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Combining risk markers improves cardiovascular risk prediction in women

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Abstract

Cardiovascular risk stratification could be improved by adding measures of atherosclerosis to current risk scores, especially in intermediate-risk individuals. We prospectively evaluated the additive value of different non-invasive risk markers (both individual and combined) for gender-specific cardiovascular risk stratification on top of traditional risk factors in a middle-aged population-based cohort. Carotid-plaques, IMT (intima–media thickness) , ABI (ankle–brachial index), PWV (pulse–wave velocity), AIx (augmentation index), CAP (central augmented pressure) and CSP (central-systolic pressure) were measured in 1367 CVD (cardiovascular disease)-free participants aged 50–70 years old. Cardiovascular events were validated after a mean follow-up of 3.8 years. AUC (area-under-the-curve) and NRI (net reclassification improvement) analyses (total-NRI for all and clinical-NRI for intermediate-risk groups) were used to determine the additive value of individual and combined risk markers. Cardiovascular events occurred in 32 women and 39 men. Traditional cardiovascular risk factors explained 6.2% and 12.5% of the variance in CVD in women and men respectively. AUCs did not substantially increase by adding individual or combined non-invasive risk markers. Individual risk markers only improved reclassification in intermediate-risk women and more than in men; clinical-NRIs ranged between 48.0 and 173.1% in women and 8.9 and 20% in men. Combined non-invasive-risk markers improved reclassification in all women and even more in those at intermediate risk; 'IMT-presence-thickness-of-plaques' showed largest reclassification [total-NRI = 33.8%, $P = 0.012$; IDI (integrated-discrimination-improvement) = 0.048, $P = 0.066$; clinical-NRI = 168.0%]. In men, combined non-invasive risk markers improved reclassification only in those at intermediate risk; 'PWV-AIx-CSP-CAP-IMT' showed the largest reclassification (total-NRI = 14.5%, $P = 0.087$; IDI = 0.016, $P = 0.148$; clinical-NRI = 46.0%). In all women, cardiovascular risk stratification improved by adding combinations and in women at intermediate risk also by adding individual non-invasive risk markers. The additive value of individual and combined non-invasive risk markers in men is limited to men at intermediate risk only, and to a lesser extent than in women.

Key words: arterial stiffness, cardiovascular disease, gender specific, intima–media thickness, non-invasive, risk stratification

INTRODUCTION

Although the number of cardiovascular deaths has decreased, an increase is anticipated again because of increasing prevalence of obesity [1,2]. Atherosclerosis is the underlying gradual process that finally leads to cardiovascular events. Despite the identification of many cardiovascular risk factors such as obesity, hypertension, lipid disorders, smoking and diabetes mellitus that promote atherosclerosis, it remains unclear why some persons develop CVD (cardiovascular disease) and why others do not. Many cardiovascular events occur in individuals who were not identified as high-risk patients according to the currently used cardiovascular risk algorithms in primary prevention [3]. Besides that, a large body of evidence showed gender-related differences in cardiovascular epidemiology with marked differences in disease prevalence and outcomes between men and women [4,5]. Therefore research over the last few years has focused on new cardiovascular risk markers, but none appeared to have

Abbreviations: ABI, ankle–brachial index; ABI-ex, ABI after exercise; AIx, augmentation index; AUC, area under the curve; CAP, central augmented pressure; CSP, central systolic pressure; CVD, cardiovascular disease; HR, hazard ratio; IDI, integrated-discrimination improvement; IMT, intima–media thickness; NRI, net reclassification improvement; PWV, pulse–wave velocity; TC/HDL-c ratio, total cholesterol/high-density lipoprotein–cholesterol ratio.

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additional value in cardiovascular risk prediction beyond traditional risk factors [6,7]. By measuring morphological and functional abnormalities non-invasively in the arterial wall, the impact of all known and unknown cardiovascular risk factors together can be determined. In individuals at intermediate risk, evidence is accumulating that several individual non-invasive risk markers may have additive value for cardiovascular risk prediction [8–10]. The different non-invasive risk markers reflect distinct aspects of the atherosclerotic damage, which is also demonstrated by the only modest correlations between several non-invasive risk markers [11,12]. Therefore it can be hypothesized that combining different non-invasive risk markers can further improve cardiovascular risk stratification. Up to now only few studies reported on the additive predictive value of non-invasive risk markers for CVD in the general population and evaluated men and women separately and the results have been conflicting [13–15].

