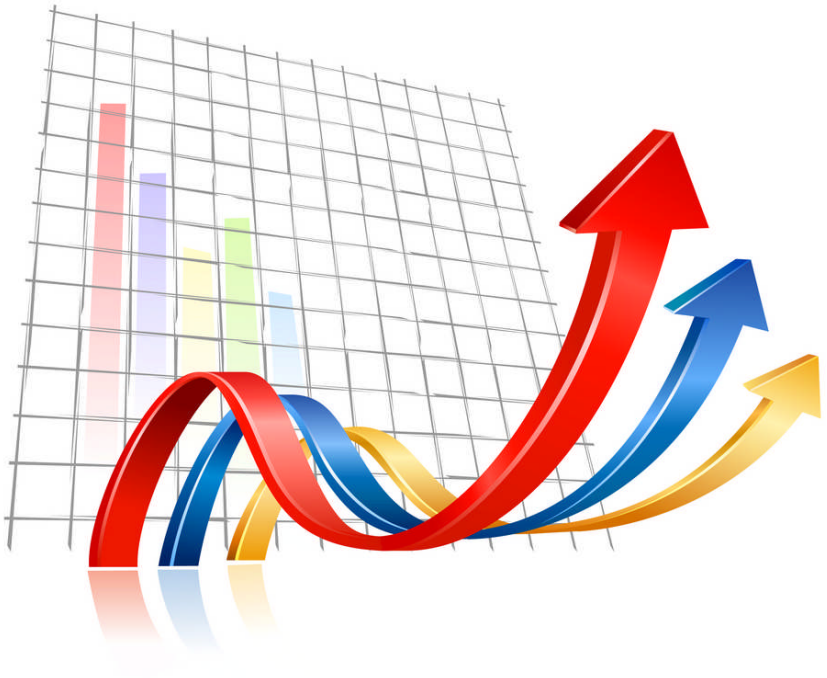
KCE REPORT 201

Federaal Kenniscentrum voor de Gezondheidszorg
Centre Fédéral d'Expertise des Soins de Santé
Belgian Health Care Knowledge Centre

NOVEL SERUM BIOMARKERS FOR THE PREDICTION OF CARDIOVASCULAR RISK



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HEALTH TECHNOLOGY ASSESSMENT

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NOVEL SERUM BIOMARKERS FOR THE PREDICTION OF CARDIOVASCULAR RISK

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AEs	Adverse Events
Apo	Apolipoprotein
AUC	Area Under the ROC Curve
BMI	Body Mass Index
CAD	Coronary Artery Disease
CHD	Coronary Heart Disease
CI	Confidence Interval
CNRI	Clinical Net Reclassification Improvement
CRP	C-Reactive Protein
CV	Cardiovascular
CVD	Cardiovascular Disease
DAHTA	German Agency for Health Technology Assessment
DIMDI	German Institute of Medical Documentation and Information
FRS	Framingham Risk Score
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HDL	High Density Lipoprotein
Hs-CRP	Highly-sensitive C-reactive Protein
HTA	Health Technology Assessment
ICER	Incremental Cost Effectiveness Ratio
IDI	Integrated Discrimination Improvement
INHATA	International Network of Agencies for Health Technology Assessment
LDL	Low Density Lipoprotein
Lp(a)	Lipoprotein(a)
LYG	Life Years Gained
MDA	Malondialdehyde
MI	Myocardial Infarction
NICE	National Institute for Health and Clinical Excellence
NND	Number needed to diagnose

NPV	Negative Predictive Values
NRI	Net Reclassification Improvement
NT-proBNP	N-terminal pro B-type Natriuretic Peptide
O_2^-	Superoxide Anion
OR	Odds Ratio
PPV	Positive Predictive Values
QALY	Quality Adjusted Life Years
RCT	Randomised Controlled Trial
ROC	Receiver Operating Characteristic
SCORE	**S**ystematic **CO**ronary **R**isk **E**valuation
SH	Sulfhydryl
SOD	Superoxide Dismutase
TNFα	Tumour Necrosis Factor α

SCIENTIFIC REPORT

1 BACKGROUND

Cardio-vascular diseases (CVD) remain the most important cause of mortality in our population. In Belgium, about a third of overall deaths are associated to CVD (circa 32 500 individuals over 104 000 in 2009; Statbel http://statbel.fgov.be/fr/binaries/FR_Tab1.3_T_internet_tcm326-80257.xls accessed 19/02/2013). Approximately 10% of the cardiovascular deaths occur before age 50 years, 23% before age 75 years. Primary prevention is thus crucial to reduce premature CVD deaths. CVD have a long asymptomatic latent period, which provides an opportunity for early preventive interventions. Atherosclerosis begins in childhood and progresses into adulthood in association with multiple coronary risk factors such as unfavourable levels of blood lipids, blood pressure, body weight and body fat, smoking, diabetes and genetic predisposition.

Risk prediction models are key components of prevention strategies by allowing the identification and appropriate management of vulnerable individuals. In Belgium, such clinical management is tailored to individuals on the basis of their absolute risk of a fatal CVD event in the following 10 years. This 10-year risk can be computed with the help of the SCORE model (**S**ystematic **CO**ronary **R**isk **E**valuation), calibrated to account for the baseline risk in our country and including traditional strong predictors of cardiovascular risk which are age, sex, smoking, systolic blood pressure, and plasma cholesterol concentrations[1, 2].

However, developing screening strategies that safely, accurately, and cost-effectively identify individuals at risk for CVD well before symptoms appear remains a challenge, partly because these traditional cardiovascular risk factors do not fully explain inter-individual variation in cardiovascular risk. For example, a case-control study in Denmark reported that only 13% of women and 50% of men who suffered a myocardial infarction were classified as high risk individuals by the Framingham risk score (FRS) 4 years before the event[3]. The highest number of CVD events occurs in individuals classified in the intermediate or lower risk groups. For instance, in the USA, at least 20% of all coronary deaths are not preceded by cardiac symptoms or diagnoses[4]. With a cut-off of 5% in 10-year mortality risk, the sensitivity of the SCORE model is 52% (13% for women, 60% for men) and its specificity is 85% (98% for women, 76% for men)[5].

The measurement of “novel” biomarkers could thus be an important component in the fight against CVD by improving the performance of risk

prediction models. Potential biomarkers are increasingly numerous, fuelled by technological advances in genomics, proteomics, and noninvasive imaging[6-8 9] (Table 1).

Up to date, inflammatory markers, lipid-related markers and markers of vascular function and neurohumoral activity are the ones which have been the most investigated.

Among inflammatory markers, the C-reactive protein (CRP), a pentameric protein synthesized in the liver, is a sensitive, nonspecific systemic marker of inflammation and tissue damage. In addition to chronic inflammation, elevated levels of CRP are associated with traditional cardiovascular risk factors[10]. CRP is a downstream marker of inflammation that has multiple effects, including complement binding, augmentation of expression of adhesions molecules, and decreased expression of the vasodilator endothelial nitric oxide synthase[9]. Additionally, CRP may stimulate the expression of the thrombotic factor PAI-1 and may induce oxidative stress and the secretion of other cytokines. A dose-response gradient between concentration of CRP and risk of CVD has been observed[11-13]. Whether CRP is involved in the pathogenesis of CVD is however still unknown[14, 15].

Among lipid-related markers, it may be interesting to improve CVD prediction beyond the measurement of total cholesterol and high density lipoprotein (HDL) cholesterol. For example, individuals treated with statins who achieve low levels of low density lipoprotein (LDL) cholesterol, but have high concentrations of either non-HDL cholesterol or apolipoprotein (apo) B, remain at increased cardiovascular risk. Similarly, individuals with low levels of either HDL cholesterol or apo A-I are also likely to experience cardiovascular events, despite having normal LDL-cholesterol levels[16]. Non–HDL-C reflects the cholesterol content of several proatherogenic lipoprotein subfractions (very low density lipoprotein, intermediate density lipoprotein, and chylomicron remnants) in addition to low-density lipoprotein cholesterol[17]. Apolipoproteins B and A1 are the main surface proteins found on proatherogenic lipoproteins and HDL, respectively. Therefore they might be more strongly related to CVD risk than the cholesterol contained in these lipoproteins. Lipoprotein Lp(a) is a cholesterol-rich plasma lipoprotein particle, the structure and composition of which closely resemble LDL. The distinguishing feature of Lp(a) is the presence of an additional large glycoprotein known as apolipoprotein-a which may competitively interfere with plasminogen action in fibrinolysis, promoting development of lipid rich plaques that are more prone to rupture than more fibrous lesions. This suggests that Lp(a) may contribute to the thrombotic and atherogenic aspects of CHD. A plasma Lp(a) threshold of 30 mg/dL has been linked to increased CVD risk in men[12]. HDL-cholesterol is integrated in all usual CVD prediction models and has recently been introduced in the SCORE as well [5]. Although HDL-C is not anymore considered an optional component of CVD risk prediction, we believe that evaluating its predictive added value remains a relevant question.

Among markers of vascular function and neurohumoral activity, homocysteine and N-terminal pro B-type natriuretic peptide (NT-proBNP) are the most documented up to date. Evidence suggests that homocysteine may promote atherosclerosis by damaging the inner lining of arteries and promoting blood clots. B-type natriuretic peptide (BNP) is a 32–amino acid polypeptide secreted by ventricular myocytes during periods of increased ventricular stretch and wall tension. This peptide is believed to play an important role in the regulation of blood pressure, blood volume, and sodium balance[18]. An increase in NT-proBNP levels is viewed by some as a response to age-related, subclinical alterations in cardiac structure or function, so this biomarker may be useful for CHD risk prediction at older ages[19]. BNP is secreted along with a 76 amino acid N-terminal fragment called NT-proBNP which is biologically inactive but has a longer half-life than BNP, and thus could also be used as a predictor of CVD.

The incremental predictive value of these biomarkers needs to be assessed as evidence evolves rapidly in this field of screening science. Therefore, this report aims at:

1. Synthesizing the statistical methods for assessing the added value of a novel risk marker.

   Novel biomarkers should add reliably to the predictive power of traditional risk factors and help in adequately (re-)classifying patients in risk categories useful for both individuals and clinicians. Being statistically associated with the occurrence of CVD does not turn automatically a biomarker into a useful predictor. Appropriate metrics are needed to quantify the added prediction offered by new markers[20, 21]. This is a rapidly evolving field of statistics with recent methodological advances. This first objective is thus also to develop a useful frame for horizon scanning in the field of CVD prevention and

for incrementing KCE expertise in the area of decision-making in primary prevention of diseases.

2. Identifying CVD biomarkers which yield an added predictive value in comparison with prediction models based on conventional risk factors, in the frame of a general screening for asymptomatic individuals at increase risk of CVD. We acknowledge that the use of some biomarkers has been proposed to identify high risk individuals in specific sub-populations (e.g. measurement of Lipoprotein(a) in individuals with a family history of premature CVD or hypercholesterolaemia[22]). However, reviewing the evidence for such sub-population screening was beyond the scope of this report. The use of biomarkers for more precise prognostic estimates in symptomatic patients was also out of our scope.
3. Reviewing evidence on the health benefit and cost-effectiveness of using CVD biomarkers identified in stage 2, if any. Reclassification based on the results of a biomarker should result in a clinical management different to what it would otherwise have been, and that is effective and cost-effective in reducing the risk for incident CVD[12]. Such evidence should be generated by high-quality RCTs and sound economical evaluations.

Table 1 – Biomarkers potentially useful for assessing cardiovascular risk

System	Biomarkers	System	Biomarkers
Lipid-related markers	Apolipoprotein A1	Inflammatory markers	C-reactive protein
	Apolipopotein B100		Fibrinogen
	Phospholipase A2 (activity or mass)		Interleukin-1B
	Paraoxonase-1		Interleukin-1 receptor antagonist
Renal function markers	Creatinine		Interleukin-6 (cytokine)
	Cystatin-C		Neopterin
	Uric Acid		Galectine-3
	NGAL		Peroxisome proliferator-activated receptor
Metabolic markers	Adinopectin		Serum amyloid A
	Ferritin		Tumor necrosis factor-α (cytokine)
	Ghrelin		White blood cell count
	Glucose (fasting or post-load)	Markers of vascular function and neurohumoral activity	(N-terminal pro) B-type natriuretic peptide
	Insulin (fasting)		C-terminal pro-vasopressin
	Leptin		C-terminal pro-endothelin-1
	Resistin		Intercellular adhesion molecule

System	Biomarkers	System	Biomarkers
	Retinol binding protein 4		Mid-regional pro-adrenomedulin
Coagulation markers	D-Dimer		Mid-regional pro-atrial natriuretic peptid
	Plasminogen activator inhibitor-1		Tissue inhibitor of metalloproteinase-1
	Tissue-type plasminogen activator		Vascular cell adhesion molecule
	Von Willebrand factor	Markers of oxidative stress	F2-isoprostanes
Angiogenesis markers	Cardiac placental growth factor		Homocysteine
Necrosis markers	Creatine kinase-MB		Myeloperoxidase
	Troponin I		

2 METHODS FOR ASSESSING THE INCREMENTAL PREDICTIVE VALUE OF BIOMARKERS (PREDICTIVE GAIN)

Accurate assessment of cardiovascular risk is essential for clinical decision making, because the benefits, risks, and costs of alternative management strategies must be compared to choose the best option. Despite its importance for optimal clinical and policy decisions, many aspects of risk assessment are still poorly understood[23]. Critical evaluation of risk markers and risk assessment methods has become crucial as novel markers of cardiovascular risk are emerging in great numbers. A major goal of developing new biomarkers in predictive medicine is to improve risk prediction. How should this improvement be measured?

Although it is obvious that a statistically significant association between a biomarker and a disease is a necessary condition for a biomarker to be clinically relevant and useful for risk prediction, such association is not sufficient[9]. Indeed, even a strong statistical association does not necessarily imply that the marker can discriminate between individuals likely to experience the outcome and those who will not[24]. Such discrepancy can occur for example when the biomarker is rare in the population or when the measure of association (odds ratio or hazard ratios) has mainly been affected by the tails of the distribution. Moreover, to yield a predictive gain, the measurement of the biomarker should add reliably to the predictive power of existing models based on traditional risk factors, such as age, sex, hypertension, body mass index (BMI), smoking status. The predictive model including the biomarker should be more discriminant than the model without the biomarker and allow subject reclassification in appropriate risk categories. Moreover, it should be well calibrated[6].

2.1 Discrimination

Discrimination is the ability of a biomarker to distinguish those who will develop a disease from those who will not. Discrimination can be characterized by the area under the ROC curve (AUC) or the *c*-statistic[a]. The *c*-statistic is a function of the sensitivity and specificity (one minus specificity) of a test across all diagnostic thresholds. It defines the probability that a randomly selected individual with a disease will have a higher test score (or that the risk factor will be more often present in the case of a binary test) than a disease-free individual. In other words, it is the probability that a given predictive model assigns a higher probability of an event to those who actually have or develop the event. The *c*-statistic ranges from 0.5 (uninformative test) to 1.0 (perfect discrimination). In general, a *c*-statistic >0.7 is considered good (the probability is 70% that a case presents the risk factor). For example, the *c*-statistic for coronary heart disease (CHD) risk evaluated with traditional risk factors (e.g., in the form of the Framingham Risk Score) is approximately 0.75[20]. Thus, the value of a new biomarker can be gauged by determining how much higher the *c*-statistic becomes with the combination of traditional risk factors and the biomarker test. A test that increases the *c*-statistic by 0.05 or more is thought to add clinically useful information[25]. There is no consensus about the importance of smaller changes in the *c*-statistic, which may depend in part on the baseline value[6].

One difficulty met with AUC statistics is its relative insensitivity to improvement. Increasing the *c*-statistics may be difficult when the usual prediction model already presents a high value. For example, Cook et al. have demonstrated that adding low-density lipoprotein concentration to a predictive model of CVD including age alone increases the *c*-statistics from 0.70 to 0.71 only, a result at odds with the well acknowledged risk of CVD associated with low-density lipoprotein[26]. Ridker et al. compared a model developed for CVD risk prediction in women using only standard risk factors with a model that also includes parental history of myocardial infarction and CRP, and observed a minimal increase in the AUC from 0.805 to 0.808[27].

