary prevention. A clinical utility analysis is recommended to identify the optimal stratification according to different public health goals.

INTRODUCTION

Current European and American guidelines for primary prevention of major coronary and stroke events recommend the use of a multivariable risk prediction method to identify high risk subjects [1, 2]. Several risk scores are available in different US [3, 4] and European [5] populations of middle-aged adults, including the Italian one [6], to estimate the risk of first fatal and non-fatal cardiovascular event over a 10 year time interval. Primary prevention however has been recently moved towards the concepts of "lifetime" [7] and "long-term" risks [8], motivated also by the increasing life expectancy in western Countries. To this extent, 10-year risk prediction models are inadequate to distinguish between those at both low short-term and long-term risks, and those at low short-term but at elevated long-term risk due to the presence of non-optimal risk factors levels [9-11]. In the Framingham Study population, an unfavorable risk factor profile led to an increased 30-year risk of first cardiovascular event, independently on the age at the risk factors assessment [10]. In a representative sample of the Italian population, about 80% of individuals classified at low 10-year risk had increased lifetime risk according to US definition (>=40%), potentially leading to a consistent number of un-prevented events that might have been prevented if lifetime risk had been considered [11]. This group was largely composed of women and young subjects, suggesting that long-term prediction models for risk stratification may be even more beneficial in populations at low incidence of cardiovascular disease [12]. To this extent, the development of a specific long-term risk prediction should be preferred with respect to re-calibration of risk models derived in high-incidence countries [13]. However, extending the range of risk prediction is not a straightforward operation. Although several studies have shown that a single measurement of risk factor is predictive of future events after 30 plus years [10, 14], behavioral changes and risk factors modification may affect model

discrimination. High-quality follow-up data, with a consistent event definition and validation over-time, are also required. Finally, subjects' stratification in risk categories is often based on arbitrary cut-points of absolute risk [15] which may show no benefit in clinical practice [16]. The evaluation of the clinical benefit of long-term prediction by means of some standard measure [17] has not been provided so far and is therefore required [8].

The aim of the present paper is to develop a 20-year risk score equation in a European population of men and women considered at low incidence of major cardiovascular events. In addition to standard model calibration and discrimination tools, we evaluate the clinical utility of the model for risk stratification.

METHODS

Study population

The Brianza population comprises residents in 173 municipalities in the area between Milan and the Swiss border, Northern Italy. The CAMUNI study includes four independent population surveys carried out between 1986 and 1994 as part of either the WHO-MONICA Project [3 surveys; 18] or the PAMELA study [19]. Participation rates were 70.1%, 67.2%, and 70.8% for the three MONICA surveys, respectively, and 64% for the PAMELA Study, with no differences between men and women. Both the baseline screening and the follow-up for all the surveys were approved by the ethical committee of the Monza Hospital.

Baseline assessment of risk factors

Cardiovascular risk factors were collected at baseline strictly adhering to the standardized procedures and quality standards of the WHO-MONICA Project [20]. Height and weight were measured on subjects without shoes and wearing light clothing. Trained technicians collected blood pressure at right arm on subjects in sitting position and at rest, using a standard mercury sphygmomanometer equipped with two side cuff bladders, for normal and

obese subjects. Systolic and diastolic blood pressure were assessed twice, at 5 minutes apart, recording the first and fifth phase of the Korotkoff sounds. The study variable for systolic blood pressure is the average of the two measurements. Venous blood specimens were taken from the ante-cubital vein on fasting subjects (12 hours or more). Serum total cholesterol, HDL-cholesterol and blood glucose were determined using the enzymatic methods; HDL-cholesterol fraction was separated using the Phosphotungstate-Mg^{++} method [20]. A standardized interview was administered to participants by trained interviewers. Information on the use of anti-hypertensive treatment in the last two weeks was dichotomised as yes/no; similarly, cigarette smoking habit was dichotomised as current versus past/never smokers. Diabetes mellitus was defined using self-reported diagnoses, information on insulin and oral hypoglycaemic treatments and fasting blood glucose exceeding 7 mmol/L (126 mg/dl). The presence at baseline of a previous history of MI, unstable angina pectoris, cardiac revascularization or stroke was defined based on self-reported information.

Study endpoint and follow-up procedures

The study endpoint is defined as the occurrence of first major coronary event (myocardial infarction, acute coronary syndrome and coronary revascularization) as well as for first ischemic stroke or carotid endarterectomy, fatal and non-fatal [13]. Data completeness for fatal events was assured through a systematic collection of death certificates provided by local health units; vital status and death certificates were available for 99% of the subjects. Suspected out-of-hospital deaths were investigated through interview of relatives. Suspected hospitalized coronary (discharge code ICD-IX 410 or 411 and ICD-IX CM 36.0-9 for coronary revascularization) and stroke events (ICD-IX 430-432, 434, 436; ICD-IX CM 38.01-39.22 or 39.50-39.52 with at least one 430-438 as discharge code, for carotid endarterectomy) were identified through deterministic and probabilistic record linkages with

regional hospital discharge databases, obtaining a satisfactory performance in case finding, as reported [18, 21]. All acute events were investigated and validated according to the MONICA diagnostic criteria [20]; the ischemic subtype for stroke was attributed after review of the available clinical information.