The aim of the present study was to determine the gender-specific additive predictive value (independent of traditional cardiovascular risk factors) of individual and different combinations of non-invasive risk markers in a community-based cohort, in order to improve cardiovascular risk stratification in clinical practice.

MATERIALS AND METHODS

Patients

In total 1517 participants from the NBS (Nijmegen Biomedical Study), aged 50–70 years, were included, as described previously [16,17]. Baseline measurements were performed from 2005 to 2008 and a follow-up questionnaire was sent between December 2010 and July 2011. For the current analyses, participants with CVD at baseline ($n = 150$), who violated the study protocol ($n = 8$) and with an ABI (ankle–brachial index)>1.4 ($n = 45$) were excluded, resulting a sample of 1314 participants.

Clinical, biochemical and non-invasive cardiovascular risk markers were determined at baseline as described previously [17]. All non-invasive measurements of (subclinical) atherosclerosis measurements were performed after an overnight fast or in the afternoon 6 h after a standardized breakfast. Participants were asked to abstain from caffeinated products and not to smoke for at least 12 h before the visit. No significant differences were reported between measurements performed in the morning and afternoon after the measurement conditions had been standardized and reproducibility was good [18]. All measurements were performed with participants in supine position after at least 10 min rest in a temperature-controlled room (23–24 °C) and performed by well-trained and certified sonographers according to highly standardized protocols.

PWV (pulse–wave velocity), AIx (augmentation index), CAP (central augmented pressure) and CSP (central systolic pressure)

Peripheral arterial pressure waveforms were recorded by applanation tonometry at the radial artery. CAP and CSP were derived and AIx was calculated using the commercially available SphygmoCor system version 7.1 (Atcor Medical). As AIx is influenced by the heart rate, an index normalized for a heart rate of 75 beats/min was used. To determine PWV, pulse waveforms were recorded at two sites sequentially (right carotid artery and left femoral artery), and wave-transit time was calculated using the R-wave of a simultaneously recorded ECG as a reference frame. All measurements had to meet the criteria of optimal quality as defined by the manufacturer.

IMT (intima–media thickness), and the presence and thickness of carotid plaques

Carotid IMT was determined using an AU5 Ultrasound machine (Esaote Biomedical) with a 7.5 MHz linear-array transducer. Actual measurement of the IMT was performed off-line by the sonographer at the time of the examination, using semi-automatic edge-detection software (M'Ath® Std version 2.0, Metris). We measured IMT of the common carotid artery and IMT was defined as the mean IMT of four measured segments of the common carotid artery: far wall left, near wall left, far wall right and near wall right. In addition, the entire common, internal and external carotid arteries were investigated on both sides to detect the presence of plaques (defined as thickening of the wall of at least 1.5× the mean IMT) and plaque thickness was measured.

ABI at rest and after exercise

Appropriately sized cuffs were placed around both arms above the elbow and around both legs just above the ankle. Resting blood pressures were measured at the left and right brachial artery and the left and right posterior tibial and dorsalis pedis arteries using an 8 MHz hand-held Doppler probe (IMEXDOPCT + ™, Biomedic). The highest of the two arm pressures was used to calculate ABI at rest for the posterior tibial and dorsalis pedis arteries. The lowest of the four ABIs was used in the analysis. In addition, participants were asked to perform a treadmill test. They were instructed to walk on a treadmill for 4 min at a speed of 2 miles/h and at an elevation of 10%. Immediately after the exercise test blood pressures were recorded at the arm with the highest pressure at rest and at both ankles. ABI-ex (ABI after exercise) was calculated for both legs. The lowest ABI-ex value was used in the analysis.