However, when they classified the predicted risks obtained using their two models into four categories (10-year CVD risk: 0–5%, 5–10%, 10–20%, >20 %) and then cross-tabulated these two classifications, they showed that around 30 per cent of individuals changed of risk category when comparing the new model with the old one[27]. Meaningful changes in AUC

[a] For binary outcomes, c is identical to AUC.

can be observed only for very large independent associations of the new biomarker with the outcome. It has been suggested that an OR of about 16 may be needed to achieve reasonable discrimination[24]. This would eliminate most risk factors currently in use for predicting cardiovascular risk.

Another metric to measure how average sensitivity improves without sacrificing average specificity as been proposed recently: the integrated discrimination improvement (IDI)[28].

$$IDI=(P_{new,events} - P_{old,events}) - (P_{new,nonevents} - P_{old,nonevents})$$

where $P_{new,events}$ is the mean of the new model-based predicted probabilities of an event for those who develop events, $P_{old,events}$ is the corresponding quantity based on the old model, $P_{new,nonevents}$ is the mean of the new model-based predicted probabilities of an event for those who do not develop events and $P_{old,nonevents}$ is the corresponding quantity based on the old model. The null hypothesis IDI=0 can be formally tested[b28].

$$Z = \frac{IDI}{\sqrt{(SEevents)2 + (SEnonevents)2}}$$

where SEevents is the standard error of paired differences of new and old model-based predicted probabilities across all event subjects, and SEnonevents the corresponding metrics for non-event subjects. The above statistic is close to the standard normal for sufficiently large sample sizes. Individual components of the IDI assessing improvement separately for integrated sensitivity and integrated specificity can be tested using the approach of paired samples[c].

The IDI and improvement in AUC are related in the sense that both can be seen as corrected average sensitivities[d]. However, empirical examples show that IDI can be significantly increased in situation where the AUC is not[28].

AUC and IDI share one additional shortcoming. Comparing different risk models does not clarify what changes occur for individual patients, i.e. patient reclassification in risk categories is not available whereas this information is crucial for clinical decision. Furthermore, these metrics do not offer information on whether the risk model is well calibrated.

2.2 Calibration

Predicted risks are the basis for most clinical decisions. It is thus important that the prediction model is accurate enough. Calibration refers to the concordance between predicted risk and observed risk. This can be assessed by comparing modelled risk estimates with actual event rates. In a well calibrated risk model, predicted and observed risks should be similar over the whole range of predicted risks. This comparison is most commonly carried out with a goodness-of-fit test, usually the Hosmer-Lemeshow statistic. Risk models with a Hosmer-Lemeshow p >0.05 are considered well calibrated because there is no significant difference between predicted and observed event rates. A convenient visual appraisal can be obtained by displaying predicted versus observed risks across the range of predicted risk for models with and without the novel biomarker.

One important parameter to account for in model calibration is the baseline level of disease risk in the population considered. Recalibration of the risk

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b Macros/program files for calculating IDI using Stata, SAS, and R can be found at http://www.ucr.uu.se/downloads

c Positive and negative predictive values (PPV and NPV) are two other important measures that complement sensitivity and specificity. However, there is no easy analog to the McNemar's test for the improvement in sensitivity, since the denominators do not stay the same (numbers of subjects classified as events will vary from model to model). The usefulness of the PPV integrated over all possible cut-offs is substantially limited by the fact that large cut-offs lead to small denominators. An improvement of 5 per cent means something very different for denominators of 100 *vs.*10. This is not the case with integrated sensitivity, where the denominator remains fixed and small values of sensitivity have small effect on the overall measure.

d The IDI is corrected by the subtracted factor assessing the undesirable increase in 'one minus specificity', and the AUC by weighting the sensitivities of the two models of interest by the corresponding derivatives of specificities.

model by adjusting the baseline risk estimates to fit the target population is necessary. For instance the SCORE-Belgium model has been calibrated for the epidemiology of CVD in Belgium[2].

As for discrimination, improvement between different models may be difficult to assess.

2.3 Reclassification

Reclassification refers to the ability of a test to change an individual's risk classification. It is a very relevant concept in clinical practice because treatment guidelines typically refer to predetermined risk categories. Reclassification can be described by estimating the proportion of individuals in a population who are reclassified based on the measurement of the biomarker under scrutiny. However, this simple metric is insufficient because it does not account for whether the reclassification is "correct" or not, i.e. if reclassifying a patient in a higher risk category corresponds to a truly higher risk of disease. Moreover, reclassification tables offer limited means of evaluating improvement in performance.

Pencina et al. have proposed a more adapted metric: the net reclassification improvement (NRI). This summarizes the net proportion of individuals with "correct" reclassification (e.g., those who develop events who were up-classified, and those who do not develop events who were down-classified) and "incorrect" reclassification (those who develop events who were down-classified, and those who don't develop events who were up-classified)[28].

$$\text{NRI} = (P_{\text{up}|D=1} - P_{\text{down}|D=1}) - (P_{\text{up}|D=0} - P_{\text{down}|D=0})$$

where "D" denotes the event indicator, "up" an up-reclassification and "down" a down-reclassification. The null hypothesis of NRI=0 can be formally tested with a simple asymptotic test[e]:

$$Z = \frac{\text{NRI}}{\sqrt{\frac{(\text{Pup}|D=1 - \text{Pdown}|D=1)}{nD=1} + \frac{(\text{Pup}|D=0 - \text{Pdown}|D=0)}{nD=0}}}$$

NRI corresponds to the sum of 'event NRI' and 'non-event NRI', which can be tested separately. These 2 metrics should also be reported in assessment studies for fuller interpretation[29]. In general, no more than 3 categories are recommended (high risk individuals who need treatment, intermediate risk individuals, low risk individuals who do not need treatment). One difficulty of the NRI is its dependence upon the number of risk categories and the choice of cut-off points. However, a "category-free" NRI, which does not depend on the existence of fixed risk categories, has recently been proposed[29]. When established risk categories are used in clinical practice to adapt the strategy of risk reduction of the communication with the patients, an NRI computed on these categories will be the most informative statistic[29].

Some authors have also introduced the concept of clinical NRI (CNRI), i.e. the amount of reclassification observed only in individuals classified in the intermediate-risk category by the reference prediction mode[30]. It calculates the amount of improvement offered by a strategy where only the individuals for whom the treatment decision could be changed by measuring a biomarker are considered. This assumes a 2-step screening strategy where individuals would first be classified based on the reference prediction model of CVD risk, and then the measurement of a biomarker would be performed only in intermediate-risk individuals. Usually, there is a risk threshold above which treatment is recommended and below which it is not[23]. Patients who have a risk that is either just above or just below a treatment threshold might be moved across the threshold and have their treatment changed by the ascertainment of additional risk information. Actually, there is often an intermediate risk zone in which the results of testing should be used to guide treatment. For very-low-risk patients, it is rational that neither testing nor treatment is needed, whereas for high-risk patients, treatment is indicated without further testing, because no test result would reduce their estimated risk below the treatment threshold.

[e] Macros/program files for calculating NRI using Stata, SAS, and R can be found at http://www.ucr.uu.se/downloads

2.4 Discussion

Assessment of cardiovascular risk in individuals is an integral part of the clinical decision-making, especially for increasing the rational use of pharmaceutical-, procedure-, or device-based therapies. A predictive model of risk should be discriminant, calibrated and allow subject reclassification into useful risk categories. None of these statistics is self-sufficient, and the 3 of them should be considered when assessing the added value of a biomarker[23]. However, among these statistics appropriate reclassification of patients in categories which might impact on the individual risk management appears to be central for both patients and clinicians[28].

These statistics should be generated through well-conducted cohort studies (although cross-sectional designs are useful for proof-of-concept studies, i.e. at the early stages of development of the biomarker). The outcome measure must be carefully defined, accurately measured, and completely ascertained to provide a reliable basis for the evaluation of the biomarker[23]. A number of issues are specific to this domain of research. First, more outcome events are needed to provide adequate statistical power for the test on whether a new risk marker adds prognostic information to established risk factors in a multivariable model than for the test on whether the new marker provides prognostic information by itself. The number of outcome events available for analysis can be increased by use of a composite end point (e.g. death or myocardial infarction, or ischemic stroke). However, composite end points may complicate the assessment of one biomarker if it is more predictive of one component of the composite end point (e.g., myocardial infarction) than of the others (e.g., stroke, death). Moreover, all outcome events do not bear the same clinical relevance, and assessment made on such composite end points may in the end be suboptimal for clinical decision-making. The number of events available for analysis can also be increased by longer follow-up, but this is appropriate only if the marker is associated with both short-term and long-term risk. Second, if a large number of potential biomarkers is tested in one study, the probability of getting positive results purely by chance increases. It is thus extremely important to validate the results of any predictive model, the strongest approach being an external validation study of the predictor in a new cohort of subjects. Data from several novel markers may be combined to form a multimarker risk score[7, 31]. In such cases, a distinction should be made between the specific markers that were included in the multimarker score and the particular scoring algorithm that was applied to the marker data to produce the final score used in risk prediction. A proper evaluation of a multimarker score includes replication of the specific proposed scoring algorithm in an independent validation sample. Third, the accuracy of biomarker measurements may vary with the laboratory techniques used[32]. This can be particularly confusing for interpreting CVD risks when risk estimation models have been established on biomarker measurements by a given technique and a new measurement technique has replaced the previous one while systematically yielding different results. For instance, in Belgium, direct measurement of HDL-C yield values higher by 20.8% in comparison with the earlier precipitation method[32].

3 PREDICTIVE INCREMENTS OF CVD BIOMARKERS

3.1 Background

We have reviewed in chapter 2 the statistics to assess the predictive increments of models including biomarkers as compared to prediction models including only conventional risk factors. In this chapter, we review the evidence on the performance of the various CVD biomarkers as measured by those statistics. We searched for information on:

Population: asymptomatic adults with no overt CVD or history of CVD

Intervention: measurement of biomarkers to predict the CVD risk beyond established risk models

Control: prediction of the CVD risk by established risk models

Outcomes: increment in discrimination, calibration and reclassification

3.2 Methods

We searched the electronic databases MEDLINE (Pubmed), EMBASE, and CRD databases with the following search strategy:

#1. “biological markers”[MeSH Terms] OR "biomarker"[All Fields]

#2. "cardiovascular diseases"[MeSH Terms]

#3. "Systematic Coronary Risk Evaluation" OR Framingham* OR Reynolds* OR ASSIGN* OR PROCAM OR QRISK1* OR QRESEARCH OR QRISK2* OR "Adult Treatment Panel III"

#4. *classifi* OR calibrat* OR discrimina* OR stratifi* OR ROC OR AUC OR *c*-statistic*

#5. #1 AND #2 AND #3 AND #4

Item #3 included the established risk prediction models for cardiovascular disease as recently reviewed by Siontis et al.[33]. We acknowledge that SCORE is the CVD risk prediction model generally recommended and used in Europe. However, we decided to broaden our search strategy, as there is obviously a correspondence between the prediction models (for example, it has been computed that a 5% SCORE risk of CVD death equates to a 10-25% Framingham risk of total CVD[34]) and we can reasonably assume that biomarkers improving the predictive power of one model will do so for other models based on similar risk factors. Item #4 included the statistics necessary to appraise the improved predictive power of adding a biomarker to an established risk prediction model as recently developed by Pencina et al.[21, 35], and explained in chapter 2.

We applied a similar search strategy in EMBASE. We also searched the CRD Databases with the following strategy: (biomarker* AND cardio*). We also screened the bibliography of the included studies.

There was no restriction on language or study design. We searched for original studies published from January 2008, as Pencina et al. published their groundbreaking paper on statistical methods to evaluate the predictive increment of biomarkers that year[28], up to November 2012. However, when a good-quality systematic review existed for one specific biomarker, we included it as such and searched for original studies published only after the date of the search carried out in the systematic review. We also searched the bibliography of included studies.

Any original study combining the 3 following criteria was considered eligible:

- Prospective study assessing the predictive increments of adding a biomarker (or combination of biomarkers) to established risk prediction models based on conventional risk factors. Studies assessing combination of biomarkers were eligible provided that the predictive increment of individual biomarkers was reported.
- Appropriate statistics for evaluating the predictive increment of biomarkers, as explained in chapter 2. Report of the NRI, or presentation of data allowing the computation of NRI, was considered a compulsory criterion.
- Reporting on prediction of first cardio-vascular event (fatal or non fatal) in the general population, i.e. reporting useful information in the field of primary CVD prevention. CVD was defined as coronary heart disease (fatal on non fatal myocardial infarction) and stroke.

Exclusion criteria were defined as:

- Studies focused only on non-serological risk markers as such risk markers will be assessed in a forthcoming KCE report.

- Studies in which the comparator is not an established risk model, as intra-study risk algorithms have likely limited external validity and render inter-study comparisons difficult.
- Studies focused on a sub-population of individuals presenting a specific risk profile (e.g. diabetes, systemic inflammatory disease).
- Studies focused on a specific cardiac outcome outside the general definition of CVD (e.g. heart failure).
- Viewpoint or editorial.

We used standardized forms to extract data on study population, sex ratio, prevalence of smoking and diabetes, levels of systolic blood pressure, total cholesterol and HDL-cholesterol, utilization of anti-hypertensive and hypo-cholesterol medications, history of family CVD, and CVD and CHD risks in the study population. For each biomarker, we extracted data on the reference prediction model, *c*-statistics changes, NRI, CNRI and IDI when the biomarker was added to the reference prediction model. When one of these metrics was not reported in the studies, we computed it when the data to do so were available in the publication.

Quality appraisal of included studies was based on the NICE checklist for prognostic studies derived from Hayden et al.[36].

3.3 Results

We applied our search strategy on October 22 2012, and identified 167 potential relevant references (Figure 1). Among those, 130 were rejected on the basis of their title and/or abstract, and 23 were further excluded after full-text was screened (reasons for exclusion are presented in Table 16). Eventually, we included 16 studies in our review. Evidence concerned in majority two main groups of biomarkers: markers of inflammation (CRP, fibrinogen, leukocyte count); and lipid-related markers (apolipoprotein A1, apolipoprotein B100, Phospholipase A2, HDL cholesterol). Biomarkers pertaining to other patho-physiological paths were also investigated but with much less convergence: homocysteine (marker of oxidative stress), NT-pro BNP (marker of vascular function and neurohumoral activity), acid uric, van Willebrand antigen, etc… The most investigated biomarker across studies was CRP (data on CRP in 12/16 studies).

3.3.1 Markers of inflammation

3.3.1.1 C-Reactive Protein

We retrieved 2 systematic reviews on the utilization of CRP in prediction models for CVD which partly overlapped [37, 38][f]. The first one included 23 studies reporting on the risk of CHD associated with high high-sensitivity CRP (hsCRP) after adjustment for all Framingham risk factors[37]. This risk was increased by 60% (95%CI: 43%; 78%) in individuals with a hsCRP>3.0 mg/L vs. <1.0 mg/L, and by 26% (95%CI: 17%; 35%) in individuals with a hsCRP between 1.0 mg/L and 3.0 mg/L vs. <1.0 mg/L, i.e. there was evidence of a dose-response gradient. Only one study included in that systematic review calculated the risk reclassification when hsCRP level was added to a predictive model that included all Framingham risk score variables [39]. In that study, 14% of participants originally classified as intermediate-risk (10-year CVD risk between 10% and 20%) were reclassified as low-risk (<10%) and 5% were reclassified as high-risk (>20%). The actual 10-year risk was 19.9% for those reclassified as high-risk and 11.5% for those who remained intermediate-risk. Unfortunately, data to re-compute NRI were not available from that study. The second systematic review included 31 studies reporting on 28 prospective cohorts[38]. A linear graded association of log-CRP values with log risk of events was noted in all studies, i.e. there was a dose-gradient relationship as in the review by Buckley et al.[37]. In the 13 studies that reported on the effect on the ROC curve or *c*-statistic of adding CRP to the Framingham-based models five reported no change and eight reported an improvement in the AUC ranging from 0.01 to 0.15. The only study reporting on overall reclassification of individuals was the one by Cook et al. mentioned above[39].

We retrieved 12 original studies published since 2008 on the predictive increment of CRP when added to a model built on traditional risk factors, totalling 223 010 individuals (Table 3). In two of these studies, the predictive increment of CRP could not be disentangled from the one of another risk factor (parental history of myocardial infarction[40] or tumour necrosis factor alpha (TNFα)[41]). The predictive increment of CRP was

[f] The systematic review by Buckley et al.[37] is also presented in a summary of systematic reviews by Helfand et al.[12]

tested against FRS in all studies (the ASSIGN model used in the study by Woodward et al. can be seen as an extended FRS[41]). Unfortunately, the usual 10-year CVD risk categories (<10%, 10%-20%, >20%) were utilized in only 5 studies[g13, 19, 41-43]. Two other studies used very close categories[44, 45], whereas other studies used more risk categories and/or different category bounds without providing a rationale for doing so. Such methodological diversity hampers a meaningful meta-analysis. CVD risk was harmonized for a 10 year follow-up period across studies, except in one study where a 7.5 year period was considered[46].