Statistical analysis

~~The CUORE Project 10-year risk equation for the Italian population [6, 18] constituted the base for the development of the 20-year risk prediction model.~~ Our 20-year risk prediction model is based on ~~We considered~~ gender-specific Cox regression models with age, total cholesterol, HDL-cholesterol, systolic blood pressure, anti-hypertensive treatment, cigarette smoking and diabetes. These predictors are core risk factors included in the CUORE Project [6, 13] as well as in other 10-year risk equations [3, 4]. After a preliminary check on linearity, total- and HDL-cholesterol were included in the model as categorical variables in four standard classes [4, 22]. The interaction between systolic blood pressure and anti-hypertensive treatment was not statistically significant (p-value 0.84 in men and 0.12 in women, respectively). There was no evidence of any cohort effect in the full model, in men (3 df test p-value: 0.2) nor in women (p-value: 0.5). Finally, no violations in the proportional hazard assumption were observed using a standard test for time-dependent variables. Model calibration was assessed through the Grønnesby-Bogan goodness-of-fit test [23]. The Area Under the ROC-curve (AUC), as well as sensitivity and specificity in the top and bottom predicted risk quintiles, were computed taking censorship into account [24]. Correction for over-optimism and confidence intervals for the AUC were obtained through 1000 bootstrapped samples [25]. To assess the hypothesis of a loss in discrimination ability due to a longer prediction period, we estimated the 10-year predicted probability of event in our database, using the same set of risk factors but with shorter follow-up period, i.e. up to

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Comment [G4]: Added; reviewer 1, comment #2

the end of 2002 for all the subjects (number of events: 234 in men, 79 in women). ~~We then compared the AUC of both models, by considering bootstrapped confidence intervals for the difference in the betas.~~ We then compared the AUC of the two models by looking at their respective bootstrapped confidence intervals.

Comment [g5]: Added; reviewer 1, comment #10

Comment [G6]: Rewording of this sentence below; reviewer 1, comment #9

To assess the clinical utility of the long-term model for risk stratification, we considered two different public health goals. One is to decrease the number of events occurring among those considered at "low-risk". If we assume that a subject classified at "high risk" will be targeted for prevention (either lifestyle intervention or treatment), any event occurring outside this category is "not-identified" or "missed" by the prevention strategy. The second strategy aims instead to reduce un-necessary treatment, by decreasing the number of non-events among those considered at "high-risk". Under the two scenarios, "high-risk "subjects are defined as those with predicted risk above a certain cut-off value. Clinical utility is defined in terms of *i)* fraction of "missed" events; *ii)* probability of event among those classified at high risk; and *iii)* false positive/true positive ratio, for several threshold values in the 20-year predicted risk. We also provide a decision curve analysis based on the net benefit: Net Benefit = (true positives - w*false positives)/n, where n is the sample size and the weight w represents the ratio between the harm of un-necessary treatment and the harm of missing a case at that given value of predicted risk [17]. All the analyses were conducted using the SAS software 9.2.

RESULTS

N=5,426 (2,703 men) subjects were enrolled in the age range 35-69 years. N=205 subjects (3.8%; n=14 events) had at least one missing data; we considered data imputation (R *transcan* function, [26]) and excluded only those with missing values in more than 4 covariates of interest (n=6 men and n=3 women). Finally n=120 men and n=45 women with a

positive history of CVD at baseline were also excluded, reducing the sample size to 2,574 men and 2,673 women.

Baseline characteristics of the study population, by gender, are shown in **Table 1**. During a median follow-up time of 15 years (interquartile range: 12-20), we observed 315 first CVD events in men (233 coronary events) and 123 in women (n=85 coronary events). The Kaplan-Meier estimate for 20-year risk was 16.1% and 6.1% in men and women, respectively.

Model development

The beta-coefficients for the 20-year risk prediction model, as well as the baseline survival term and the calibration slope [25], are provided in the Supplementary Material (**Table S1)**. All the risk factors were statistically significant, except for anti-hypertensive treatment, though its point estimate reflected a 30% increase in hazard in both men and women; the variable was retained in the model for comparability with the short-term CUORE model [6]. There were no significant differences in the set of beta estimates for the 20-year model as compared to those from the 10-year risk model for the risk factors in the model (data not shown). The model calibration was satisfactory, in men (Grønnesby-Bogan goodness-of-fit chi-square 6.7, p-value 0.67) and in women (chi-square 9.6, p-value 0.38); calibration plots are available as supplementary material (**Figure S1**).

Comment [G7]: Added; reviewer 1, comment #8

We found no statistically significant difference in the overall discrimination ability between long- and short-term prediction models, in men (0.736 vs. 0.731) and in women (0.801 vs. 0.816; **Table 2)**. Only 5% of 20-year events in men occurred among subjects with a predicted risk below the 20th percentile (bottom ~~quintile~~fifth); the corresponding figure in women is 2%. The relative risk of event for being above the 80th percentile vs. below the 20th percentile of 20-year risk was 9.5 (i.e. 35.1/3.7) in men and 22.4 (i.e. 20.2/0.9) in women. Finally, the value of the 80th percentile for 20-year risk was more than twice as high than the similar

Comment [g8]: Reviewer 1, comment #7

percentile for 10-year risk in men (26.8 vs. 10.8) and more than three times as high in women (10.1 vs. 3.0). A similar consideration holds for the 20th percentile of risk or the median value.