Cardiovascular events reported in the follow-up questionnaires were evaluated by hospital and general practitioner records; only verified CVD based on objective measures [such as ECG, coronary angiography, CT (computed tomography), MRI (magnetic resonance imaging) and ABI] were used in the analyses. Physicians were blinded for the non-invasive risk marker findings during the validation process. Events were classified according to the International Classification of Diseases, 10th edition (http://apps.who.int/classification/icd10). Clinical end points were determined as verified CHD (coronary heart disease) (codes I21-I46-I25-I50-R96-I20-I22-I24), cerebro-vascular disease (codes G45–I63) and PAD (peripheral arterial disease) (code I73). The Medical Ethics Committee of the Radboud University Nijmegen Medical Centre

approved the study protocol (which is in accordance with the Declaration of Helsinki) and all participants provided written informed consent.

Statistical analyses

Clinical characteristics and non-invasive risk markers are shown as means ± S.D. (Table 1). To determine the independent predictive value of the non-invasive risk markers we used Weibull proportional hazard models with adjustment for traditional cardiovascular risk factors, including age, gender, current smoking, systolic blood pressure and TC/HDL-c (total cholesterol/high-density lipoprotein-cholesterol) ratio, that is, the baseline-model (Table 2). Gender-specific cut-off values of the non-invasive risk markers were determined as values above the 75th percentile for IMT, PWV, AIx, CAP and CSP. The models of AIx, CAP and CSP were additionally adjusted for height. For ABI, the clinical cut-off value of 0.9 was used.

To determine the additive value of the individual and different combinations of non-invasive risk markers for future CVD on top of traditional cardiovascular risk factors, we compared AUCs (area-under-the-curves) for models with and without non-invasive risk markers added to the baseline model. To assess calibration, we compared explained variance (R^2). Model fit was evaluated using log-likelihood ratio test for global model fit and a Hosmer–Lemeshow test (Tables 3a and 3b for men and women respectively). The incremental effect of adding non-invasive risk markers was additionally evaluated using the NRI (net reclassification improvement) (Table 4). Risk groups were categorized as low (<10%), moderate (⩾10% to <20%) and high (⩾20%) risk. We evaluated whether there would be improvement in reclassification; that is, whether reclassification would assign persons who developed CVD to a higher risk category and those who did not develop CVD to a lower risk category [19]. Clinical NRI represents reclassification in intermediate-risk individuals. The IDI (integrated discrimination improvement) was determined to evaluate whether the increased explained variance between the models was significant. The IDI does not depend on the selection of risk categories that is inherent in reclassification tables and may be used as an objective indicator of reclassification improvement [19]. P values of <0.05 (two-sided) were considered significant. All analyses were performed using STATA 11.0 (StataCorp LP).

RESULTS

Baseline characteristics

Participants with missing data on future CVD were excluded ($n = 72$). Of the remaining 1242 participants, 46.9% were male. Subject characteristics are shown in Table 1, stratified by gender and CVD. During a mean follow-up of 3.8 years (range, 1 month–5.6 years), 39 men and 32 women developed CVD. The majority included CHD in both men (61.5%) and women (59.4%). A total of 29 participants died during follow-up and four died from CVD. Both men and women with CVD showed worse non-invasive risk markers; they had lower ABIs, thicker IMT and increased stiffness parameters compared with those without CVD. Men additionally had more and thicker carotid plaques.

Independent predictive value of individual non-invasive risk markers

The independent predictive value of individual non-invasive risk markers on top of traditional cardiovascular risk factors is shown in Table 2. In men, only CAP [HR (hazard ratio) = 2.2, $P = 0.043$], whereas in women ABI at rest (HR = 7.2, $P = 0.002$), ABI after exercise (HR = 2.6, $P = 0.039$) and IMT (HR = 2.7, $P = 0.012$) independently predicted future CVD.

Additive value of individual and combinations of non-invasive risk markers

Tables 3(a) and 3(b) summarize the measures of discrimination and calibration for individual and combined non-invasive risk markers added to the baseline-model for men and women respectively (models showing the largest improvements). In both men and women, adding individual or combined non-invasive risk markers did not result in substantial increases in AUC or explained variance. Traditional cardiovascular risk factors together explained 12.5% of the variance in future CVD in men, and only 6.2% in women, and the AUCs were 0.748 and 0.691 respectively.