Quite consistently across studies, adding CRP to the FRS or ASSIGN models resulted in a significant albeit modest overall reclassification of study participants (NRI ranged from 1.52% to 11.8%) for the predicted 10-year risk categories of low (<10%), intermediate (10% to <20%), and high (≥20%). However, among the 7 studies using these 3 reference categories, two reported a NRI not significantly different from 0[19, 45]. CNRI was consistently greater than NRI (range: 6.5% to 31.4%), except in one of the 6 studies allowing such comparison[7]. For example, in Kaptoge et al., by far the largest study of this review as it included 166 596 individuals from 52 prospective cohorts, the CNRI among those who developed a cardiovascular event was 23.8%, whereas the CNRI in noncases was 6.7%[42].

NRI was also consistently higher when the event under scrutiny was "coronary heart disease" versus "all cardiovascular event", the lowest NRI being observed for "stroke". Changes in the *c*-statistics provided results consistent with those based on NRI, whereas IDI was rarely reported.

Kaptoge et al. have stratified their analysis by sex and smoking status[42]. The use of information on CRP improved cardiovascular risk discrimination in men but not in women (P≤0.001 for the interaction). In 15 studies that involved both men and women with at least 10 years of follow-up (19 467 men with 2 784 first cardiovascular events and 25 157 women with 2 323 first cardiovascular events), the NRI with measurement of CRP among men was 1.24% (95%CI: 0.20%, 2.69%; P = 0.09), and the net improvement among women was 0.36% (95%CI: 0.70%, 1.42%; P = 0.51).

CRP also had greater predictive value in current smokers than in nonsmokers (P<0.001 for the interaction). There were no significant differences in cardiovascular risk discrimination in other clinically relevant subgroups, including age group. The differences in the improvement of cardiovascular risk discrimination according to sex with the use of information on CRP levels persisted in further analyses in which participants were stratified according to smoking status (or in which smokers were omitted) and in analyses in which women who were known to be receiving hormonal treatment at baseline were excluded, although information on such treatments was incomplete. The difference of the predictive increment of CRP by sex was also apparent in the study by Blankenberg[7], although not formally tested.

3.3.1.2 Other markers of inflammation

We retrieved 2 studies in which the predictive increment of fibrinogen was tested (Table 4), and one on the added value of leukocyte count (Table 5). The findings were quite similar to what was observed for CRP, i.e. a modest overall NRI (not reaching statistical significance in the study by Kavousi[19], and a greater CNRI. At odds with CRP however, the CNRI was mainly due to noncases who were down-classified.

In Kaptoge et al., adding fibrinogen in the prediction model on top of hsCRP did not result in changing the C-index more than when either marker was used alone[42]. In that study, improvements observed in the C-index when leukocyte count was added to the analysis did not differ significantly from those observed with the addition of CRP or fibrinogen, although concomitant information on CRP, fibrinogen, and at least one other biomarker of inflammation was available for only about one third of the participants.

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g The study by Woodward et al. used 4 10-year risk categories initially (<10%, 10%-<15%, 15%-<20%, ≥20%), but data allowing computation of NRI based on 3 categories was available from the paper [41]

Figure 1 – Flow chart of evidence retrieval

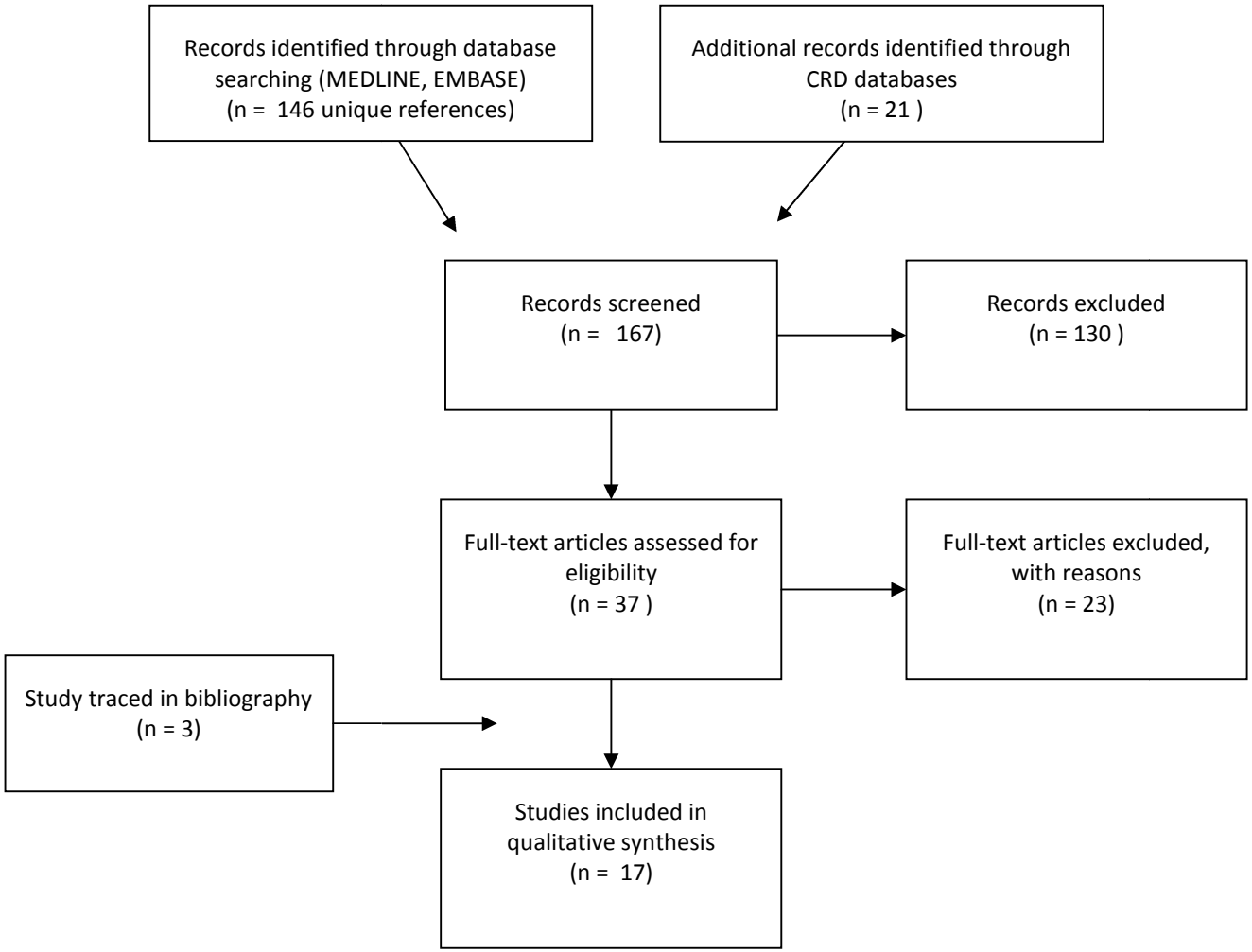


Table 2 – Description of studies

Study	Nb	Age years	Male %	Current smoker %	History diabetes %	Systolic BP Mm Hg	Total cholesterol Mg/dL	HDL-C Mg/dL	BMI Kg/m2	Statins %	Antihypertensives %	Family history of premature CHD	Median follow-up years	CHD %	CVE %
Kavousi 2012[19]	5933	69.1±8.5	40.6	17.5	12.9	143±21	225±38	54.0±15	27±4	10.2	23.5	NR	6.8h	5.8	NR
Kaptoge 2012[42]	246 669i	59.7±8.6	49	21	6	136±19	228±42	51.0±15	NR	NR	NR	NR	8.8j	5.3	8.1%
Di Angelantonio 2012[17]	165 544k	56.4±8.4	49.1	26.7	5.7	135±18	226±42	51±15	NR	NR	NR	NR	10.4 (IQR: 7.6;14)	NR	9.1%l
Ridker 2008[40]	10 724	63 (IQR: 57;70)	100	3.2	0	128 (IQR: 120;135)	203 (IQR: 180; 227)	42.5 (IQR: 34.4; 52.4)	NR	17.3	24.2	10.8	10.8 (IQR: 7.8; 11.2)	10.0%	12.1%m
Woodward 2010[n41]	1 836	43.6±11.5	48	40	2.1	127±19	227±44	51±15	NR	NR	NR	34	10.5 (median)	NR	11.4
Wilson 2008[o44]	3 006	46±9	47	35	4	127±15	207±36	43±11	27±4	NR	11	NR	12	7.5	14.4
Möhlenkamp 2011[p13]	3 966	63±8	70	27.5	17.6	143±23	238±38	54±17	28±4	11.0	47.3	NR	5.1±0.3	2.3	NR
Cooney 2009[5]	104 961	*q													2.3r

h 10-year CHD risk estimated through a parametric Weibull proportional hazards regression model

i From 52 studies

j Median; 5th percentile=2.9 y; 95th percentile=23.3 y

k From 37 prospective cohorts

l Computed by us =15 126/165 544

m Computed by us =1 294/10 724

n Although this paper considered 5 biomarkers (CRP, Fibrinogen, IL-6, IL-18, TNFα), only the reclassification for CRP+ TNFα is reported. Therefore, we also present the results only for CRP+ TNFα

o Figures presented in this table are for men. The corresponding information for women are as follows: age=45±9; current smoker=37%; diabetes=1%; systolic BP=119±17; total cholesterol=203±40; HDL cholesterol=54±14; BMI=25±5; antihypertensive therapy=9%; CHD incidence=1.4%; incidence of CVE=5.1%

p Figures presented in this table are for study participants with a coronary event. The corresponding figures for participan ts who did not develop a coronary event were age=59±8; male=47%; current smokers=22.5%; diabetes=7.0%; systolic BP=113±21; total cholesterol=231±39; HDL-C=59±17; statins=9.1%; antihypertensive medications=31.3

q Authors have been contacted to get the study population characteristics

r CVD deaths only

Study	Nb	Age years	Male %	Current smoker %	History diabetes %	Systolic BP Mm Hg	Total cholesterol Mg/dL	HDL-C Mg/dL	BMI Kg/m2	Statins %	Antihypertensives %	Family history of premature CHD	Median follow-up years	CHD %	CVE %
Merry 2011[47]	20 055	41±11	47	39.8	0.9	119±14	209±42	50±12	24±2	0.5	4.5	30	10.9	3.9	NR
Blankenberg 2010[st7]	3 870	49±14	100	26.4	5.6	139±19	213±54	48±15	27±4	3.0	13.0	24	10	6.9	9.7
	4 045	47±13	0	17.2	5.1	132±20	209±58	58±19	26±4	1.5	10.3	25	10	2.9	4.0
	2 551	55±3	100	23.3	1.8	134±20	225±51	45±15	26±3	1.1	8.7	17	10	8.3	10.2
Yeboah 2012[46]	1 330	64±9.5	67	16.5	0	130±20	197±35	46.5±12	28±5	14.1	38.2	42.6	7.6(IQR: 7.3; 7.8)	7.1	9.2
Shah 2009[11]	3 012 M (NPHS-II)	NR	100	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	5.4	NR
	1 592 (EAS)	NR	51	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	9.2	NR
Pencina 2008[28]	6 528	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	5.6
Melander 2009[45]	5 067	58±6	40	27	8	141±19	216	54±15	26±4	NR	16	NR	12.8 (IQR: 12.1; 13.5)	7.8	4.4
Schneider 2012[u31]	3 967	50 (IQR: 36, 63)	48	38.2	7.7	141 (IQR: 129, 153)	222 (IQR: 193, 251)	48 (IQR: 41, 58)	NR	NR	26.3	NR	10 (IQR: 9.3, 10)	2.6 CVD death	NR
Rutten 2010[48]	5 063	68±8	40	17.6	9.1	143±21	228±39	54±15	27±4	NR	NR	NR	NR	NR	8.3
Wannamethee 2011[v43]	2 893	68w	100	12.4	10.6	150	232	39	26.7	NR	NR	NR	9 (IQR: 8, 10)	6.7	13.9

s Figures presented are from: 1st line, the Men FINRISK 97 study; 2nd line, the Women FINRISK 97 study; 3rd line, the PRIME Men Belfast Study Men

t Measurement of total cholesterol and HDL-cholesterol are median±IQR

u Characteristics presented here are for men. Corresponding values for women are median age=48, % smokers=28.6, % diabetes=6.7% , SBP=126 (IQR: 114, 142), total cholesterol=218 mg/dl, HDL cholesterol=60 mg/dl, %anti-hypertensive medication=26.3

v This included 6 649 men with or without CVD at baseline. We present here only the results on the 2 893 participants free of CVD at baseline

w Derived by us from the data in table 1 (p. 58 of the paper)

Table 3 – C-Reactive Protein

Study	N	CRP Mg/L	Reference Model	Outcome	C-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	3 029[x]	2.3 (1.2, 4.4)[y]	FRS (<10%, 10%-20%, >20%)	CHD	0.00 (-0.01; 0.00)	2.0 (-2.3; 6.4)	9.2 (0.2; 18.0)[z]	NR
Kaptoge 2012[42]	166 596[aa] Accounting for 1.6 106 person-years	logCRP=0.59±1.09	FRS (<10%, 10%-20%, ≥20%)	MI or fatal CHD or stroke	0.0039 (0.0028; 0.0050)	1.52[bb] (0.78; 2.27)[cc]	30.6[dd]	0.0036(0.0028;0.0043)
	id	id	id	CHD	0.0051(0.0035; 0.0066)	NR	NR	NR
	id	id	id	Stroke	0.0016 (0.0003; 0.0030)	NR	NR	NR
Möhlenkamp 2011[ee13]	3 966	2.1(IQR:0.9; 4.8)[ff]	FRS (<10%, 10%-20%, ≥20%)	CHD	0.023 (p=0.12)	10.5[gg] (p=0.026)	NR	0.0015 (p=0.32)
Woodward 2010[hh41]	1 319	1.30 (IQR: 0.64-3.22)	ASSIGN[ii] (<10%, 10%-<20%, ≥20%)	CVD	0.006[jj]	8.2[kk]	31.4[ll]	11.2 (3.06; 19.4)[mm]

x CRP was measured in 3 029 individuals among the 5 933 included in the cohort. Authors state that this subpopulation did not differ significantly from the whole group

y Median (25th, 75th percentiles)

z NRI in cases was 1.9%. NRI in noncases was 7.3%

aa CRP was measured in 166 596 individuals over the 246 669 included in the cohort

bb NRI and IDI assessed on 72 574 participants from 22 studies

cc The NRI was 1.46 (95% CI: 0.73; 2.19) in those who developed CVD within 10 years (5.48% appropriately reclassified, 4.02% ina ppropriately reclassified). The NRI was 0.06% (95%CI: -0.09; 0.22) in those who remained event free at 10 years (2.12% appropriately reclassified; 2.06% inappropriately reclassifie d)

dd Computed by us from data in supplementary appendix table 6. The NRI in cases was 23.84% (82/344), and the NRI in non-cases was 6.74% (561/8324)

ee Figures presented in this table are for study participants with a coronary event

ff CRP in individuals who develop a coronary event (40.7% had a CRP>3 mg/l). The corresponding figure in those who did not devel op a coronary event was 1.4 (IQR: 0.7; 2.9) (22.2% had a CRP>3mg/l)

gg Dose-response visible

hh This study reported only on reclassification when CRP together with tumour necrosis factor alpha (TNFα) was added to the ASSIGN model. Therefore all the indicators reported in the table also relate to changes of indicators when adding CRP together with TNFα

ii Includes age, sex, systolic blood pressure, total cholesterol, HDL cholesterol, cigarettes/day, Scottish index of multiple de privation, diabetes and family history of CHD

jj 95%CI of AUC for ASSIGN (0.790 ; 0.809) and for ASSIGN+CRP+TNFα (0.793; 0.813) overlap

kk Computed by us. The NRI in cases was 8.64% (7/81). The NRI in non-cases was -0.43% (-4/929)

ll Computed by us. The NRI in cases was 27.6% (8/29). The NRI in non-case was 3.8% (8/207)

mm This result relates to the Relative Integrated Discrimination Improvement (RIDI)