Clinical utility

Table 3a and **Table 3b** describe strategies for the identification of high-risk subjects, based on predicted 20-year risk, in men and women respectively. A cut-off value of 10% twenty year risk in men would result in a 9% of "missed" events (i.e. events among those with predicted risk below the cut-point), with a probability of event of 23% and one true positive for every 3.4 false positive subjects (**Table 3a**). In the second scenario, by choosing the 20% twenty year risk threshold value, the fraction of missed events was 36%. Note that about 30% of events occurred for a predicted 20-year risk between 20% and 30%. Finally, using the number of risk factors to define high risk subjects would result in a higher fraction of missed events, with no changes in specificity or in the prevalence of subjects considered at high risk. Among women, a cut-off value of 2% would result in a 5% of missed events, with a probability of event of 9% and a true positive for every 10.1 false positive women (**Table 3b**). In the second scenario, the probability of event among those with absolute risk greater than 10% was 20.4%, with a true positive for every 3.9 false positive subjects. However, the fraction of missed events would be 32%; this number can be reduced by lowering the cut-off value to 8%. By considering at high risk those with 2 or more risk factor would result in a higher fraction of missed events, with no gain in specificity or in the probability of event in the group. **Figure 1** illustrates the decision curve analysis based on the Net Benefit [17], for men (left) and women (right). The figure suggests a greater net benefit for the predicted risk with respect to the number of risk factors over the whole range of values, thus generalizing the findings from Table 3a and Table 3b.

DISCUSSION

In this paper we present the 20-year prediction model of first major coronary or ischemic stroke event in a Northern Italian population of men and women aged 35 to 69 years at baseline. To our knowledge, this is the first long-term prediction model in a low-incidence, European population. The discrimination ability of the long-term model did not significantly drop with respect to a 10-year risk prediction model derived on the same population. Risk stratification based on the predicted 20-year risk can be modulated according to different prevention aims, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment. Under both scenarios, the predicted 20-year risk showed an overall better Net Benefit with respect to a risk stratification based on the number of risk factors.

Our data confirmed previous findings on predictiveness of a single measurement of risk factors on long-term CVD risk, in the Italian [27] as well as in other populations [10, 14]. Event discrimination for the 20-year risk prediction model did not change significantly from 10-year's, although in women it decreased from 0.814 to 0.801. In the Framingham Offspring Study updating the baseline measurement of blood pressure and lipids with a later assessment poorly affected model discrimination and reclassification [28] and cardiometabolic risk factors clustering has been found to be quite stable over time [29].

As in the Framingham population, in our study the long-term predicted risk was more than simply *n*-times the short-term risk prediction [10]. In addition in the age range 35 to 49 years, the long-term predicted risk in subjects with 1 or more non-optimal or elevated risk factors (defined as in [7]) was 3-times the short-term risk in men, and 4-times in women (see **Figure S~~1~~2** in the Supplementary Material). This conveys the importance of long-term prediction for early identification of young subjects and women at increased likelihood of

Comment [g9]: Calibration plot has been added as Figure S1, and this becames Figure S2

event during their remaining lifespan. We observed in our data a modest net reclassification improvement (computed as in [24]) for the 20-year risk prediction model over the re-calibrated 10-year risk, in men (1.8%) and in women (4.5%). The net reclassification increased when we considered subjects with a low 10-year predicted risk but a cluster of 2 or more risk factors (5.4% and 7.6% in men and women, respectively; data not shown). Subjects' stratification is often based on arbitrarily-chosen thresholds of predicted risk [15], which may limit the clinical utility of risk prediction models [16]. We considered two strategies for the identification of "high-risk" subjects with contrasting goals, either to decrease the fraction of missed events or to decrease un-necessary treatment. These can be implemented by choosing threshold values for the predicted risk driven by either sensitivity or by specificity, respectively. Despite the lowering costs of statin treatment with respect to the costs of one un-prevented event, the high sensitivity scenario was not cost-effective over a 10-year period [30]. ~~might not be cost-effective [30].~~ These two scenarios might be combined to adopt a more complex risk stratification, as often present in clinical practice [1-2, 12]. For instance, if we consider at "low-risk" the 36% of men with 20-year absolute risk less than 10%, the fraction of missed events would be 9%, i.e. 31 first events in 20 years. About 31% of men with absolute risk between 10% and 20% could be addressed for lifestyle modification or treatment according to the presence of specific risk factors; this category accounts for about 20% of cases. Finally, the 33% of men with predicted risk above the 20% could be targeted with treatment intervention; they account for 68% of events, and out of 3.2 treated men, one is a case. A similar stratification can be provided for women, with different threshold values reflecting gender-specific underlying risk as for the cardiovascular age assessment [15].