Table 4 summarizes the results of NRI and IDI analyses. Analysing all risk groups together (total NRI), in both men and women, none of the individual non-invasive risk markers resulted in substantial reclassification when added to the baseline model, except for plaque thickness in women. Analysing only individuals at intermediate risk (clinical NRI), in men adding CSP (clinical NRI = 20%), plaque thickness (clinical NRI = 19.2%), presence of plaque (clinical NRI = 16.7%) and ABI at rest (clinical NRI = 13.6%) resulted in substantial reclassification. In intermediate-risk women, all individual risk markers resulted in substantial reclassification. Highest reclassification was observed for the models with plaque thickness or IMT added (clinical NRI of 173.1 and 102% respectively). In general, total NRI's and clinical NRIs were larger in women compared with men.

Combined non-invasive risk markers did not result in substantial reclassification in all men (total NRI), but only in intermediate-risk men, approximately half of the combinations showed clinical NRIs over 20%. Largest reclassification in men was observed for the combination of PWV-AIx-CSP-CAP-IMT with a total NRI of 14.5% ($P = 0.087$), an IDI of 0.016 ($P = 0.148$) and a clinical NRI of 46%. Combined risk markers in all women resulted in substantial reclassification in approximately half of the combinations. Adding IMT-presence-thickness-of-plaques combined resulted in the largest reclassification showing a total NRI of 33.8% ($P = 0.012$), an IDI of 0.048 ($P = 0.066$) and a clinical NRI of 168%. In women at intermediate risk, clinical NRIs were very high, with over half showing a clinical NRI over 100%.

DISCUSSION

The novel aspect of the present prospective population-based study is the evaluation of the additive value of combining different non-invasive risk markers for gender-specific cardiovascular risk stratification. In our extensive evaluation according to the

Table 1 Baseline characteristics of the men and women without a cardiovascular event versus those who developed CVD during follow-up

Continuous variables are presented as the mean followed by S.D. Dichotomy variables are presented as *n* followed by the percentage. Hypertension was defined as systolic and diastolic blood pressure ⩾ 140 mmHg and/or ⩾ 90 mmHg respectively, or anti-hypertensive therapy. Diabetes was defined as fasting glucose level ⩾7 mmol/l or diabetic therapy. *$P < 0.05$.