Study	N	CRP Mg/L	Reference Model	Outcome	C-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Wilson 2008[nn44]	1 430 M 1 576 W	2.67±5.17 2.28±4.49	FRS (<6%, 6–20%, >20%	CHD	0.002	11.8 (p=0.009)	NR	NR
	id	id	id	CVD	0.004	5.6 (p=0.014)	NR	NR
Melander 2009[45]	4 852	1.3 (IQR: 0.7, 2.7)	FRS (<6%, 6-19%, ≥20%)	CVD	0.003 (p=0.14)	1.1 (p=0.57)	NR	p=0.39
Ridker 2008[oo40]	10 724	0.86 (IQR: 0.43; 1.71)	Age, blood pressure, smoking status, total cholesterol and HDL-C (<5%, 5%-<10%, 10%-20%, ≥20%)	CVD	0.007 (p<0.001)	3.2 (p=0.006)	6.5 (p<0.001)	NR
	id	id	id	CHD	0.010 (p<0.001)	4.3 (p=0.003)	7.8 (p<0.001)	NR
	id	id	id	All CVE	0.004	5.6 (p=0.014)	NR	NR
Blankenberg 2010[pp7]	3 870 M	1.1 (IQR:0.6;2.3)	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%)	CVD[qq]	0.0031 (p=0.11)	NR	NR	0.010 (p=0.0008)
	4 045 F	1.1 (IQR:0.5;2.5)	id	id	0.0015	NR	NR	0.0068 (p=0.0068)
	2 551 M	1.6 (IQR:0.8;3.2)	id	id	0.014 (p=0.044)	7.2±3.0 (p=0.0148)	7.8[rr]	0.0085 (p=0.0032)
Yeboah 2012[ss46]	1 330	1.62(IQR: 0.79-3.68)	FRS (7.5-year risk: 2.0%- 15.4%)	CHD[tt]	0.017 (p=0.03)	NR	7.9 (p=0.79)	NR

nn The reference 10-year risk categories were 0-5%; 6-20%; >20% for both CHD and all CVE

oo This study looked at the added information value of hsCRP together with parental history of myocardial infarction before age 60 years

pp Figures presented are from: 1st line, the Men FINRISK 97 study; 2nd line, the Women FINRISK 97 study; 3rd line, the PRIME Men Belfast Study Men

qq Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarction

rr Computed by us. The NRI in cases was -1.8% (-2/111). The NRI in non-cases was 9.6% (68/705)

ss This study included only individuals at intermediate risk. To account for the fact that actual follow-up was less than 10 years, the authors redefined the risk in terms of 7.5-year risk when calculating the NRI, using a logistic regression model with probability weighting to reflect the sampling from the overall cohort. Based on the new model, intermediate 7.5-year risk categories for CHD and CVD were defined as 2.0% to 15.4% and 3.4% to 21.1%. With the addition of each novel risk marker to the base model, participants were considered to be reclassified to high risk if their estimated risks for CHD and CVD were greater than 15.4% and 21.1%, and reclassified to low risk if their estimated risks were lower than 2.0% and 3.4% for CHD and CVD

tt CHD defined as myocardial infarction, angina followed by revascularization, resuscitated cardiac arrest, or CHD death. CVD additionally included stroke or CVD death

Study	N	CRP Mg/L	Reference Model	Outcome	C-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
	id	id	FRS (7.5-year risk: 3.4% to 21.1%	CVD	0.017 (p=0.03)	NR	3.7 (p=0.037)	NR
Shah 2009[11]	3 012 M (NPHS-II)	2.5±2.5[uu]	FRS (<5%, 5% to <10%, 10% to <15% and ≥15%)	CHD	0.02 (NS)	8.5 (-1.3, 18.3)	NR	NR
	id	id	FRS (<15% or ≥15%)	CHD	0.02 (NS)	4.9 (0.8, 9.0)	NR	NR
	1 592 (EAS)	1.9±3.0[vv]	FRS (<5%, 5% to <10%, 10% to <15% and ≥15%)	CHD	0	8.8 (-1.3 to 18.9)	NR	NR
	id	id	FRS (<15% or ≥15%)	CHD	0	3.0 (3.0, 9.2)	NR	NR
Schneider 2012[ww31]	3 967	1.2 (IQR: 0.6, 2.8)	FRS (<2%, 2%-9%, >9%)	CVD death	0.008	4.7 (-2.7, 15.9)	NR	NR
Wannamethee 2011[xx43]	2 893	1.7[yy]	FRS (<10%, 10%-20%, >20%)	CVD	0.009 (p=0.06)	3.8 (p=0.07)	20.5[zz]	0.32 (p=0.14)

uu Geometric mean

vv Geometric mean

ww Characteristics presented here are for men. Corresponding values for women are median age=48, % smokers=28.6, % diabetes=6.7%, SBP=126 (IQR: 114, 142), total cholesterol=218 mg/dl, HDL cholesterol=60 mg/dl, %anti-hypertensive medication=26.3

xx This included 6 649 men with or without CVD at baseline. We present here only the results on the 2 893 participants free of CVD at baseline

yy We derived the CRP concentration by summing the geometric means presented by quartiles of NT-proBNP concentrations and by dividing it by the total numbers of participants

zz CNRI in cases was 17.4% (23/132) and CNRI in noncases was 3.1% (27/873)

Table 4 – Fibrinogen

Study	N	Fibrinogen µmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5933	11.2 (9.7;12.9)[aaa]	FRS[bbb] (<10%, 10%-20%, >20%)	CHD	0.00 (0.00; 0.01)	2.9 (-0.2; 6.0)	10.2 (4.5; 15.9)[ccc]	NR
Kaptoge 2012[42]	185 892[ddd]	9.2±2.2	FRS (<10%, 10%-20%, ≥20%)	MI or fatal CHD or stroke	0.0027 (0.0018; 00036)	0.83 (0.16;1.50)	6.4[eee]	0.0027(0.0021;0.0033)
	id	id	id	CHD	0.0112(0.0090; 0.0134)	NR	NR	NR
	id	id	id	stroke	0.0003 (-0.0002; 0.0007)	NR	NR	NR

Table 5 – Leukocyte count

Study	N	Leukocytes 10^9cells/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5 933	6.8 (1.9)	FRS (<10%, 10%-20%, >20%)	Any CHD event	0.01 (-0.00; 0.02)	1.5 (-1.5; 4.6)	9.3 (3.2; 15.4)[fff]	NR

aaa Median (25th, 75th percentiles)

bbb Authors refitted a model similar to the Framingham model in their own study population

ccc NRI in cases was 5.8%. NRI in noncases was 4.4%

ddd Fibrinogen was measured in 185 892 individuals from over the 246 669 included in the total cohort

eee Computed by us from data in supplementary appendix table 6. NRI in cases was 1.44% (28/1941) and NRI in non cases was 4.94% (446/9028)

fff NRI in cases was 5.1%. NRI in noncases was 4.2%

3.3.2 Lipid-based markers

We retrieved 6 original studies on the predictive increment of lipid-based markers fulfilling our inclusion criteria, totaling 447 499 individuals. Four studies focused on the added value of integrating measurement of HDL-cholesterol either in SCORE[5, 47] or in FRS[28, 42]. Two other studies reported on apolipoproteins A1 & B1[7, 17]. Only one of those also assessed lipoprotein(a). The body of evidence on the predictive increment of lipid-based biomarkers appears thus rather thin and diverse. This said, the study by Di Angelantonio et al. and by Kaptoge et al. were pooled analyses of 37 and 40 previously published prospective cohorts, respectively[17, 42].

Studies assessing the predictive increment of HDL-cholesterol consistently reported an NRI significantly different from 0, although there were large variations in the size of this increment from 1.7%[42] to 12.1%[28] (Table 6). Such variation may be explained by different outcomes (CVD deaths in study by Cooney et al.5 vs. CHD in the 2 other studies) and various10-year risk categories. Whatsoever, HDL cholesterol appeared to increase the performance of the prediction models independently of total cholesterol. The predictive increment of HDL-C in intermediate-risk individuals could be recomputed in only one study[28], yielding a CNRI much higher than the NRI (CNRI in cases=9.5%,; CNRI in noncases=13.3%.)

Cooney et al. have stratified their analysis by sex and risk category of countries (low vs. high risk)[5]. They reported that the NRI was higher in women (5.4%, p=0.014) than in men (1.5%, p=0.082), and particularly in women from high-risk countries (11.5%, p=0.015).

None of the other lipid-based biomarkers improved significantly the risk reclassification in comparison to existing models which already included total cholesterol and HDL-cholesterol (Table 7, Table 8 Table 9 and Table 10). Di Angelantonio et al. also showed that risk discrimination or reclassification for CVD or CHD did not improve or even worsened when these lipid-related markers replaced total cholesterol and HDL-C in prognostic models[17]. Interestingly, the authors reported some of the interactions mentioned by Kaptoge et al.[42] for CRP, i.e. the addition of apolipoprotein A1 and B1 could preferentially improve CVD risk discrimination in men, and risk discrimination for CHD but not for stroke. Other interactions were reported. Apolipoprotein A1 and B1, as well as lipoprotein(a), could improve CVD prediction more in individuals with higher total cholesterol or in people initially classified at 10% to less than 20% predicted 10-year risk ($p<0.001$ and $p=0.02$, respectively). The authors rightly recommended caution in interpreting these results given the multiplicity of comparisons made.

Table 6 – HDL cholesterol

Study	N	HDLcholesterol mmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Cooney 2009[5]	104 961[ggg]	NR[hhh]	SCORE <5%-≥5%	CVD death	0.006[iii] (p<0.0001)	2.2[jjj] (p=0.006)	NR	NR
	id		SCORE ≤2%, 3-4%, 5-9%, ≥10%	id	NR	3.8	NR	NR
Merry 2011[47]	11 250	50±12	SCORE[kkk] (<2%, 2-5%, 5-10%, ≥10%)	CHD	0.003 (p=0.802)	6.0 (p<0.001)	NR	NR
Pencina 2008[28]	3 264	NR	Age, sex, smoking, diabetes, SBP, total cholesterol (<6%, 6%–20%, >20%)	CHD	0.012 (p=0.092)	12.1[lll] (p<0.001)	22.8[mmm]	0.009 (p=0.008)
Kaptoge 2012[42]	185 892[nnn]	1.32±0.39	Age, smoking, SBP, diabetes, total cholesterol (<10%, 10%-20%, ≥20%)	MI or fatal CHD or stroke	0.010 (0.0083; 0.0116)	2.47 (1.57;3.346)	NR	NR

ggg In 12 studies, accounting for 991 058 person-years

hhh Authors have been contacted to get this information

iii The C-statistics difference was 0.008 (p=0.1244) for women, 0.033 (p<0.0001) for women from high-risk countries, -0.006 (p=0.4516) for women from low-risk countries, 0.006 (p=0.0001) for men, 0.009 (p=0.0001) for men from high-risk countries, and 0.005 (p=0.0572) for men from low-risk countries

jjj NRI=5.4% (p=0.014) for women, NRI=11.5 (p=0.015) for women in high-risk countries, NRI=0.7 (p=0.683) for women in low risk countries, NRI=1.5 (p=0.082) for men, NRI=1.6 (p=0.153) for men in high-risk countries, NRI=0.9% (p=0.498) for men in low-risk countries

kkk SCORE re-estimated using individual data on risk factors and CHD incidence from the Dutch Cardiovascular Registry Maastricht (CAREMA). The reference SCORE model included total/HDL cholesterol ratio, whereas the alternative model included total and HDL cholesterol separately

lll The NRI in cases was 12% (22/183). The NRI in non-cases was 0.03% (1/3081)

mmm Computed by us. The NRI in cases was 9.5% (10/105). The NRI in non-cases was 13.3% (117/882)

nnn From 40 prospective cohort studies

Table 7 – Apolipoprotein A1

Study	N	Apolipoproteins mg/dL	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Di Angelantonio 2012[17]	139 581[ooo]	146±32	FRS (<10%, 10%-<20%, ≥20%)	CVD	0.0005 (0.0002; 0.0007) p<0.0001	-0.020 (-0.49;0.08) p=0.166	NR	0.0001 (0.0000, 0.0002)
Blankenberg 2010 [ppp7]	2 551	137 (IQR: 122; 153)	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%)	CVD[qqq]	0.0011 (p=0.69)	1.6±1.9 (p=0.40)	NR	0.0010 (p=0.20)

Table 8 – Apolipoproteins B1

Study	N	Apolipoproteins mg/dL	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Di Angelantonio 2012[17]	139 581[rrr]	110±29	FRS (<10%, 10%-<20%, ≥20%)	CVD	0.0001 (-0.0002; 0.0003) p=0.64	-0.17 (-0.52;0.18) p=0.34	NR	0.0002 (0.0000, 0.0004)
Blankenberg 2010 [sss7]	2 551	113 (IQR: 96;131)	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%	CVD[ttt]	-0.001 (p=0.45)	-0.2±1.1 (p=0.83)	NR	0.000 (p=0.98)

ooo From 26 studies

ppp Figures presented are from the PRIME Men Belfast Study Men

qqq Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarcti on

rrr From 26 studies

sss Figures presented are from the PRIME Men Belfast Study Men

ttt Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarction

Table 9 – Lipoprotein-associated phospholipase A2

Study	N	Phosp. A2	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Di Angelantonio 2012[17]	28 567[uuu]	167±40 nmol/min/ml (activity)	FRS (<10%, 10%-<20%, ≥20%)	CVD	0.0001 (-0.0003; 0.0006)	0.21 (-0.45; 0.86)	NR	-0.0002 (-0.0006, 0.0002)
	28 494	421±228 µg/l (mass)	id	id	0.0018 (0.0010; 0.0026) p<0.0001	0.81 (-0.15; 1.77) p=0.099	NR	0.0016 (0.0008, 0.0024)

Table 10 – Lipoprotein(a)

Study	N	Lipoprotein (a) mg/dL	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Di Angelantonio 2012[17]	133 502[vvv]	10.9 (IQR: 4.4;28.0)	FRS (<10%, 10%-<20%, ≥20%)	CVD	0.0016 (0.0009; 0.0023) p<0.0001	0.05 (-0.59;0.70) p=0.871	NR	0.0009 (0.0004, 0.0014)

[uuu] From 8 studies

[vvv] From 24 studies

3.3.3 Other CVD biomarkers

We retrieved 5 original studies which assessed the predictive increment of other CVD biomarkers, among which NT-pro-BNP, homocysteine, uric

Table 11, Table 12, Table 13, Table 14, Table 15). Among those biomarkers, NT-proBNP, a marker of vascular function, was the only one to improve substantially discrimination and reclassification when added to FRS, in 4 studies over 5[www].

Worth mentioning, Blankenberg et al. developed a score consisting of the 3 biomarkers the most strongly associated with CVD in their study but weakly correlated with one another, i.e. CRP, NT-proBNP, and troponin I. This score resulted in an NRI=11% (p=0.0008; 6% of cases classified up; 5% of noncases classified down)[xxx7].

[www] Since we applied our search strategy, we learned that a sixth study on the incremental prediction of N-terminal pro-BNP was published[49]. This study showed that the continuous NRI was 19.8% (95% CI: 13.6%; 25.9%) for N-terminal proBNP.