Comment [G10]: Modified. See reviewer 2, comment #3

Among the study strengths and limitations, our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. The underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. A major limitation is the lack of an external validation. External validation for long-term prediction models is in general an issue [10]; we provide the over-optimism adjusted AUC as well as the ~~, although we provide As Supplementary Material we provide the baseline survival term as well as a~~ calibration slope [25] to allow applying our equation to different contexts (see Supplementary Material). ~~However, a validation study in a different population might be desirable to investigate the generalizability of our findings.~~ We also mention a high-quality of follow-up procedures, including case ascertainment for non-fatal events [21] and a consistent event validation according to MONICA criteria, resulting in a Standardized Incidence Rate for the study cohorts above 1 over the whole follow-up period [18]. Finally, the study endpoint reflects the clinical need to treat the "global" ischemic risk of a given patient, and not its separate components [3].

In conclusions, we provide a model to predict long-term risk of first major ischemic cardiovascular event in a low-incidence population. Risk stratification based on long-term risk can be clinically useful, especially for young subjects and women. A clinical utility analysis is required to identify the optimal stratification, according to different public health goals.

Comment [G11]: Modified; see reviewer 2, comment #5

Comment [G12]: Deleted; see reviewer #2, comment #5

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Competing Interests

None.

Contributors

Conception and design: MMF, GM, GC and GV. All authors interpreted the data and critically reviewed the paper. Statistical analyses: GV, WC. GV drafted the manuscript and is the guarantor. All authors have read and approved the final version of the manuscript.

Data sharing

There is no additional data available

ARTICLE SUMMARY

Article focus

- Primary prevention of cardiovascular disease (CVD) has been recently moved towards the concepts of "lifetime" and "long-term" risk, especially in young subjects and women.
- There is no long-term risk prediction model available for low-incidence Southern European populations; in addition, the evaluation of the clinical benefit of long-term prediction has not been provided so far.
- We aim to develop a 20-year risk score equation in a northern Italian population of men and women considered at low incidence of major cardiovascular events; and to evaluate the clinical utility of the model for risk stratification in primary CVD prevention program.

Key Messages

- In our population, the 20-year risk model had satisfactory discrimination ability as compared to short-term risk prediction. The importance of long-term prediction for early

identification of young subjects and women at increased likelihood of event during their remaining lifespan is confirmed.

- Risk stratification based on the predicted 20-year risk had a better clinical Net Benefit with respect to a stratification based on the number of risk factors, in men and women.
- In both genders, the optimal treatment allocation based on 20-year risk can be determined according to different public health strategies, i.e. either to reduce the fraction of events potentially un-prevented or to avoid un-necessary treatment.

Strengths and limitations of this study

- Our sample comprises subjects drawn from a representative northern Italian population, with a satisfactory participation rate. We also mention the high-quality of follow-up procedures, including case ascertainment for non-fatal events and a consistent event validation according to MONICA criteria over the whole follow-up period.
- Our 20-year risk prediction model is the first attempt to characterize long-term risk of first coronary or ischemic stroke event in a low-incidence European population. A limitation of our study is the lack of a formal external validation, although we provide a cross-validation analysis. ~~To allow applying our equation to different populations, as Supplementary Material we provide the baseline survival term as well as the calibration slope. However, an external validation study might be desirable.~~ We also remind that our underlying population is characterized by high levels of industrialization and urbanization, with one of the highest average incomes in Italy. Caution is therefore required before generalizing our findings to different contexts.

Comment [G13]: Added; see reviewer 2, comment #5

Comment [G14]: Deleted; see reviewer 2, comment #5

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Tables and figures

Table 1. Baseline characteristics (mean (SD) or %) of the study population and number of incident events, by gender. Men and women, 35-69 years old, CVD-free at baseline.

	Men (n=2574)	Women (n=2673)
Age (years)	50.8 (9.1)	50.3 (9)
Years of schooling	8.5 (4.2)	7.3 (3.4)
Total Cholesterol (mmol/L)	5.8 (1.1)	5.8 (1.1)
HDL-Cholesterol (mmol/L)	1.3 (0.3)	1.6 (0.4)
Body Mass Index (Kg/m^2)	26.2 (3.5)	25.6 (4.7)
Systolic Blood Pressure (mmHg)	134.8 (19.3)	131.6 (20.2)
Diastolic Blood Pressure (mmHg)	85.9 (10.6)	82.8 (10.8)
Anti-hypertensive treatment (%)	11.8	16.0
Fasting plasma glucose (mmol/L)	5.4 (1.3)	5.1 (1.2)
Diabetes (%)	6.7	4.0
Current smoker (%)	37.1	19.6
Incident coronary event (n)	233	85
Incident ischemic strokes (n)	99	43
Incident CVD event (n)	315	123
20-year absolute risk of CVD^	16.1	6.1

^: Kaplan-Meier estimate.