	Men		Women	
Characteristic	**CVD − (*n*=543)**	**CVD + (*n*=39)**	**CVD − (*n*=627)**	**CVD + (*n*=32)**
CVD (*n*)				
Angina		17 (43.6%)		17 (53.1%)
Myocardial infarction		8 (20.5%)		2 (6.3%)
Transient ischaemic attack		2 (5.1%)		6 (18.8%)
Cerebrovascular accident		3 (7.7%)		3 (9.4%)
Peripheral arterial disease		9 (23.1%)		4 (12.5%)
Age (years)	61.0 (5.9)	64.8 (5.8)*	60.3 (5.8)	62.9 (5.3)*
Pack years (years)	10.4 (14.9)	21.5 (19.6)*	9.2 (13.6)	11.5 (18.1)
Current smoking (*n*)	81 (14.9%)	13 (33.3%)*	105 (16.8%)	6 (18.8%)
Exercise sessions (per week)	1.16 (1.29)	1.31 (1.52)	1.31 (1.37)	1.16 (1.22)
Body mass index (kg/m^2)	26.7 (3.5)	27.0 (3.2)	26.3 (4.2)	26.4 (3.7)
Waist (cm)	98.7 (10.2)	100.5 (9.2)	88.8 (11.2)	88.5 (10.8)
Systolic blood pressure (mmHg)	129.3 (13.4)	139.6 (21.4)*	126.5 (15.9)	137.0 (18.1)*
Diastolic blood pressure (mmHg)	81.0 (9.2)	82.5 (9.9)	75.2 (10.5)	79.2 (11.3)*
Hypertension (*n*)	200 (36.8%)	21 (53.9%)*	195 (31.1%)	18 (56.3%)*
Anti-hypertensive therapy (*n*)	100 (18.4%)	13 (33.3%)*	142 (22.7%)	13 (40.6%)*
Total cholesterol (mmol/l)	5.7 (1.0)	5.7 (0.9)	6.1 (1.1)	6.1 (1.0)
Triacylglycerols (mmol/l)	1.45 (0.77)	1.65 (0.89)	1.31 (0.66)	1.49 (0.73)
High-density lipoprotein cholesterol (mmol/l)	1.29 (0.31)	1.30 (0.28)	1.57 (0.39)	1.50 (0.36)
TC/HDL-c ratio	4.61 (1.27)	4.54 (1.17)	4.08 (1.20)	4.27 (1.30)
Low-density lipoprotein cholesterol (mmol/l)	3.76 (0.91)	3.65 (0.79)	3.93 (0.96)	3.92 (0.94)
Apolipoprotein B (g/l)	1.00 (0.22)	1.00 (0.24)	1.01 (0.23)	1.05 (0.23)
Lipid-lowering therapy (*n*)	56 (10.3%)	8 (20.5%)*	55 (8.8%)	4 (12.5%)
Glucose (mmol/l)	5.3 (0.9)	5.7 (1.8)*	5.1 (0.8)	5.0 (0.5)
Type 2 diabetes (*n*)	26 (4.8%)	4 (10.3%)*	26 (4.2%)	0 (0.0%)
ABI at rest	1.13 (0.09)	1.06 (0.18)*	1.10(0.08)	1.04 (0.10)*
ABI after exercise	1.13 (0.14)	1.01 (0.28)*	1.11 (0.13)	1.04 (0.23)*
Mean IMT (mm)	0.85 (0.11)	0.93 (0.14)*	0.81 (0.10)	0.90 (0.17)*
Presence of plaque (*n*)	222 (40.8%)	27 (69.2%)*	195 (31.1%)	14 (43.8%)
Plaque thickness (mm)	2.34 (0.83)	2.67 (0.79)*	2.32 (0.77)	2.59 (0.76)
PWV (m/s)	9.9 (2.5)	11.3 (3.2)*	9.6 (2.4)	11.5 (4.5)*
Augmentation index	1.21 (0.08)	1.26 (0.07)*	1.30 (0.07)	1.34 (0.09)*
CAP (mmHg)	11.0 (5.8)	16.5 (8.1)*	16.4 (6.4)	20.1 (7.0)*
CSP (mmHg)	124.3 (14.7)	138.0 (20.6)*	124.0 (16.9)	133.7 (17.7)*

Table 2 Independent predictive value of the individual non-invasive risk markers for future CVD

Values are adjusted for age, gender, current smoking, systolic blood pressure and TC/HDL-c ratio.

	Men			Women		
Parameter	***n* (%)**	**HR**	***P***	***n* (%)**	**HR**	***P***
ABI at rest (⩽0.9)	7 (1.2)	1.9	0.350	9 (1.4)	7.2	0.002
ABI after exercise (⩽0.9 or difference between rest and exercise ⩾0.15)	64 (11.0)	1.3	0.528	56 (8.5)	2.6	0.039
IMT (men, ⩾0.92 mm and women, ⩾0.87 mm)	145 (24.9)	1.8	0.097	155 (23.6)	2.7	0.012
Presence of plaque	249 (42.7)	1.9	0.067	207 (31.6)	1.2	0.650
PWV (men, ⩾11.2 m/s and women, ⩾11.0 m/s)	136 (23.8)	1.0	0.990	161 (24.9)	1.4	0.448
Augmentation index (men, ⩾ 1.27 and women, ⩾1.35)	150 (26.0)	1.0	0.930	179 (27.5)	1.1	0.876
CAP (men, ⩾14.98 mmHg and women, ⩾20.51 mmHg)	141 (24.4)	2.2	0.043	161 (24.7)	0.9	0.737
CSP (men, ⩾135.5 mmHg and women, ⩾ 134.6 mmHg)	139 (24.1)	1.3	0.577	169 (25.9)	1.1	0.816

Table 3 Additive value of individual and combinations of non-invasive risk markers in men (a) and women (b) for the prediction of CVD on top of the traditional cardiovascular risk factors, indicated by measures of discrimination and calibration

The five models for individual and combinations of non-invasive tests showing the largest increase in AUC are shown. LR X^2, likelihood ratio X^2. HL X^2, Hosmer–Lemshow X^2. CVRF, cardiovascular risk factors; including age, gender, current smoking, systolic blood pressure and TC/HDL-c ratio. ABIR, ABI at rest.