[xxx] When applied to 3 risk categories (<6%, 6%-20%, ≥20%), the NRI of the score based on multiple biomarkers was 7.3% (p=0.006)

Table 11 – NT-proBNP

Study	N	NT-proBNP pmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5 933	9.5 (IQR: 5.1; 4.4)	FRS (<10%, 10%-20%, >20%)	CHD	0.02 (0.01; 0.04)	7.6 (2.8; 12.5)	33.0 (23.4; 42.6)[yyy]	NR
Blankenberg 2010[zzz7]	3 870	0.03 (IQR:0.01;0.06)	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%)	CVD[aaaa]	0.0032 (p=0.072)	NR	NR	0.0157 (p=0.0002)
	4 045	0.05 (IQR:0.03;0.09)	id	id	0.0073 (p=0.0023)	NR	NR	0.0194 (p<0.0001)
	2 551	0.03 (IQR:0.02;0.06)	id	id	0.0114 (p=0.0427)	6.4 (p=0.025)	13.8[bbbb]	0.0080 (p=0.0017)
Melander 2009[45]	4 713	0.06 (IQR: 0.05; 0.09)	FRS (<6%, 6-19%, ≥20%)	CVD	0.004 (p=0.12)	0.4 (p=0.84)	NR	p=0.08
	id	id	id	CHD	0.006 (p=0.21)	1.2 (p=0.60)	NR	p=0.08
Rutten 2010[48]	2 032 men	6.8 (IQR: 3.7; 13.6)	Age, SBP, total cholesterol, HDL-C, diabetes, smoking	CVD[cccc]	0.033 (0.012; 0.052)	9.2% (3.5%; 14.9%)	23%[dddd]	9.1%[eeee] (5.0%; 12.9%)
	3 031 women	10.2 (IQR: 5.8;	id	CVD	0.032	13.3%	34.1%[ffff]	16.8%

yyy NRI in cases was 12.6%. NRI in noncases was 20.4%

zzz Figures presented are from: 1st line, the Men FINRISK 97 study; 2nd line, the Women FINRISK 97 study; 3rd line, the PRIME Men Belfast Study Men

aaaa Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarcti on

bbbb Computed by us. The NRI in cases was 0.9% (1/112). The NRI in non cases was 12.9% (91/704)

cccc Coronary heart disease, heart failure, ischemic stroke

dddd Computed by us. The NRI in cases was 24.1% (13/54). The NRI in non cases was -1.1% (-7/783)

eeee Relative IDI

Study	N	NT-proBNP pmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
		18.0)			(0.016; 0.068)	(5.9%; 20.8%)		(10.9%; 23.2%)
Wannamethee 2011[gggg43]	2 893	NR	FRS (<10%, 10%-20%, >20%)	CVD	0.018 (p=0.01)	8.8 (p=0.0009)	26.1[hhhh]	2.33 (p<0.0001)

Table 12 – Homocysteine

Study	N	Homocysteine µmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5 933	13.5 (IQR: 11.4; 16.6)	FRS (<10%, 10%-20%, >20%)	CHD	0.00 (0.00; 0.00)	-0.3 (-3.0; 2.3)	4.7 (-0.9; 10.3)[iiii]	NR
Blankenberg 2010 [jjjj7]	3 870	12.4 (IQR:10.7;14.6)	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%)	CVD[kkkk]	0.0009 (p=0.21)	NR	NR	0.001 (p=0.22)
	4 045	10.1 (IQR:8.6;12.1)	id	id	-0.0004 (p<0.0001)	NR	NR	-0.0004 (p<0.0001)
	2 551	11.8 (IQR:9.9;14.4)	id	id	0.0039 (p=0.21)	2.7±2.0 (p=0.19)	NR	0.0013 (p=0.20)

ffff Computed by us. The NRI in cases was 15.4% (10/655). The NRI in non cases was 18.7% (162/867)

gggg This included 6 649 men with or without CVD at baseline. We present here only the results on the 2 893 participants free of CVD at baseline

hhhh CNRI in cases was 17.2% (23/134) and CNRI in noncases was 8.9% (77/865)

iiii NRI in cases was -0.2%. NRI in noncases was 4.9%

jjjj Figures presented are from: 1st line, the Men FINRISK 97 study; 2nd line, the Women FINRISK 97 study; 3rd line, the PRIME Men Belfast Study Men

kkkk Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarction

Table 13 – Uric acid

Study	N	Uric acid µmol/L	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5 933	300 (IQR: 260;360)	FRS (<10%, 10%-20%, >20%)	CHD	0.00 (0.00; 0.00)	0.8 (-0.5; 2.1)	2.6 (1.0; 4.2)[llll]	NR

Table 14 – von Willebrand factor antigen

Study	N	vWF IU/mL	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Kavousi 2012[19]	5 933	1.2 (IQR: 0.9;1.6)	FRS (<10%, 10%-20%, >20%)	CHD	0.00 (0.00; 0.00)	0.4 (-1.7; 2.5)	4.0 (-0.2; 8.1)	NR

Table 15 – Troponin I

Study	N	%≥0.032 ng/mL	Reference Model	Outcome	*C*-statistic change	NRI (95%CI)	CNRI (95%CI)	IDI (95%CI)
Blankenberg 2010 [mmmm7]	3 870	1.7	FRS (<5%, 5%-<10%, 10% -<20%, ≥20%)	CVD[nnnn]	0.0045 (p=0.0028)	NR	NR	0.0077 (p=0.0002)
	4 045	0.9	id	id	0.0009 (p=0.3950)	NR	NR	0.0037 (p=0.009)
	2 551	0.7	id	id	0.0039 (p=0.21)	1.4 (p=0.43)	NR	0.0019 (p=0.16)

[llll] NRS in cases was 0.8%. NRI in noncases was 1.8%

[mmmm] Figures presented are from: 1st line, the Men FINRISK 97 study; 2nd line, the Women FINRISK 97 study; 3rd line, the PRIME Men Belfast Study Men

[nnnn] Acute MI, coronary death, unstable angina pectoris, cardiac revascularization, unclassifiable death, likely cerebral infarcti on

Table 16 – Excluded studies

Study	Reasons for exclusion
Agarwal 2012[50]	4% of participants were suffering of CHD at baseline. Predictive increments of biomarkers were tested in comparison with the ARIC model which differs from SCORE or FRS on various factors, i.e. it includes prevalent coronary heart disease, use of blood pressure-lowering medication, heart rate and BMI. NT-proBNP had a NRI=13.5% (95%CI: 10.2%; 19.9%) (ARIC model with 4 10-year risk categories <5%; 5-10%; 10-20%; ≥20%)
Brouwers 2012[51]	6.5% of participants had MI at baseline, and 0.8% had an history of stoke. Results were not stratified on history of CVD
Cao 2008[52]	No direct comparison of the performance of a prediction model with and without a biomarker.
Cross 2012[53]	The authors compared the predictive value of an algorithm combining age, sex, diabetes, family history of myocardial infarction, and multiple biomarkers biomarkers (CTACK, Eotaxin, Fas Ligand, HGF, IL-16, MCP-3, and sFas) to the Framingham model, i.e. the 2 models were very different and the study did not provide direct evidence of adding a specific biomarker to currently used and validated risk prediction model.
Finckh 2012[54]	Biomarkers applied in a specific sub-population of patients suffering from rheumatoid arthritis. The anti-apo A-I had an IDI=175% (p<0.001) in comparison with the FRS only.
Hamer 2009[55]	No NRI computed and data to compute it were not reported
Herder 2011[56]	Study population includes only diabetic patients. No NRI is computed.
Kim 2010[57]	This study had a case-control design and relatively small numbers (321 patients and 743 controls). The computation of reclassification was done relatively to 3 categories where high risk was mixed with moderate risk (<5%, 5%-10%, ≥10%). Moreover this computation compared a prediction model with traditional risk factors with one containing all biomarkers, with no possibility to disentangle the predictive increment of individual biomarker.
Kofler 2012[58]	Focus on APOE genotype. No NRI reported.
LLuis-Ganella 2012[59]	Focus on a genetic risk score, not on biomarkers
Nordestgaard 2010[3]	This study had a nested case-control design. Moreover, no NRI was reported and data to compute it were not reported
Pikula 2012[60]	8.8% of participants were suffering of cardiovascular disease at baseline
Rana 2009[61]	This study was a nested case-control, and as the authors stated it “Because NRI quantifies the combined effect of reclassification in cases and controls combined, our design may have an impact on NRI”
Rana 2012[62]	• The authors assessed the predictive increment of a set of multiple biomarkers (C-reactive protein, interleukin-6,

	myeloperoxidase, B-type natriuretic peptide, and plasminogen activator-1), and it was not possible to disentangle the performance of each biomarker beyond changes in c-statistics. • The follow-up duration was only 4.1±0.4 years. The risk categories were unusual (<2.4%, 2.4%-8%, >8%) corresponding respectively to 10-year risk categories of <6%, 6% to 20%, >20% according to the authors.
Sattar 2010[63]	• The reference model was different from the usual prediction model SCORE and FRS as it was based on childhood and adult social class, physical activity, fasting lipids (not more detailed) and glucose, waist to hip ratio, diabetes, statin and aspirin use. • NRI not reported and no data to compute it was reported • Case-control design
Sehestedt 2010[64]	Authors attempted to assess the predictive increments of indicators of subclinical organ damages when combined with the risk factors of the SCORE. However, the 4 indicators of subclinical organ damages (left ventricular mass index, atherosclerotic plaques in the carotid arteries, carotid/femoral pulse wave velocity, urine albumin/creatinine ratio) were used in undescribed combinations (it appears in table 2 that not all indicators were measured in every participant). The paper does not allow disentangling the predictive increment per indicator of subclinical organ damage.
Shah 2012[65]	Study carried in patients undergoing cardiac catheterization, i.e. no screening for primary cardiovascular prevention
Tsimikas 2010[66]	No NRI was reported and data to compute it were not reported
Tsimikas 2012[67]	10% of participants were suffering of CVD at baseline (25% in the CVD group). Unusual cut-offs were used (<15%, 15% to 30%, >30% 10-year CVD risk). Sample size was relatively modest (n=765).
Volpe 2012[68]	Low-quality narrative review ("our literature search to support a viewpoint, based on meta-analyses and the most representative and large studies, reflects overall a straightforward approach"). No results on NRI presented.
Wang 2012[69]	• The authors assessed the predictive increment of a set of multiple biomarkers (sST2, GDF-15, hsTnI, BNP, and hsCRP), and it was not possible to disentangle the performance of each biomarker • CVD at baseline was prevalent in 5 % of participants
Woodward 2009[70]	• Characteristics of the study population are not reported. • The way the NRI was computed is not clearly explained and seemed different from the common definition of it ("NRI measured the improvement in sensitivity plus specificity in the two-way table of true CVD event status versus expected CVD risk"). • The reference risk categories had only 1 cut-off (20% 10-year risk of CVD)
Woodward 2011[71]	No usual biomarkers of incident CVD were measured (fatty acids in adipose tissue after a biopsy)

3.4 Discussion

CRP has been the most studied CVD biomarker so far. Its predictive increment has been assessed mainly against the Framingham risk model (which predicts CVD risk in the next 10 years), and we retrieved no study involving directly the SCORE (which predicts CVD death risk in the next 10 years). However, there is no straightforward argument to assume that a biomarker improving CVD risk prediction in comparison with the FRS model would behave differently in comparison with the SCORE. Overall, the predictive increment of CRP was relatively modest in comparison with the risk prediction based on the traditional factors included in the FRS. A number of hypotheses can be put forward to explain such modest added value in spite of the strong evidence that CRP is associated with CVD. First, CRP may not be a direct risk factor of CVD[14, 15] but rather associated with the development of other CVD risk factors such as high BMI[72], hypertension[73], diabetes[74], and smoking. These factors are already captured in the FRS. Whether CRP is on the causal pathway or a mere covariate of these other risk factors, the contribution of CRP to prediction models is subsequently reduced. The study by Kaptoge et al. gives a nice illustration of this. The NRI of CRP is 3.29% (95%CI: 2.28%, 4.30%) when compared to a non-lipid-based FRS model, but only 1.52% (0.78%, 2.27%) after total and HDL-cholesterol have been accounted for[42].

Second, the prediction models integrate CRP as a continuous variable and CRP is log-normally distributed in the general population[11]. A substantial proportion of all CVD events occur among the large number of individuals with near average levels of CRP. It might be more discriminant to use a cut-off of CRP above which the risk of CVD would greatly increase. For example, Blankenberg et al. estimated that the CRP cut-off point giving the best discrimination by IDI, which is equivalent to the increase in average sensitivity given no changes in specificity[28], was 6.81 mg/L[7]. This value is much higher than the current cut-off to define high concentration of CRP (>3.0 mg/L). Third, accuracy and precision of measurements might be suboptimal. We have already discussed in chapter 2 that different laboratory techniques may yield results with various accuracy[32]. The day to day variability of CRP measurements must also be accounted for especially when one considers that 10-year CVD probability is usually based on a single measurement[75].

In individuals identified at moderate 10-year risk of CVD by the FRS, the measurement of CRP resulted in a much greater CNRI than previously reported. Very few studies directly assessed the effect of CRP on risk reclassification in intermediate-risk persons up to date. One of the reasons of this higher CNRI might be that a proportion of intermediate-risk individuals classified by FRS have indeed an absolute CVD risk close to the upper bound, in which case using a lower CVD risk cut-off could also help in up-reclassifying individuals at high risk. Unfortunately, none of the studies included in our review assessed how the actual CVD risk within usual risk categories (e.g. 10%-20% risk of CVD in the next 10 years) influenced reclassification. Another explanation for a CNRI greater than the CRI would be that in intermediate-risk individuals, other risk factors such as smoking or age are less prevalent, letting more room for a larger contribution of CRP to explanatory models.

Besides CRP, we found consistent evidence that HDL-cholesterol, already integrated in most CVD risk prediction models, improves CVD risk prediction independently of total cholesterol, although the NRI was modest across studies. The NRI for HDL-cholesterol was highly variable across studies as a likely result of different reference prediction models and various clinical outcomes assessed. Other lipid-based biomarkers presented no added value. There is also emerging evidence that NT-proBNP could be a biomarker allowing a better discrimination and classification of individuals. The above considerations on the information and shortcomings of CRP studies however also apply to these biomarkers.

Overall, the quality of evidence was moderate. Validation in a population different from the one used to establish the prediction model was seldom used[7], although several studies corrected their estimates for over-optimism. A selection bias in the population under scrutiny was also often difficult to assess: characteristics of individuals with missing measurements were rarely compared to those of the eligible study population, and existence of lost-to-follow up was poorly described. Lastly, it is unfortunate that CNRI in intermediate-risk individuals is still rarely reported in studies. It is also unfortunate that the risk-categories in a number of studies differ from what is currently recommended in clinical practice, hampering direct translation of evidence, as NRI is sensitive to the number of categories considered[28].

It is desirable that in further studies crude numbers of individuals in risk categories is systematically presented so as to allow re-computation of reclassification indexes. In all cases, the NRI without categories should also be reported to facilitate comparison across studies.

It is worth mentioning that the predictive increment of some biomarkers is modified by the presence of other parameters. The NRI of CRP was higher in men than in women in 2 studies[7, 42]. This was also the case for apoliproteins A1 and B[17], whereas the opposite was retrieved in a study assessing the added value of HDL-cholesterol in SCORE[5]. Age could also be an effect modifier[51]. Whereas these interactions are not fully understood and could be partly due to chance, such analysis should be provided in future studies if the overall goal of risk prediction models is to adapt as much as possible CVD prevention to each individual situation.

It is also interesting to note that conventional risk factors have not yet been used to their full potential. Conventional risk factors have been chosen because they were available in the majority of the cohort studies which served to establish the prediction models and because their definition was quite standardized. This also facilitates the utilization of these models in the form of clinical scores. Such approach generates two difficulties though; First, the dose-response gradient of risk factors is not accounted for. For example, current smoking might bear a very different CVD risk for consumption of 5 cigarettes a day for 5 years vs. 25 cigarettes per day for 25 years. Duration of diabetes is another example. Second, some risk factors for which information is easy to collect during clinical consultation are not included because of difficulties in their standardization. This is for example the case of physical inactivity, or family history of CVD, which is included in some prediction models, but not in FRS or SCORE. So-called conventional risk factors still need to be further understood and developed.

Finally, there is no good-quality evidence showing that using an established risk prediction model integrating a novel biomarker results in better clinical management and outcomes in primary CVD prevention. To the best of our knowledge, there was no RCT assessing this crucial question. The JUPITER trial[oooo] on rosuvastatin for primary prevention of cardiovascular events in 17,802 men and women with elevated (>2 mg/L) CRP levels, low-density lipoprotein cholesterol levels and no other indication (such as diabetes) for statin therapy was not designed to assess the added value of measuring CRP on top of conventional risk factors for deciding clinical treatment[76]. Furthermore, there is still no obvious treatment or strategy to improve many of the biomarkers reviewed. Before such biomarkers are integrated in risk prediction models, good-quality evidence is needed on the usefulness of such strategy for motivating lifestyle changes or for guiding therapy.

[oooo] The JUPITER trial (Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin)

4 COST-EFFECTIVENESS OF CVD BIOMARKERS

4.1 Introduction

This chapter provides an overview of studies evaluating the use of novel serum biomarkers in primary prevention of cardiovascular disease from an economic perspective. In spite of the lack of robust clinical evidence in favour of a strong predictive increment by use of the biomarkers reviewed in chapter 3, cost-effectiveness studies of CVD biomarkers are increasingly published. It is therefore important to review and critically appraise such studies, to assess how methodologically robust they are and what specific data inputs remain a challenge. Moreover, some of the biomarkers here analyzed (e.g. CRP) have already been included in some prediction algorithms, such as the Reynolds score in the USA, and thus reviewing all available evidence (clinical and economic) may facilitate a better understanding of the basis for such decisions.