Table 2. Discrimination ability for the 10-year and the 20-year risk prediction models.
Men and women, 35-69 years old, CVD-free at baseline

	Men		Women	
	10-year risk	**20-year risk**	**10-year risk**	**20-year risk**
AUC (95% CI)	0.731 (0.702; 0.761)	0.737 (0.713; 0.764)	0.814 (0.779; 0.853)	0.801 (0.771; 0.833)
Subjects with predicted risk below the 20th percentile				
20th percentile of risk	2.3	6.3	0.3	1.1
Fraction of events* (%)	4.4	5.1	1.4	2.0
Probability of event in the group^ (%)	0.8	3.7	0.2	0.9
Subjects with predicted risk above the 80th percentile				
80th percentile of risk	10.8	26.8	3.0	10.1
Sensitivity* (%)	49.9	45.6	68.7	62.0
Specificity (%)	82.4	85.5	81.1	83.1
Probability of event in the group^ (%)	19.4	35.1	7.5	20.2

The Area Under the ROC-curve (AUC) was estimated taking censorship into account, and adjusting for over-optimism (n=1000 bootstrap).
*: Probability of belonging to the group, given that the subject is a case. ^: Kaplan-Meier estimate of the probability of event in the group.

Table 3a. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment. Men, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2574	100.0	0.0	-	16.1	5.2
1+ Major Risk Factor[#]	1842	71.6	13.7	32.5	19.5	4.1
20-year absolute risk > 10%	1645	63.9	9.1	41.2	22.9	3.4
20-year absolute risk > 15%	1169	45.4	22.1	60.9	27.7	2.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors[#]	828	32.2	50.4	73.6	24.9	3.0
20-year absolute risk > 20%	841	32.7	35.7	73.7	31.7	2.2
20-year absolute risk > 30%	415	16.1	62.6	88.9	37.4	1.7

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Table 3b. Identification of high risk subjects based on the 20-year risk prediction model with respect to the number of risk factors, according to strategies aiming to *i)* reducing the fraction of missed events; and *ii)* reducing un-necessary treatment.
Women, 35-69 years old, CVD-free at baseline

	Subjects at high risk		Fraction of missed events (%)	Specificity (%)	Probability of event* (%)	FP/TP Ratio
	n	%				
***Strategy a*: reduce the fraction of missed events**						
All subjects	2673	100.0	0.0	-	6.1	15.3
1+ Major Risk Factor[#]	1654	61.9	17.7	40.1	8.2	11.3
20-year absolute risk > 2%	1733	64.8	4.5	37.4	9.0	10.1
20-year absolute risk > 5%	1067	39.9	14.7	63.2	13.1	6.6
***Strategy b*: reduce un-necessary treatment**						
2+ Major Risk Factors[#]	640	23.9	42.3	79.5	14.8	5.8
20-year absolute risk > 8%	698	26.1	22.7	77.1	18.2	4.5
20-year absolute risk > 10%	545	20.4	32.1	82.7	20.4	3.9

"Missed" events are events occurring among subjects not classified at "high risk", i.e. with 20-year absolute risk (or a number of risk factors) below the cut-off point.
*: Kaplan-Meier estimate of the probability of event in the group (positive predicted value).
FP = Number of False Positives; TP = Number of True Positives
#: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Figure 1: Decision curve for the 20-year risk prediction model in the CAMUNI population, Northern Italy. Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

Net Benefit: (TP-w*FP)/n, where TP = True Positive; FP = False Positive; w = (Absolute risk threshold)/(1- (Absolute risk threshold)); n=sample size
Number of risk factors: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

Supplementary material

Table S1: Beta-coefficients, standard errors and baseline survival for the 20-year risk prediction model in Northern Italy. Men and women, 35 to 69 years old, free of CVD at baseline.

	Men			Women		
	Beta	**SE**	**p-value**	**Beta**	**SE**	**p-value**
Age (years)	0.058	0.008	<.0001	0.084	0.014	<.0001
Total Cholesterol^						
200-240 mg/dl	0.388	0.161		0.553	0.281	
240-280 mg/dl	0.690	0.167	<.0001	0.607	0.316	0.027
> 280 mg/dl	0.923	0.198		0.996	0.328	
HDL-Cholesterol°						
<45 mg/dl	0.403	0.160		0.804	0.256	
45-50 mg/dl	0.367	0.186	0.013	0.364	0.309	0.015
50-60 mg/dl	0.024	0.177		0.261	0.227	
Systolic Blood Pressure (mmHg)	0.011	0.003	0.0003	0.015	0.005	0.001
Anti-hypertensive treatment (yes/no)	0.247	0.154	0.11	0.267	0.209	0.20
Smoking (yes/no)	0.521	0.117	<.0001	0.994	0.216	<.0001
Diabetes (yes/no)	0.744	0.163	<.0001	1.020	0.245	<.0001
Baseline 20-year survival ($S_0(20)$)*****		0.94168			0.98502	
G(μ)		4.35638			6.20915	
Calibration Slope		0.948			0.937	

SE = Standard Error. ^: reference group: total cholesterol<=200 mg/dl. °: reference group: HDL-cholesterol >60 mg/dl. *: at the mean value for continuous RFs, and at the reference class for categorical variables. 20-year risk: 1- $S_0(20)^{\exp(\sum \beta X - G(\mu))}$. Calibration slope: correction term to be used in different population to shrink the beta-coefficients. See reference [25] for more details. The risk model should be used within the following range for continuous risk factors: total cholesterol 135-330 mg/dl; HDL-cholesterol 30-100 mg/dl; systolic blood pressure 100-190 mmHg.