(a) Men

Model	AUC CVRF and risk markers	Δ	*P* Δ	R^2 CVRF + risk markers (%)	LR X^2	*P*	HL X^2	*P*
Traditional cardiovascular risk factors	0.748			12.5	35.85	<0.001	7.08	0.528
Individual risk markers								
Presence of plaque	0.763	0.019	0.160	13.2	38.42	<0.001	8.90	0.351
CSP (mmHg)	0.761	0.019	0.240	14.2	41.34	<0.001	4.06	0.852
CAP (mmHg)	0.759	0.016	0.330	13.8	40.01	<0.001	12.83	0.118
Mean IMT (mm)	0.752	0.009	0.470	13.3	38.64	<0.001	5.68	0.683
PWV (m/s)	0.752	0.002	0.590	12.4	34.57	<0.001	6.42	0.601
Combined risk markers								
IMT, ABIR, PWV, ABI-ex and plaque	0.777	0.022	0.200	15.2	41.71	<0.001	2.74	0.950
IMT, ABIR, PWV and CAP	0.777	0.022	0.190	15.4	42.21	<0.001	2.07	0.979
IMT, ABIR, PWV, CAP and ABI-ex	0.776	0.022	0.200	15.5	42.29	<0.001	2.06	0.979
IMT, ABIR and plaque presence	0.775	0.028	0.110	15.2	43.46	<0.001	3.94	0.862
IMT, ABIR, ABI-ex and plaque	0.775	0.027	0.120	15.2	43.5	<0.001	3.97	0.860

(b) Women

Model	AUC CVRF and risk markers	Δ	*P* Δ	R^2 CVRF + risk markers (%)	LR X^2	*P*	HL X^2	*P*
Traditional cardiovascular risk factors	0.691			6.2	15.81	0.003	9.26	0.321
Individual risk markers								
ABI at rest	0.726	0.036	0.260	9.6	24.55	<0.001	14.75	0.064
Augmentation index	0.717	0.014	0.600	7.9	19.79	0.001	9.31	0.317
PWV (m/s)	0.717	0.013	0.540	7.8	19.30	0.002	12.96	0.113
CAP (mmHg)	0.711	0.009	0.490	7.3	18.08	0.003	6.09	0.637
CSP (mmHg)	0.706	0.003	0.740	7.1	17.67	0.003	3.77	0.875
Combined risk markers								
IMT, ABIR, PWV, CSP and ABI-ex	0.760	0.056	0.120	13.8	34.23	<0.001	12.70	0.123
IMT, ABIR, PWV, ABI-ex and plaque	0.760	0.056	0.160	14.2	35.24	<0.001	3.34	0.911
IMT, ABIR, PWV, CAP and ABI-ex	0.760	0.056	0.120	13.8	34.26	<0.001	6.04	0.643
IMT, ABIR, PWV, AIx and ABI-ex	0.759	0.056	0.120	14.1	35.12	<0.001	7.09	0.527
IMT, ABIR, PWV, ABI-ex and plaque	0.759	0.055	0.120	13.8	34.27	<0.001	14.73	0.065

scientific statement from the American Heart Association [20], in all women, many combinations of non-invasive risk markers showed additive value, which was even much higher in women at intermediate risk. The additive value of combined risk markers in men is limited to men at intermediate risk only, and to a much lesser extent compared with women.