4.2 Methods

4.2.1 Search strategy

A systematic search for relevant publications was carried out with the consultation of electronic reference databases up to January 2013.

Medline (though OVID), EMBASE, Econlit (through OVID), the NHS EED (CRD) and the NHS HTA (CRD) were searched to retrieve primary full economic evaluations (studies comparing at least two competing alternatives in terms of both costs and outcomes) and reviews of economic evaluations (i.e. secondary economic evaluations). An overview of the search strategy is given in the appendix.

Furthermore, the websites of Health Technology Assessment (HTA) institutes listed on the INHATA website (International Network of Agencies for Health Technology Assessment) were consulted to capture HTA reports on the use of novel serum biomarkers in cardiovascular disease prevention.

4.2.2 Selection procedure

To identify potentially relevant studies for our analysis we first went through all titles and abstracts in order to exclude any obvious studies that did not match our research subject. All articles that appeared to be interesting or for which there were some doubts were read in full in order to select those relevant for inclusion in our review.

Reference lists of the selected primary and secondary economic evaluations found via our search were checked for additional references worth adding to our analysis.

All studies finally included in our review were critically appraised by using an adaptation of the checklist developed by Drummond et al[77]. A summary of the studies, their characteristics and overall results is provided in the appendices in an individual tabular form.

4.2.3 Selection criteria

Although studies evaluating the addition of a novel serum biomarker to current, well validated, risk classification systems such as the Systematic Coronary Risk Evaluation (SCORE) or the Framingham systems would be most interesting, a deliberately broader selection strategy was used given the novelty of the topic and the focus of this review (economic evaluations). Thus, to ensure capturing a good overview of what has been published so far, all full economic evaluations adding biomarkers to any other way of classifying adults according to risk of cardiovascular disease were included in our review. Analyses performed on patients already diagnosed with cardiovascular disease (i.e. secondary prevention) or at high risk of suffering from cardiovascular (CV) events were excluded.

Cost descriptive analyses or cost comparisons not taking into consideration effectiveness were also excluded. Similarly we decided to exclude publications in the form of letters, editorials or notes, since these would not offer enough information to include them in our analysis and critically appraise their findings. An overview of the inclusion/exclusion criteria is given in Table 17.

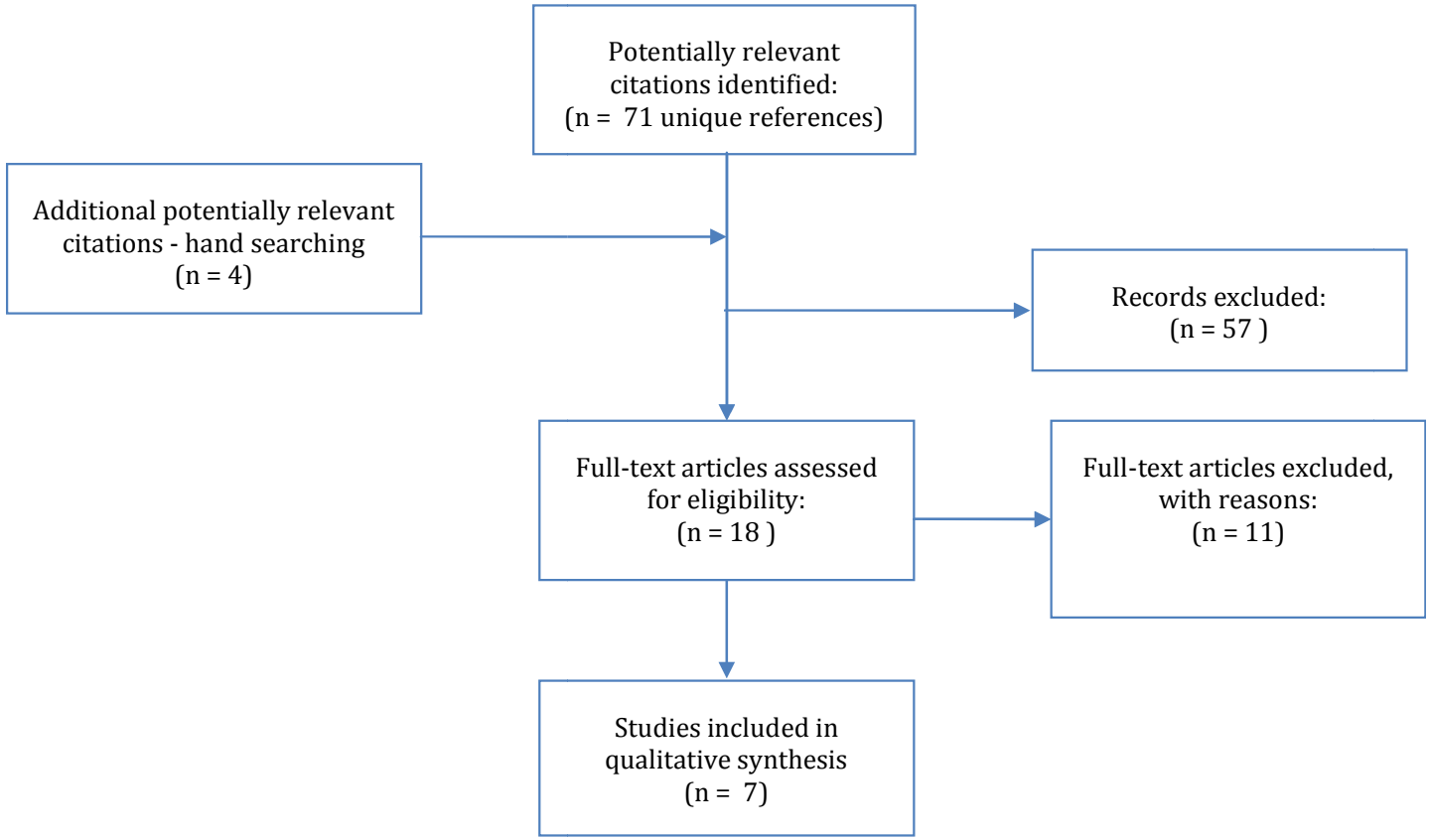
Table 17 – Selection criteria for economic evaluations

Selection criteria	Inclusion criteria	Exclusion criteria
Population	General adult population	Patients already diagnosed with cardiovascular disease or at high risk of suffering CV events
Intervention	Novel serum biomarkers	Any other marker
Comparator	Alternative patient risk scoring systems	Absence of risk stratification system
Design	Full economic evaluations (primary or secondary)	Cost descriptive analysis, cost comparisons
Type of publication	Articles or reviews	Letters, editorials, notes

Our search returned 71 unique citations, after eliminating duplicates. Of those 57 did not meet our inclusion criteria based on a review of their title and/or abstract. Of the 18 citations left, 11 were excluded after reading their full text. Amongst these, there were two conference abstracts referring to theoretical models[78, 79]. The lack of detailed information in the abstracts did not allow us to include these studies in our analysis, which left us with seven full primary economic evaluations[80-86].

The flow chart in figure 2 illustrates our literature selection process.

Figure 2 – Flow chart selection procedure – economic literature



4.3 Overview of economic evaluations

Table 18 – Overview of economic evaluations of biomarkers in primary prevention of CVD

Author	Year	Country	Type of analysis	Perspective	Biomarker	Time horizon (in years)	Discount rate; both costs and outcomes (%)
Long-term evaluations including treatment resulting from screening							
Choudhry[82]	2011	USA	Cost-utility	Healthcare	CRP	Lifetime	3
Lee[86]	2010	USA	Cost-utility	Healthcare	CRP	Lifetime	3
Blake[80]	2003	USA	Cost-utility/cost-effectiveness	Healthcare	CRP	Lifetime	3
Ess[84]	2002	France, Spain, Switzerland	Cost-effectiveness	Healthcare	CRP	5	NA
Ess[83]	2001	Germany and Italy	Cost-effectiveness	Healthcare	CRP	5	NA
Short-term evaluations on diagnosis accuracy							
Lakić[85]	2010	Serbia	Cost-effectiveness	Healthcare	SH, O_2^-, MDA, SOD, Lp(a), apoB, apoA-I, CRP, apo(a) isoform	NA	NA
Bogavac-Stanojević[81]	2007	Serbia	Cost-effectiveness	Healthcare	Lp(a), apo(a), apoB, apoA-I, CRP	NA	NA

apo= apolipoprotein; CRP= C-reactive protein; Lp= lipoprotein; MDA= malondialdehyde; O_2^-= superoxide anion; SH= sulfhydryl; SOD= superoxide dismutase

As shown in Table 18 three studies were undertaken in the USA[80, 82, 86], two in Western European countries[83, 84] and two in Eastern Europe (Serbia)[81, 85]. The two studies looking at costs in Western European countries were undertaken by the same main author, with the most recent looking at costs in France, Spain and Switzerland and the other focusing on Germany and Italy.

All studies were published after 2000, with three of them, published very recently (2010 or 2011). All but two studies were model-based (decision-analytic or Markov models). The two Serbian economic evaluations by Lakić et al. and Bogavac-Stanojević et al.[81, 85] were built on case control studies.

4.3.1 Type of economic evaluation

Two of the studies performed cost-utility analyses[80, 82, 86], with outcomes expressed in quality-adjusted-life-years (QALYs), while Blake et al.[80] carried out both cost-utility and cost-effectiveness analyses with outcomes in QALYs and life-years gained (LYG). The remaining four studies were cost-effectiveness analyses, two of them with outcomes expressed in LYG[83, 84] and the other two simply looking at accuracy in coronary artery disease (CAD) diagnosis and expressing their outcomes in terms of number of successfully diagnosed CAD cases[81, 85].

4.3.2 Time frame of analyses and discounting

The three cost-utility studies included in this analysis[80, 82, 86] looked at costs and outcomes over a patient's lifetime, while two cost-effectiveness analyses limited their time frame to five years[83, 84], a study period too short to truly capture the long-term consequences of the intervention of focus. Indeed, CV events tend to happen in the long-term, years or even decades after the screening was performed.

The remaining two studies[81, 85] consisted of regression analyses looking at the association between laboratory tests and the presence of CAD, the only relevant timeframe for both studies was thus limited to the time required for the completion of the laboratory tests.

From the five studies with a time frame of over a year only three[80, 82, 86] discounted both cost and outcomes at an equal rate of 3%. The studies using discounting were also the most recent ones.

The two studies[83, 84] which did not apply discounting had a time spam of five years. They did not attempt to extrapolate their results over a longer time span and did not specify either the year to which their costs referred to.

4.3.3 Perspective

All studies were performed from a healthcare perspective. However, five studies[80, 82-84, 86] presented their analyses as following a societal perspective but when analysed in detail, they appeared to only cover direct medical costs. No study captured productivity losses, travel costs or family costs.

4.3.4 Population

Five studies identified in our review were model-based[80, 82-84, 86] and two were built from case control studies[81, 85]. The sample size of the population-based studies was of 385 patients (188 CAD patients and 197 asymptomatic, CAD free patients attending a regular annual medical check-up) for the study by Lakić et al.[85] and 510 patients (221 CAD patients and 289 asymptomatic patients) for the study by Bogavac-Stanojević et al.[81]

The hypothetical patient cohorts used in the model-based studies consisted of adults aged 40-70 in Lee et al.[86], 35-85 in Blake et al.[80] and men ≥50 and women ≥60 years of age in Choudhry et al.[82]. The remaining two evaluations[83, 84] modelled hypothetical cohorts of men dividing them into three separate age groups: 35-44; 45-54 and 55-64. The modelled cohorts presented in all cases normal lipid levels of cholesterol and no signs of cardiovascular disease.

4.3.5 Focus and intervention

The evaluations included in this study can be separated in two groups depending on their aim and approach.

The first group consists of the two Serbian studies[81, 85] aimed at selecting amongst a number of available serum biomarkers the most cost-effective combinations which could, added to current risk-stratification methods (*i.e.* Framingham score system), offer better value for money in terms of their discriminative abilities as CAD predictors. Their focus is thus, restricted to the role that new screening methods could play in better diagnosing

patients and their perspective is limited to the short-term. The biomarkers evaluated included the following: lipoprotein a (Lp (a)), apolipoprotein a (apo (a)), apolipoprotein B (apoB), apolipoprotein A1 (apoA-I) and C-reactive protein (CRP) in Bogavac- Stanojević et al.[81] and sulfhydryl (SH), O_2^- (superoxide anion), malondialdehyde (MDA), superoxide dismutase (SOD), the combination of SOD and O_2^-, Lp (a), apoB, apoA-I, CRP and apo (a) isoform in Lakić et al.[85].

A second group of studies looks instead at the cost-effectiveness of implementing primary prevention for patients at an increased risk of suffering from CV events in the future, according to the results from new risk stratification methods involving serum biomarkers. Contrarily to what we saw for the first group, this second group is more interested in the long-term costs and outcomes that could come associated both with the introduction and implementation of a new patient stratification system and the treatment that may derive from it for specific patient groups. As many as five studies fell within such group[80, 82-84, 86].

Although the aim of our review was to identify studies looking at the cost-effectiveness of any novel serum biomarker in primary prevention of cardiovascular disease, all seven studies included one specific biomarker, CRP (see chapter 1 for more details on this biomarker), which was the main focus in as many as five studies[80, 82-84, 86]. The cut-off points used for "high levels" of CRP at which treatment would be started varied from one study to another from a low of 1.6mg/l[80] to a high of 3mg/l[83, 84]. Three of the studies which focused on CRP looked at treatment with statins for patients with low levels of cholesterol but high levels of CRP, while the remaining two considered both statins and aspirin as potential treatment strategies[83, 84].

4.3.6 Comparators used

"Usual" risk assessment methods were, in all cases, the comparator used. However, the description of those methods, most often, did not correspond to well recognised and validated current patient classification systems (*i.e.* Framingham, SCORE, Adult Treatment panel III, Reynolds, ASSIGN, PROCAM or QRISK). Instead, these were either described as "no testing and no treatment" and purely based on cholesterol levels or included, in addition to cholesterol levels, the presence or not of other risk factors. More specifically, the most recent study[82], described their comparator as "no testing and no treatment", while Blake et al.[80] compared CRP testing to both dietary counselling alone in patients with low cholesterol levels and treatment with statins for all patients. The two cost-effectiveness studies by Ess et al.[83, 84] described their comparator as current risk assessment methods based on cholesterol levels and presence or not of other risk factors (*i.e.* age).

Only three studies compared biomarkers with one of the specific, well recognised, patient classification systems previously mentioned (*i.e.* the Framingham risk score system)[81, 85, 86].

4.3.7 Cost and outcome inputs

Costs were derived from the published literature but while in the two European cost-effectiveness studies inputs represented country-specific market prices, for two of the three cost-utility US studies[82, 86] these were taken from Medicare payment rates, while Blake et al.[80] did their modelling based on costs as opposed to charges.

Age-specific QoL values for the three cost-utility studies were taken from different published sources with Lee et al[86]. using a US medical expenditure survey and adjusting the values by weights for cardiovascular health states and Blake et al. and Choudhry et al.[80, 82] referring to US published literature.

4.3.8 Modelling

Three studies consisted of Markov models with annual cycles[80, 82, 86], while the remaining two were decision-analytic models not described in detail[83, 84]. All of them focussed on CRP screening.

Although the model design was transparent for two of the studies[82, 86], the three remaining did not clearly explain their model structure[80, 83, 84]. The model by Choudhury et al.[82], and even more so, that of Lee et al.[86] presented a complex structure which was, in both cases, supported by additional information in the form of appendices to increase transparency and facilitate model reproducibility.

The main weakness in all of these models is the existing gaps in the literature from which inputs are taken, most of which relate to the efficacy of treatment following the screening of patients. The two cost-effectiveness studies[83, 84] included assumptions not well backed-up with literature about

the prevalence of high CRP values amongst apparently healthy individuals and the difference in efficacy of statins versus aspirin for preventing future CV events. Although the three cost-utility studies based the assumptions regarding the probabilities of events and the transition from one state to another on meta analyses or clinical trial data, a specific problem was the lack of evidence for the long-term effectiveness of statins in populations with high CRP levels, since the only study up to date providing limited evidence in this regard is the JUPITER trial [76], although this study was not designed to assess the effect of rosuvastatin in individuals with and without an elevated CRP and some of its methods was heavily questioned criticized [87]. While any modelling exercise looking at the impact of a CRP screening strategy should ideally encompass costs and outcomes over a patient's lifetime, the median follow-up time in the JUPITER was of just 1.9 years. Uninformed assumptions had thus to be made on the transition of treatment effects over time for those studies which did use the JUPITER trial as an important source of their model inputs. While this study was not available at the time Blake et al. or Ess et al. performed their evaluations[80, 83, 84], it was taken into consideration as an important source of data both in Lee et al.[86] and, even more so, in Choudhry et al.[82, 86], who used the JUPITER trial as their main data source. Extrapolation methods were made explicit for the latter in which the base-case assumption was that treatment effects seen in the JUPITER trial would persist for 15 years to then tamper off after 25 years.