Figure S1: Calibration plot for the final 20-year risk model. Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

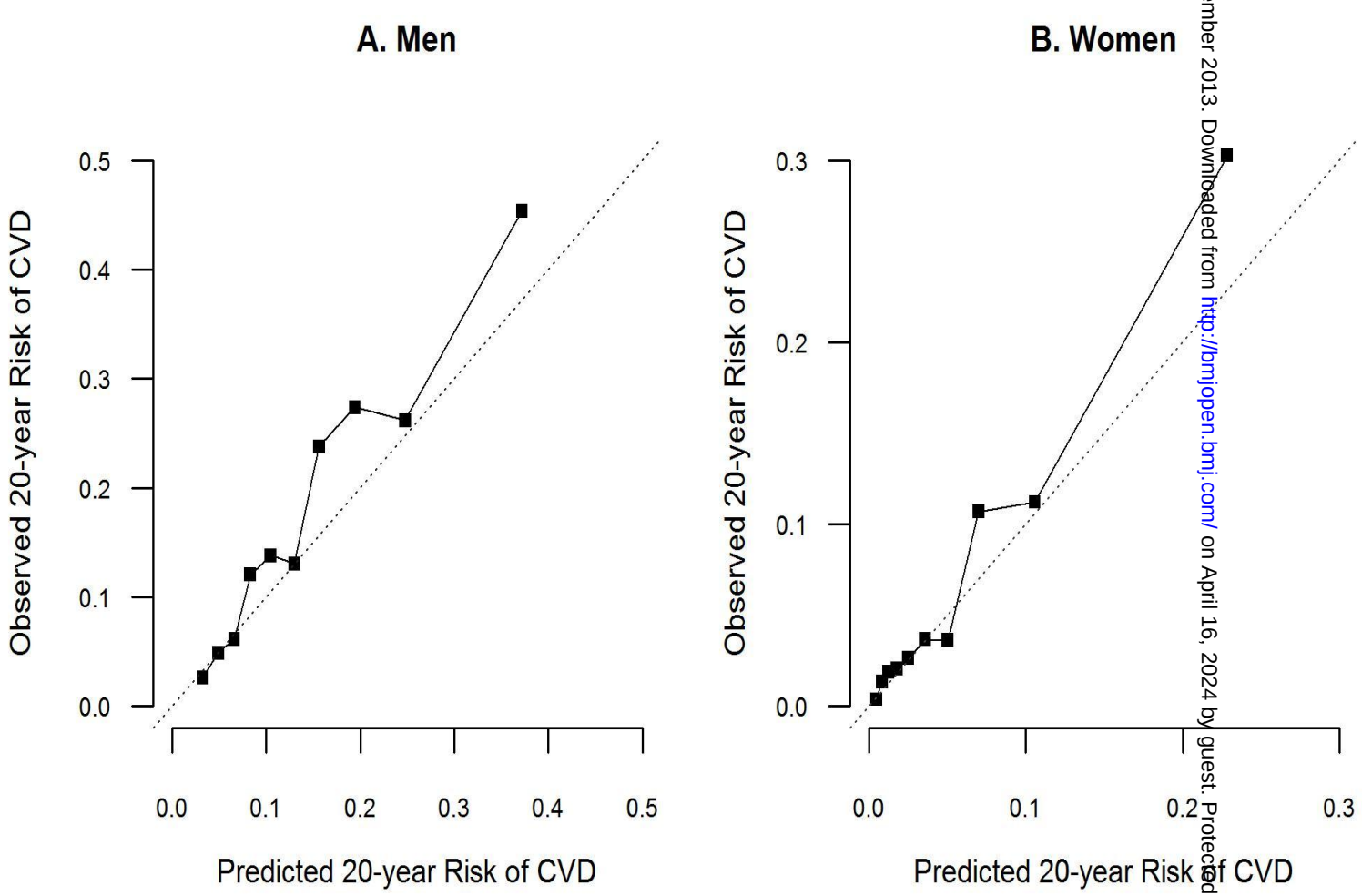
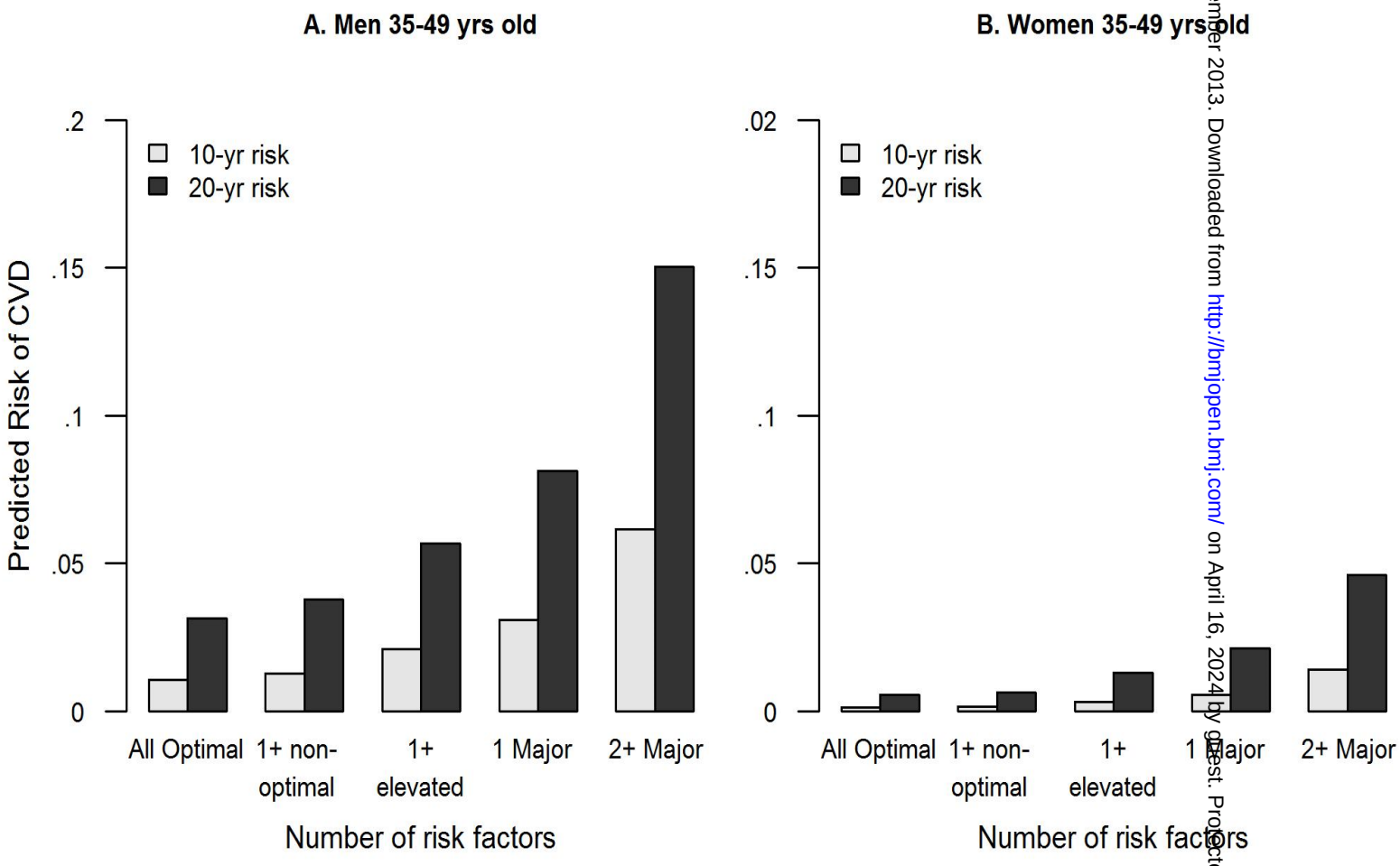