Additive value of non-invasive risk markers

Conflicting results have been reported on the additive value of most individual risk markers to current standard care. Recently, Peters et al. [10] summarized the mostly modest NRIs for IMT (between −1.4 and 12.0%) and plaques (between 8 and 11%). All NRIs were largest in individuals at intermediate risk. In the present study, in intermediate risk women more than in men, substantial reclassification was observed. This is also consistent with a meta-analysis that concluded that measuring mean common carotid IMT may be worthwhile in intermediate-risk individuals, but no gender differences were reported [21]. In the ARIC (Atherosclerosis-Risk-In-Communities) study, ABI at rest did not improve risk stratification [22], whereas a recent meta-analysis showed that adding ABI to the Framingham risk score resulted in substantial reclassification of 19% in men and 36% in women [23]. The present ABI results are concordant with this meta-analysis: reclassification was larger in women than in men. The Framingham heart study showed that PWV improved cardiovascular risk prediction when added to standard risk factors [24], whereas in elderly of the Rotterdam study [14] PWV had no additive value. The latter reported similar values for men and women [14], whereas especially in intermediate risk, we report marked differences between men and women. Data on the additive value, as determined by NRI analyses of ABI after exercise, AIx, CAP and CSP in population-based cohorts are lacking to

Table 4 Additive value of the five individual and five combinations of non-invasive risk markers on top of the traditional cardiovascular risk factors showing the largest reclassification

Clinical-NRI, NRI in intermediate-risk category. CVRF, cardiovascular risk factors, including age, gender, current smoking, systolic blood pressure and TC/HDL-c ratio. ABIR, ABI at rest.

(a) Men

Parameter	Total NRI (%)	*P*	IDI	*P*	Clinical NRI (%)
Individual risk markers					
CSP (mmHg)	3.3	0.574	0.010	0.197	20.0
Plaque thickness (mm)	1.5	0.891	0.005	0.598	19.2
Presence of plaque	0.2	0.982	0.003	0.589	16.7
Mean IMT (mm)	−0.2	0.980	0.010	0.166	8.9
ABI at rest	−1.1	0.686	0.013	0.263	13.6
Combined risk markers					
PWV, AIx, CSP, CAP and IMT	14.5	0.087	0.016	0.148	46.0
IMT and presence and thickness plaque	10.1	0.399	0.018	0.226	22.7
PWV, AIx, CSP and CAP	7.1	0.376	0.006	0.523	31.9
IMT and plaque thickness	7.0	0.572	0.018	0.228	12.7
IMT, ABIR, PWV and CSP	6.2	0.429	0.028	0.086	41.0

(b) Women

Parameter	Total NRI (%)	*P*	IDI	*P*	Clinical NRI (%)
Individual risk markers					
Plaque thickness (mm)	30.2	0.020	0.036	0.068	173.1
ABI at rest	15.9	0.056	0.025	0.087	60
Mean IMT (mm)	9.4	0.186	0.026	0.066	102
ABI after exercise	8.9	0.207	0.036	0.105	48
PWV (m/s)	2.9	0.607	0.007	0.352	50.2
Combined risk markers					
IMT and presence and thickness plaque	33.8	0.012	0.048	0.066	168
IMT and plaque thickness	28.0	0.009	0.047	0.061	169.2
IMT and ABI-ex	22.5	0.007	0.054	0.032	130
IMT, ABIR, PWV, CSP and ABI-ex	22.1	0.024	0.071	0.006	106.9
IMT, ABIR and PWV	21.6	0.027	0.061	0.009	96.7

the best of our knowledge. The present study therefore extends current evidence.

Some reports compared many different risk factors including some non-invasive risk markers, but, so far, only a few studies reported on the combination of two or three risk markers and not all included full analyses of HR, AUC, NRI and IDI. Some studies reported that adding plaque combined with IMT improved cardiovascular risk stratification, especially in intermediate risk [25,26]. Nambi et al. [15] reported higher total- and clinical-NRIs in women compared with men for the additive value of IMT and presence of plaque combined. Concordant with these results, we observed larger additive value in women compared with men for all individual and combined non-invasive cardiovascular risk markers, whereas others showed that adding other measures to IMT did not further improve risk stratification [27].