A further challenge was that the potential for CRP levels to discriminate between patients in which statin therapy would be effective from those in which it would not work has not been assessed, given that the JUPITER trial presented a scope limited to individuals with high CRP levels. Only the study by Lee et al.[86] presented a scenario analysis in which the authors tested a scenario assuming statins are effective independently of CRP levels (equal-effects scenario) as well as a scenario assuming statins are not effective in individuals with low CRP levels (differential scenario). Both scenarios resulted in weak, unclear messages, showing that until new data becomes available it is difficult to truly measure the additional value of CRP testing followed by statin treatment versus (or added to) other risk-stratification methods. Similarly, the same authors tested different possibilities linked to the adverse events (AEs) of statins since there is a further important gap in the literature regarding the long-term use of these agents and their potential AEs over a patient's life time.

All of these issues make the modelling exercises performed too theoretical and so their results need to be interpreted with caution.

4.3.9 Results

4.3.9.1 Incremental costs

Table 19 shows the average costs obtained in the seven studies included in our review. Comparisons between studies are difficult primarily because of the different grouping systems used (gender, age, risk groups). While, Lee et al.[86] reported costs depending on gender, age (40-70 years) and risk level (*i.e.* no risk, hypertension only or hypertension + smoking), others such as Blake et al.[80] analysed costs purely by gender and age group (35-80 years).

Overall, costs appear to decrease when CRP is applied to higher risk groups or older individuals. Also in all studies looking at both genders, a strategy including CRP screening in women was more expensive than in men.

4.3.9.2 Incremental outcomes

Table 20shows that all studies focussing on the addition of CRP to "usual" methods for stratifying patients according to their risk to suffer a CV event in the future showed positive effects when compared to usual stratification methods alone.

Direct comparisons of incremental gains in Quality Adjusted Life Years (QALYs) are challenging since each study grouped their populations according to different characteristics.

The results from the two studies looking at test accuracy appear to vary from one biomarker to another although most biomarkers offered positive effects in terms of effective diagnoses of new CAD cases over and above the Framingham score system. While such effects were measured by the AUC in the case of Lakić et al.[85], Bogavac-Stanojević et al.[81] measured effectiveness in terms of number needed to diagnosed (NND), calculated as the reciprocal value of the proportion of positive tests in the group with the disease (sensitivity) minus the proportion of positive tests in the group without the disease (NND=1/(sensitivity-(1-specificity)).

4.3.9.3 Incremental cost-effectiveness ratios (ICERs)

Table 21shows that, overall, the studies looking at the value of CRP in primary prevention of CV events report that such strategy could be cost-effective, in particularly for men, at relatively low willingness-to pay thresholds. Choudhry et al.[82] reported an overall ICER of US$25 198/QALY for CRP testing followed (if high levels detected) by statins, versus no test and no treatment in a cohort of men ≥50 and women ≥60 years of age. Blake et al.[80] found an ICER of US$48 100/QALY for an average 58-year old men, although this went up to US$94 400 for women of the same age.

Although the results for the base case scenario from Lee et al.[86] show that both CRP screening followed by statin treatment for those with high CRP levels, and treatment with statins for all patients presenting certain risk characteristics (considering age, hypertension and smoking status) could be cost-effective for men at relatively low willingness to pay thresholds (see table 5 for details), ICERs for CRP screening appear to be lower. The overall pattern showed ICERs being influenced not just by risk group category (*i.e.* lower ICERs for hypertensive smokers than for patients in the no risk or hypertension only groups) but also by age (*i.e.* lower ICERs for older individuals). Due to the lower risk of CV events presented by women ICERs in this group for both CRP screening and treatment with statins for all, showed to be considerably higher than those seen in men.

Two further studies by the same author[83, 84] performed in men aged 35-64, showed that testing CRP levels followed by treating those with high levels with aspirin over a 5-year period is cost-effective when compared to “usual risk assessment methods” (based on cholesterol levels and presence or not of other risk factors) in Western European countries. The same studies showed that a strategy involving testing for CRP followed by aspirin or statins would still be cost-effective when compared to “usual risk assessment methods”, but not as attractive as the option of testing for CRP levels followed by treatment with aspirin for all patients presenting high CRP levels.

With regards to the cost per additional accuracy in CAD prediction, the study by Lakić et al.[85] showed the combination of Framingham scoring test, followed by SOD and O2 testing to be the preferred option. By contrast, Bogavac-Stanojević et al.[81] concluded that a scoring system using Framingham risk score as first line, then adding apoA-I testing as second line, would offer the best value for money. It is important to mention that these studies have an important limitation in that they only look at accuracy in diagnosis but do not follow the patient over time to assess the long-term impact of the treatment on costs and outcomes. They, therefore, only offer a partial picture of their potential cost-effectiveness.

Table 19 – Costs of novel serum biomarkers in CVD prevention

Study	Costing year	Time horizon (years)	Costs included	Intervention	Costs/patient over study period (as reported)
Long-term evaluations including treatment resulting from screening					
Choudhry 2011 USA	**2009**	**Lifetime**	**CRP and liver function tests, drug costs, acute hospitalisation, physician services, post-acute and ongoing care**	**Intervention: CRP screening ± statins**	US$27 616
				Comparison: "Usual care"	US$19 717
Lee 2010* USA	**2008**	**Lifetime**	**Lab tests, medication, AEs with statins, treatment and intervention for MI, stroke, post MI and post stroke and end-of-life**	**Intervention:**	
				ATP-III guidelines	**No risk: m:** US$250 409
				Age 70 CRP screening	**No risk: m:** US$251 703
				Age 65 CRP screening	**No risk: m:** US$252 261
				Age 70 start statins	**No risk: m:** US$252 351
				Age 60 CRP screening	**No risk: m:** US$252 834
				Age 65 start statins	**No risk: m:** US$253 246
				Age 55 CRP screening	**No risk: m:** US$253 448
				Age 50 CRP screening	**No risk: m:** US$254 109
				Age 60 start statins	**No risk: m:** US$254 182
				Age 45 CRP screening	**No risk: m:** US$254 842
				Age 55 start statins	**No risk: m:** US$255 189
				Age 40 CRP screening	**No risk: m:** US$255 599
				Age 50 stat statins	**No risk: m:** US$256 288
				Age 45 start statin	**No risk: m:** US$257 562
				Age 40 start statins	**No risk: m:** US$259 054
				Comparison: all alternatives compared	NA

Study	Costing year	Time horizon (years)	Costs included	Intervention	Costs/patient over study period (as reported)
Blake 2003 **USA**	**2000**	**Lifetime**	**CRP testing, physician visits, acute and chronic costs of MI and stroke**	**Intervention:** **1. CRP screening ± statins** **2. Statins for all**	 **58-yr old** **m:** US$14 600; **w:** US$14 400 **58-yr old** **m:** US$21 100; **w:** US$22 000
				Comparison: "usual care"	**58-yr old** **m**: US$9 500**;** **w:** US$7 500
Ess 2002 **France, Spain & Switzermand**	**NA**	**5**	**CRP testing, statin and aspirin treatment, MI, other CV events**	**Intervention:** **1. CRP screening ± statins or aspirin** **2. CRP screening ± aspirin**	 NA NA
				Comparison: usual risk-assessment methods	NA
Ess 2001 **Germany & Italy**	**NA**	**5**	**CRP testing, statin and aspirin treatment, MI, other CV events, CV deaths**	**Intervention:** **1. CRP screening ± statins or aspirin** **2.CRP screening ± aspirin**	 NA NA
				Comparison: usual risk-assessment methods	NA
Short-term evaluations on diagnosis accuracy					
Lakic 2010 **Serbia**	**2008**	**NA**	**Cost of tests, materials and labor fees /100 tests**	**Intervention:** **Framingham** **Framingham + SH** **Framingham + O_2^-** **Framingham + MDA** **Framingham + SOD** **Framingham + SOD + O_2^-** **Framingham+ Lp(a)** **Framingham + apoB**	 €506.7 €543.4 €558.5 € 568 €570.5 €622.2 €767.1 €1 020.9

Study	Costing year	Time horizon (years)	Costs included	Intervention	Costs/patient over study period (as reported)
				Framingham + apoA-I **Framingham + CRP** **Framingham + apo (a) isoform**	€1 035 €1 139.4 €1 261.3
				Comparison: all alternatives compared	NA
Bogavac-Stanojević 2007 Serbia	**NA**	**NA**	**Costs of materials tests and labor fees/100 tests**	**Intervention:** **Framingham +Lp(a)** **Framingham +apo(a)** **Framingham +apoB** **Framingham + apoA-I** **Framingham +CRP**	 €228.35 €689.63 €70.99 €85.06 €189. 5
				Comparison: all alternatives compared	NA

** Results from baseline model (equal-scenario)*

AEs=Adverse Events; apo= apolipoprotein; ATP=Adult Treatment Panel; CRP= C-Reactive Protein; CV= Cardiovascular; Lp= lipoprotein; m=men; MDA= malondialdehyde; MI=Myocardial Infarction;

NND= Number Needed to Diagnose; O2-= superoxide anion; QALY=Quality Adjusted Life Years; SH= sulfhydryl; SOD= superoxide dismutase; w=women

Table 20 – Outcomes of novel serum biomarkers in CVD prevention

Study	Time horizon (years)	Outcomes considered	Intervention	Results
Long-term evaluations including treatment resulting from screening				
Choudhry 2011 **USA**	**Lifetime**	**QALYs**	**Intervention: CRP screening ± statins**	10,61
			Comparison: "Usual care	10,29
Lee 2010* **USA**	**Lifetime**	**QALYs**	**Intervention:** **ATP-III guidelines** **Age 70 CRP screening** **Age 65 CRP screening** **Age 70 start statins** **Age 60 CRP screening** **Age 65 start statins** **Age 55 CRP screening** **Age 50 CRP screening** **Age 60 start statins** **Age 45 CRP screening** **Age 55 start statins** **Age 40 CRP screening** **Age 50 stat statins** **Age 45 start statin** **Age 40 start statins**	 **No risk: m:** 19.012 **No risk: m:** 19.051 **No risk: m:** 19.067 **No risk: m:** 19.069 **No risk: m:** 19.084 **No risk: m:** 19.095 **No risk: m:** 19.099 **No risk: m:** 19.112 **No risk: m:** 19.120 **No risk: m:** 19.120 **No risk: m:** 19.145 **No risk: m:** 19.125 **No risk: m:** 19.166 **No risk: m:** 19.181 **No risk: m:** 19.191
			Comparison: all alternatives compared	NA
Blake 2003 **USA**	**Lifetime**	**QALYs**	**Intervention:** **1. CRP screening ± statins** **2. Statins for all**	 **58-yr old m:** 12.323; **w:**13.983 **58-yr old m:** 12.336; **w:** 13.995
			Comparison: "usual care"	**58-yr old m:** 12.217; **w:** 13.910
Ess 2002 **France, Spain & Switzerland**	**5**	**CV events/100 000 men**	**Intervention:** **1. CRP screening ± statins or aspirin**	 **France:** **35-44yr=**670**;** **45-54yr=**1 923**;**

Study	Time horizon (years)	Outcomes considered	Intervention	Results
			2. CRP screening ± aspirin	**55-64yr=**4 453; **Spain:** **35-44yr=**655; **45-54yr=**2 088; **55-64yr=**5 790; **Switzerland:** **35-44yr=**560; **45-54yr=**2 197; **55-64yr=**4 925 **France:** **35-44yr=**714; **45-54yr=**2 000; **55-64yr=**4 748; **Spain:** **35-44yr=**708; **45-54yr=**2 172; **55-64yr=**6 173; **Switzerland:** **35-44yr=**596; **45-54yr=**2 285; **55-64yr=**5 251
			Comparison: usual risk-assessment methods	**France:** **35-44yr=**766; **45-54yr=**2 334; **55-64yr=**5 666; **Spain:** **35-44yr=**760; **45-54yr=**2 534; **55-64yr=**7 366; **Switzerland:** **35-44yr=**640; **45-54yr=**2 660; **55-64yr=**6 266

Study	Time horizon (years)	Outcomes considered	Intervention	Results
Ess 2001 **Germany & Italy**	**5**	**CV events/300 000 men**	**Intervention:** **1. CRP screening ± statins or aspirin**	**Italy:** **35-44yr=**658; **45-54yr=**2 414; **55-64yr=**6 500; **Germany:** **35-44yr=**842; **45-54yr=**3 483; **55-64yr=**9 000
			2.CRP screening ± aspirin	**Italy:** **35-44yr=**699; **45-54yr=**2 629; **55-64yr=**6 986; **Germany:** **35-44yr=**882; **45-54yr=**3 690; **55-64yr=**9 440
			Comparison: usual risk-assessment methods	**Italy:** **35-44yr=**740; **45-54yr=**3 134; **55-64yr=**8 351; **Germany:** **35-44yr=**934; **45-54yr=**4 400; **55-64yr=**11 000
Short-term evaluations on diagnosis accuracy				
Lakic 2010 **Serbia**	**NA**	**AUC**	**Intervention:** **Framingham** **Framingham + SH** **Framingham + O_2^-** **Framingham + MDA** **Framingham + SOD** **Framingham + SOD + O_2^-**	 0.854 0.858 0.875 0.854 0.906 0.924

Study	Time horizon (years)	Outcomes considered	Intervention	Results
			Framingham + Lp(a) **Framingham + apoB** **Framingham + apoA-I** **Framingham + CRP** **Framingham + apo (a) isoform**	0.854 0.855 0.858 0.887 0.866
			Comparison: all alternatives compared	NA
Bogavac-Stanojević 2007 **Serbia**	**NA**	**NND**	**Intervention:** **Framingham +Lp(a)** **Framingham +apo(a)** **Framingham +apoB** **Framingham + apoA-I** **Framingham +CRP**	 **Low-risk:** 8.270**; intermediate:** 8.480**; high risk:** NA **Low-risk:** 6.260**; intermediate:** NA**; high risk:** NA **Low-risk:** 3.730**; intermediate:** NA**; high risk:** NA **Low-risk:** 3.090; **intermediate:** 3.480**; high risk:** 2.640 **Low-risk:** 2.730**; intermediate:** 3.380**; high risk:** 3.780
			Comparison: all alternatives compared	NA

** Results from baseline model (equal-scenario)*

apo= apolipoprotein; ATP=Adult Treatment Panel; AUC= Area Under the Curve; CRP= C-Reactive Protein; CV= Cardiovascular; Lp= lipoprotein; m=men; MDA= malondialdehyde;

NND= Number Needed to Diagnose; O2-= superoxide anion; QALY=Quality Adjusted Life Years; SH= sulfhydryl; SOD= superoxide dismutase; w=women

Table 21 – ICERs for novel serum biomarkers in CVD prevention

Study	Time horizon (years)	Intervention	ICER	ICER (as reported)
Long-term evaluations including treatment resulting from screening				
Choudhry 2011 **USA**	**Lifetime**	**CRP screening ± statins**	**Cost/QALY**	US$25 198
Lee 2010[1,2] **USA**	**Lifetime**	**ATP-III guidelines** **Age 70 CRP screening** **Age 65 CRP screening** **Age 70 start statins** **Age 60 CRP screening** **Age 65 start statins** **Age 55 CRP screening** **Age 50 CRP screening** **Age 60 start statins** **Age 45 CRP screening** **Age 55 start statins** **Age 40 CRP screening** **Age 50 stat statins** **Age 45 start statin** **Age 40 start statins**	**Cost/QALY**	**No risk: m:** Dominated **No risk: m:** US$33 328 **No risk: m:** US$34 066 **No risk: m:** Dominated **No risk: m:** US$35 474 **No risk: m:** Dominated **No risk: m:** Dominated **No risk: m:** Dominated **No risk: m:** US$36 588 **No risk: m:** Dominated **No risk: m:** US$40 862 **No risk: m:** Dominated **No risk: m:** US$51 438 **No risk: m:** US$86 172 **No risk: m:** US$151 821
Blake 2003[2] **USA**	**Lifetime**	**CRP screening ± statins** **Statins for all**	**Cost/QALY**	**58-yr old:** **m:** US$48 100; **w:** US$94 400 **58-yr old:** **m:** US$506 100; **w:** US$637 500
Ess 2002	**5**	**CRP screening ± statins or aspirin**	**Cost/LYS**	**France:** **35-44yr=**€39 430; **45-54yr=**€25 134; **55-64yr=**€18 413; **Spain:** **35-44yr=**€36 620; **45-54yr=**€22 915; **55-64yr=**€13 790;