Figure S2: Distribution of predicted 10-year and 20-year risk of first major CVD event, according to the number of risk factors. Men (left) and women (right), 35 to 49 years old, free of CVD at baseline.



Risk factors stratification derived from Lloyd-Jones [7].
All optimal: total cholesterol <180 mg/dl, HDL-Cholesterol >= 40 mg/dl [men] or >= 50 mg/dl [women], blood pressure <120/80 mmHg, non smoker, non diabetic;
1+ non-optimal: total cholesterol 180 to 199 mg/dl, systolic blood pressure 120 to 139 mmHg, diastolic blood pressure 80 to 89 mmHg, non smoker, non diabetic
1+ elevated: total cholesterol 200 to 239 mg/dl, systolic blood pressure 140 to 159 mmHg, diastolic blood pressure 90 to 99 mmHg, non smoker, non diabetic
Major risk factor: total cholesterol >=240 mg/dl, HDL-Cholesterol <40 mg/dl [men] or <50 mg/dl [women], systolic blood pressure>=160 mmHg or treatment, diastolic blood pressure >=100 mmHg, smoker, or diabetic

Table S2: Baseline characteristics of the study population, by study cohort, for risk factors included in the risk prediction model. Men and women, 35 to 69 years old, free of CVD at baseline.

	Cohort study name			
	MONICA 1	**MONICA 2**	**PAMELA**	**MONICA 3**
Recruitment period	1986-87	1989-90	1990-93	1993-94
Number of subjects	1259	1255	1442	1291
Age, years	49.4 (8.6)	49.6 (8.8)	52.5 (9.8)	50.3 (8.7)
Men, %	48.0	49.2	49.9	49.0
Total cholesterol, mg/dl	216.5 (43.3)	215.7 (42.7)	228.6 (42.3)	229.9 (41.7)
HDL-cholesterol, mg/dl	55.5 (14.4)	56.2 (14.6)	55.9 (15.9)	57.1 (15.1)
Systolic Blood Pressure, mmHg	136 (20)	132.3 (19.3)	133.6 (20)	130.6 (19.7)
Anti-hypertensive treatment, %	11.0	13.7	17.6	12.8
Current smokers, %	30.7	27.5	28.3	26.3
Diabetes, %	6.4	6.5	4.4	4.3

Data are mean values (SD) for continuous variables, and % for dichotomous risk factors.