Evaluating analyses to determine the additive value of risk markers

The question arises of whether NRI and IDI analyses provide explicit evidence on the additive value of biomarkers or measures of target organ damage, such as the non-invasive risk markers presented. NRI provides information on the proportion of correctly reclassified individuals, but, from a clinical point of view, all individuals who are reclassified, for whom a non-treatment strategy would change in a drug treatment strategy is important. We observed that reclassification occurs as appropriate in women; mostly upward reclassification in those who developed CVD and downward reclassification in those who did not develop CVD. In contrast, in men who developed CVD, a large proportion was falsely reclassified downwards (15–46%, results not shown). Taken together, we conclude that in the present middle-aged population-based cohort, risk stratification using traditional cardiovascular risk factors works reasonably well in men, but fails in women. Most importantly, in all women, combining non-invasive cardiovascular risk markers improved cardiovascular risk stratification.

Comparing NRI values from different studies is complicated, because many studies use different risk scores or take other risk factors into account. We used traditional cardiovascular risk factors continuously in the models to obtain the best fit. We extended our analyses by adding waist, BMI (body mass index), anti-hypertensive treatment and diabetes mellitus to the SCORE variables. Although some small differences were found, the conclusions remained the same (results not shown).

Another aspect that makes comparison of studies almost impossible is the use of different cut-off values of the non-invasive risk markers. Several guidelines already recommend some of the non-invasive risk markers to improve risk stratification in intermediate-risk individuals, including IMT, the presence of plaque, PWV and ABI. The cut-off values provided by these guidelines include an IMT value >0.9 mm or the presence of plaque as a sign of target organ damage [28,29]. In our population, we used gender-specific cut-off values above the 75th percentile as mentioned in the statistics update of the American Heart Association [30]; for men and women the 75th percentile of IMT was 0.92 and 0.87 mm respectively. For PWV they recommend a cut-off of 12 m/s; in our population the 75th percentile was 11.2 m/s for men and 11.0 m/s for women. Using the cut-off values according to the guidelines did not change the results (results not shown).

Limitations

Interpretation of the present results must be within the context of some limitations. A small number of events occurred during follow-up. Participants received lifestyle advice after their baseline visit, including information about their lipids, glucose and blood pressure. Whenever necessary, they received advice to discuss medical treatment with their general practitioner. There may be some bias towards the healthier individuals that participated in our sample, resulting in less advanced atherosclerosis, although over 70% of the participants showed at least one deteriorated risk marker, reflecting subclinical atherosclerosis, and over 80% of participants had one or more cardiovascular risk factors. Effect sizes, however, may therefore have been compromised. We only included participants aged 50–70 years old at baseline, and most were Caucasians, so the results cannot be extrapolated to other age or ethnic groups.

CLINICAL PERSPECTIVES

- Many cardiovascular events occur in individuals who were not identified as high risk patients according to the currently used cardiovascular risk algorithms in primary prevention. A large body of evidence showed gender-related differences in cardiovascular epidemiology, with marked differences in disease prevalence and outcomes between men and women. By measuring morphological and functional abnormalities non-invasively in the arterial wall, the impact of all known and unknown cardiovascular risk factors together can be determined.
- The present study shows that cardiovascular risk prediction using current CVD risk scores differs between men and women. The current risk scores seem to work reasonable well in men (although still not perfect), but in women the algorithms seem to fail. Furthermore, this study highlights the need for additional research to unravel the mechanisms in CVD in men and women separately, with a special focus on how CVD risk stratification can be improved in women; which risk factors do account for the events in women and better predict CVD?
- Until then, clinicians could use non-invasive risk markers to evaluate the women's risk, especially IMT, presence of plaques and ABI can provide clinical information on the presence of subclinical atherosclerosis in women at intermediate risk. If the measures are above the thresholds mentioned in several guidelines, the clinician might consider this patient to be at relatively higher risk.

AUTHOR CONTRIBUTION

Jacqueline de Graaf and Anton Stalenhoef were responsible for conception and design of the study and interpreted the data. Martin den Heijer and Suzanne Holewijn analysed and interpreted the data. Lambertus A. Kiemeney interpreted the data. Suzanne Holewijn drafted the paper. All authors revised the paper critically for important intellectual content and have read and approved the submitted paper.

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