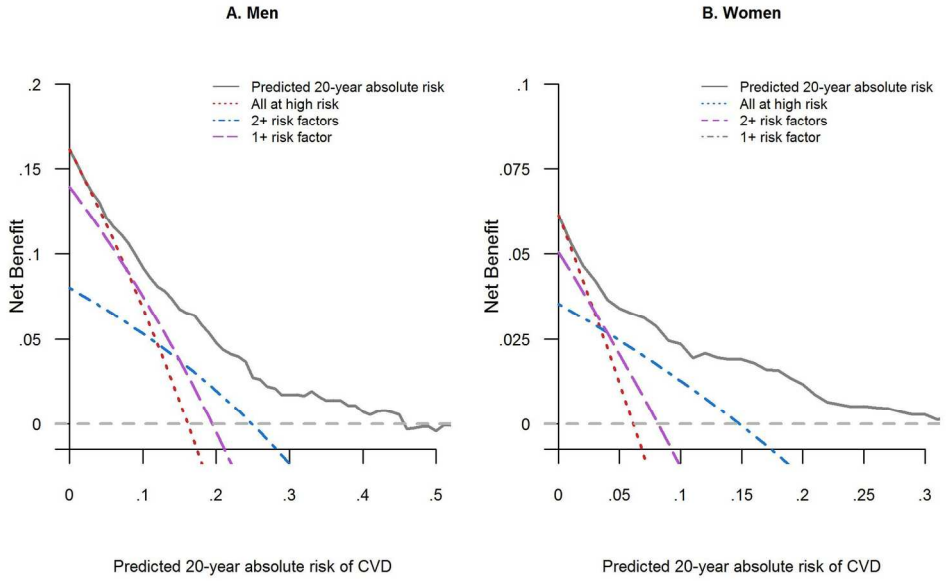


Figure 1: Decision curve for the 20-year risk prediction model in the CAMUNI population, Northern Italy. Men (left) and women (right), 35 to 69 years old, free of CVD at baseline.

Net Benefit: (TP-w*FP)/n, where TP = True Positive; FP = False Positive; w = (Absolute risk threshold)/(1-(Absolute risk threshold)); n=sample size
Number of risk factors: total cholesterol>240 mg/dl; HDL-cholesterol <40 [men] or <50 [women] mg/dl; systolic blood pressure >160 mmHg; smoking; diabetes

180x120mm (300 x 300 DPI)

Supplementary material for the paper:

Long-term prediction of major coronary or ischemic stroke event in a low-incidence European population: model development and evaluation of clinical utility.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Actions
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	The study setting is clearly stated in the abstract.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Done
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	See the introduction section at pages 3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	See page 4, end of introduction section
Methods			
Study design	4	Present key elements of study design early in the paper	See the Methods section (pages 4-7)
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Relevant information on cohorts setting, location and periods of recruitment are provided in the paragraphs "Study population" (page 4), "Baseline assessment of risk factors" (page 4) and "Study endpoint and follow-up procedures" (page 5).
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	See the paragraphs "Study population" (page 4) and "Study endpoint and follow-up procedures" (page 5).
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not applicable
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	See the paragraph "Statistical analysis", page 6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	See paragraphs "Baseline assessment of risk factors" (page 4), and "Statistical analysis" (page 6-7). Exposure group: not applicable for this analysis.
Bias	9	Describe any efforts to address potential sources of bias	See the Methods section.
Study size	10	Explain how the study size was arrived at	See the first period in the "Results" section (page 7)
Quantitative	11	Explain how quantitative variables were	See the "Statistical Analysis" paragraph

variables		handled in the analyses. If applicable, describe which groupings were chosen and why	(page 6)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	See the "Statistical Analysis" paragraph (page 6)
		(*b*) Describe any methods used to examine subgroups and interactions	See the "Statistical Analysis" paragraph (page 6)
		(*c*) Explain how missing data were addressed	See the first line in the "Results" section (page 7)
		(*d*) If applicable, explain how loss to follow-up was addressed	See the "Statistical Analysis" paragraph (page 6) for details on the survival analysis techniques
		(*e*) Describe any sensitivity analyses	Not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Participation rates are reported in the paragraph "Study population" (page 4). Exposure group: not applicable for this analysis.
		(b) Give reasons for non-participation at each stage	Not applicable
		(c) Consider use of a flow diagram	Not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	See Table 1. Exposure group: not applicable for this analysis.
		(b) Indicate number of participants with missing data for each variable of interest	See the "Results" section, first period (page 7). Exposure group: not applicable for this analysis.
		(c) Summarise follow-up time (eg, average and total amount)	"Results" section, second period (page 7). Exposure group: not applicable for this analysis..
Outcome data	15*	Report numbers of outcome events or summary measures over time	Number of events, by type, are reported in Table 1. Exposure group: not applicable for this analysis.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	The study model is reported in Table S1, supplementary material; the analysis is multivariable by nature.
		(*b*) Report category boundaries when continuous variables were categorized	Not applicable
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Not applicable

Discussion			
Key results	18	Summarise key results with reference to study objectives	See the first part of the Discussion section, page 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Study limitations are reported and discussed at pages 11-12.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Done
Generalisability	21	Discuss the generalisability (external validity) of the study results	See pages 11-12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Source of funding is reported at page 12.

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.