Study	Time horizon (years)	Intervention	ICER	ICER (as reported)
France, Spain & Switzerland		**CRP screening ± aspirin**		**Switzerland:** **35-44yr=**€39 390; **45-54yr=**€18 590; **55-64yr=**€13 327 **France:** **35-44yr=**€9 260; **45-54yr=**€ 513; **55-64yr=**dominant; **Spain:** **35-44yr=**€11 370; **45-54yr=**€1 950; **55-64yr=**dominant; **Switzerland:** **35-44yr=**€9 170; **45-54yr=€** 270; **55-64yr=**dominant
Ess 2001 **Germany & Italy**	**5**	**CRP screening ± statins or aspirin** **CRP screening ± aspirin**	**Cost/LYS**	**Italy:** **35-44yr=**€36 270; **45-54yr=**€16 950; **55-64yr=**€9 905; **Germany:** **35-44yr=**€44 630; **45-54yr=**€10 217; **55-64yr=**€7 760 **Italy:** **35-44yr=**€11 203; **45-54yr=**dominant; **55-64yr=**dominant; **Germany:** **35-44yr=**€5 318; **45-54yr=**dominant; **55-64yr=**dominant

Study	Time horizon (years)	Intervention	ICER	ICER (as reported)
Short-term evaluations on diagnosis accuracy				
Lakić 2010[2] **Serbia**	**NA**	**Framingham** **Framingham + SH** **Framingham + O_2^-** **Framingham + MDA** **Framingham + SOD** **Framingham + SOD + O_2^-** **Framingham + Lp(a)** **Framingham + apoB** **Framingham + apoA-I** **Framingham + CRP** **Framingham + apo (a) isoform**	**Cost/AUC**	NA €9 167.00 €885.00 Dominated €389.00 €2 873.00 Dominated Dominated Dominated Dominated Dominated
Bogavac-Stanojević 2007[2] **Serbia**	**NA**	**Framingham +Lp(a)** **Framingham +apo(a)** **Framingham +apoB** **Framingham + apoA-I** **Framingham +CRP**	**Cost/NND**	**Low-risk:** €18.88**; intermediate:** €19.36**; high risk:** NA **Low-risk:** €118.81**; intermediate:** NA**; high risk:** NA **Low-risk:** -€57.10**; intermediate:** NA**; high risk:** NA **Low-risk:** € 2.53**; intermediate:** -€8.46**; high risk:** -€9.14 **Low-risk:** €24.47**; intermediate:** €122.85**; high risk:** €7.16

1.Results for Lee 2010 from baseline model (equal-scenario)

2. Incremental analysis performed

apo= apolipoprotein; AUC= Area Under the Curve; CAD=Coronary Artery Disease; ICER= incremental cost effectiveness ratio; CRP= C-Reactive Protein; m=men; Lp= lipoprotein; LYS=Life Years Saved;

MDA= malondialdehyde; NND= Number needed to diagnose; O2-= superoxide anion; QALY=Quality Adjusted Life Years; SH= sulfhydryl; SOD= superoxide dismutase; w=women

4.3.10 Sensitivity analysis

Uncertainty is intrinsic to any economic evaluation and should therefore always be accounted for. All studies included in our review performed sensitivity analyses in order to assess the robustness of their results. However, from the seven studies included, only two[82, 86] included probabilistic sensitivity tests. While all included one-way sensitivity analyses, all but two[83, 84] performed multi-way sensitivity analysis.

Results appeared to be mostly sensitive to the efficacy of statin therapy in reducing cardiovascular events as well as to their cost. Some sensitiveness to the rate and severity of adverse events (AEs) from statin therapy was also seen in the study by Lee et al.[86] which included extensive sensitivity analyses (i.e. one-way, multi-way and probabilistic).

4.3.11 Conflict of interest

Only two studies[81, 85] did not report a conflict of interest of some kind (mostly funding from the industry) which could affect the objectivity of the study results, although there is, up to date, no hard evidence on this. The two studies which reported no conflict of interest were the Serbian studies looking at diagnosis accuracy which were financially supported by the Serbian Ministry of Sciences and Environmental Protection.

4.4 Discussion

Despite the fact that overall the studies found appear to show that biomarkers, and more specifically CRP, could be cost-effective at willingness to pay thresholds lower than US$50 000 for the primary prevention of cardiovascular disease, only the long-term economic evaluation by Lee et al.[86], taking into consideration both the screening methods and the long-term treatment that could derive from them, compared the result of a strategy using CRP testing to a well validated, currently used, patient screening method: the Framingham score system. The authors made use of a cost-utility model (Markov model) for individuals starting at 40 years of age with normal lipid levels and no clinical signs of coronary artery disease, peripheral arterial disease or diabetes mellitus. Clear descriptions of the three competing alternatives were provided and assumptions were made explicit. Thus, the authors modelled full adherence to ATP-III and compared it to the addition of statin therapy for also those patients showing elevated levels of CRP (>2mg/l). A third option studied was what the authors called “risk-based statin treatment without CRP screening”. This last arm looked at the option of treating all patients presenting certain risk characteristics (considering age, hypertension and smoking status) with statins.

The model was well constructed and explained, and all assumptions were backed-up whenever possible with published literature. Although the results appear to favour the use of either CRP screening or risk-based statin treatment without CRP when compared to using the Framingham score system alone, CRP screening resulted in lower ICERs.

The results showed to be highly sensitive to the rate and severity of AEs experienced when following statin treatment as well as to the effects on risk reduction from statin therapy in patients with normal CRP levels. Thus, a small increase in the number or severity of AEs would make the use of CRP screening a more attractive option. Similarly, the efficacy of statins in patients with normal CRP levels has not been studied. If statins were not effective in those patients then the use of CRP would become more cost-effective since it would discriminate amongst those patients who would respond to therapy and those who will not (or who would respond to a lesser extent), avoiding wastage. Further analyses on these matters are required in order to obtain a clearer picture of the value of CRP as a valid marker to be used in patient discrimination preventive processes.

An additional limitation of this study is the need to rely on the results from the JUPITER trial[76] which has been criticized on a number of points [87, 88] for the lipid levels of all patient cohorts and for the relative risk for MI and stroke in the differential scenario (statins not effective in individuals with low CRP levels).

Despite the important uncertainties surrounding this evaluation and its results which make the internal validation of the study poor, the possibility of adapting the model once new data become available is its main strength, showing good external validity. Thus, as new evidence becomes available the precision of estimates may be improved.

The authors of this review believe that the potential value of modelling the Belgian situation would be, at present, very limited since it would suffer from the same drawbacks discussed in this report for the currently available evidence and knowledge.

5 GENERAL DISCUSSION AND RECOMMENDATIONS

The various statistical measures discussed in the second chapter are useful in gauging the information provided by a new risk marker. However, pure statistical measures do not assess the clinical importance of the information provided by a new risk marker, which might be assessed by its effect on clinical decisions and ultimately on clinical outcomes. The value of risk information in this framework depends on the absolute accuracy of the risk measurement, the effectiveness of the available treatments in improving clinical outcomes, and the costs of alternative treatment approaches[23](Table 22).

Table 22 – Evaluating the usefulness of a new biomarker for improving CVD risk prediction

Biomarker	Association with CVD	Change in *c*-statistics	Better calibration	Appropriate risk reclassification	Change clinical management	Small Number Needed to Screen	Cost-effective
Ideal biomarker	+	+	+	+	+	+	+

This review focused on the predictive increment of novel biomarkers added to SCORE for the general screening of CVD risk in asymptomatic individuals. Although the use of some biomarkers has been proposed to identify high risk individuals in specific sub-populations (e.g. measurement of Lipoprotein(a) in individuals with a family history of premature CVD or hypercholesterolaemia[22]), reviewing the evidence for such sub-population screening was beyond the scope of this report.

This review has shown that biomarkers, notably CRP and NT-proBNP, yield a moderate prediction increment when added to CVD predictive models in the general population. Whether this increment is clinically significant is unknown as evidence is lacking on the impact of using such biomarkers on risk management (risk communication, lifestyle intervention, or drug therapy) and patient outcomes. Moreover, although Lee et al.[86] concluded that CRP screening followed by statin treatment in patients with high CRP levels but low cholesterol levels who would not qualify for such treatment under the current Framingham system could be cost-effective when compared to the Framingham stratification method alone, their results were not robust. Given these elements, we strongly recommend not to measure the biomarkers described in this report for screening for CVD in asymptomatic individuals. This is consistent with the recent recommendations of the European guidelines on cardiovascular disease prevention[34]. It is also important to emphasize here that any genuine patient-based appraisal of CVD risk should account for individual characteristics which are not included in prediction algorithms, and are unlikely to ever be because their standardization is difficult, such as diet quality, physical activity, or psychosocial factors. Clinical skills remain central to adapt risk evaluation and management according to each individual situation.

We also found converging evidence that CNRI could be substantial. This opens avenues for a 2-step screening process, biomarkers being measured only in patients at intermediate CVD risk according to conventional risk models. This would allow to detect more high-risk patients than with conventional risk models, and to potentially impact their clinical management and health outcomes. However, up to date, there is insufficient evidence to recommend one specific biomarker or combination of biomarkers over the others, and not enough evidence on the benefit of such 2-step screening. It is also unknown how the prediction increment differs from the one that could be obtained by conventional risk factors not integrated in prediction algorithms (e.g. food intake, physical activity or precise tobacco consumption levels). Before such evidence becomes available, we can only make the weak recommendation (following GRADE terminology) that biomarkers such as CRP may be measured in individuals at intermediate 10-year CVD risk when their doctor feels it would be useful to refine their risk assessment[34, 89].

APPENDICES

APPENDIX 1. QUALITY APPRAISAL OF CLINICAL STUDIES INCLUDED

	Study identification	Kavousi 2012[19]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Unclear The study population is 6948 participants at the third examination of the original Rotterdam Study-I. What this number represents in term of the original cohort is not described. Moreover CRP was measured in only 3029 of participants. The authors state that the general characteristics of that subpopulation did not materially differ from those of the larger population, but numbers are not presented.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Yes Only 20 patients lost-to-follow up
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Unclear for CRP
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes "We obtained information on study outcomes from general practitioners and from letters and discharge reports from medical specialists. Events were classified by study physicians. Incident CHD was defined as a definite nonfatal or fatal myocardial infarction or death due to CHD. Definite and possible fatal CHD were coded by using the definitions applied within the Cardiovascular Health Study and Atherosclerosis Risk in Communities Study. Only first CHD events were included in the analyses
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NR
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes? - Because median follow-up in the cohort was 6.8 years and most CHD risk prediction

		instruments, including the FRS, predict 10-year CHD risk, we used a parametric Weibull proportional hazards regression model to estimate 10-year CHD risk from data available over a shorter follow-up period for each person. - Information on some markers and covariables was missing in up to 13% of participants. We performed multiple imputations of the missing values by using the Hmisc library of R

	Study identification	Kaptoge 2012[42]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Not reported This is a meta-analysis of 52 prospective cohorts
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Not reported
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Not reported
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Not reported
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Not reported
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Di Angelantonio 2012[17]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Not reported This is a meta-analysis of 37 prospective cohorts Data from the AMORIS study could not be incorporated because it did not measured HDL-C, blood pressure, smoking, BMI, diabetes. Reclassification index was computed in studies with at least 10 years follow-up. These studies are not separately described
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Not reported
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Not reported
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Ridker 2008[40]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Cohort of US men 50 years and <80 years with no diabetes and cancer at baseline
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Not reported
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Not reported
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	"For the NRI and CNRI analyses, observations censored before 7 years were excluded. All risk calculations were extrapolated to 10 years". How this extrapolation was done and what its validity tested is not explained.

	Study identification	Woodward 2010[41]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Not properly addressed. Of the 1836 study participants, 1319 had valid measures for all variables. How these 2 groups differ is not explained in the paper.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Only 2 participants lost-to-follow up
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes

1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Yes
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	**Wilson 2008[44]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Yes
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Not reported
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Yes
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	**Cooney 2009[5]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Yes

1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Probably HDL-cholesterol was measured by the precipitation method. The authors discussed that new direct methods may result in higher estimates. Calculating an individual's risk based on HDL measured with new methods and a risk estimation system that includes older HDL measurements will result in underestimation of the risk. It is unlikely that this affect the comparative predictive value of models with and without HDL measurement.
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	(only CVD deaths)
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NA
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	**Merry 2011[47]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	yes
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Poor 13% were lost to follow up, and no sensitivity analysis to assess the impact of this on results was performed
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	yes

1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Yes The influence of other easy-to-get info was tested (parental history of MI, physical inactivity, level of tobacco consumption…) on the model predictive power was tested
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	yes

	Study identification	**Yeboah 2012[pppp46]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Yes. The full cohort includes 6814 women and men aged 45 to 84 years without known CVD, recruited from 6 US communities. The race/ethnic breakdown of MESA participants was 38% white, 28% black, 22% Hispanic, and 12% Chinese adults. However, The final study population included 1330 participants without diabetes mellitus, with an FRS of more than 5% to less than 20%, and with complete data on all 6 of the novel risk markers. The number of individuals excluded because of incomplete data on biomarkers is unknown.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Loss to follow-up not reported
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes

pppp This study included only individuals at intermediate risk. To account for the fact that actual follow-up was less than 10 years, the authors redefined the risk in terms of 7.5-year risk when calculating the NRI, using a logistic regression model with probability weighting to reflect the sampling from the overall cohort. Based on the new model, intermediate 7.5-year risk categories for CHD and CVD were defined as 2.0% to 15.4% and 3.4% to 21.1%. With the addition of each novel risk marker to the base model, participants were considered to be reclassified to high risk if their estimated risks for CHD and CVD were greater than 15.4% and 21.1%, and reclassified to low risk if their estimated risks were lower than 2.0% and 3.4% for CHD and CVD.

1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NA
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	**Melander 2009[45]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	The Malmö Diet and Cancer (MDC) study is a population-based, prospective epidemiologic cohort of 28,449 persons enrolled between 1991 and 1996. From this cohort, 6,103 persons were randomly selected to participate in the MDC Cardiovascular Cohort, which was designed to investigate the epidemiology of carotid artery disease Biomarkers were measured in 4 936 subjects. Authors state that subjects with measurement of biomarkers did not differ from eligible subjects
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Traditional risk factors of the FRS+BMI+diastolic blood pressure
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Möhlenkamp 2011[13]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	The Heinz Nixdorf Recall (HNR) study is a populationbased cohort study designed to assess the predictive value of novel markers of risk when used in addition to traditional risk. Participants were randomly selected from mandatory city registries in Essen, Bochum, and Mülheim, and invited to participate in the study as previously reported. Physician- or self-referral was not allowed to avoid selection bias. A total of 4,814 subjects aged 45 to 75 years (50% females) were included between December 2000 and August 2003.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	Of the remaining participants, 34 (0.8%) were lost to follow-up, in n 94 (2.1%), we were unable to obtain 5-year primary end point information, and in 244 (5.4%), 1 or more measurements of cardiovascular risk factors, hsCRP, or CAC were unavailable. Subjects with hsCRP. 10 mg/l suggesting acute inflammation were excluded (n=149, 3.3%), leaving 3,966 subjects (53% women) for this analysis.
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NR
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Shah 2009[38]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Yes.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	NR
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NA
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Pencina 2008[28]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	NR
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	NR
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	NR
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NR
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	Schneider 2012[31]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	The Study of Health in Pomerania (SHIP) is a longitudinal, representative, population-based cohort study in West Pomerania, a region in northeast Germany. Baseline data was collected from 1997 through 2001. A total of 4,308 subjects participated.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes

1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	We included the following parameters as conventional risk factors: age (continuous); sex (binary); systolic blood pressure (continuous); antihypertensive medication (binary); HDL cholesterol (continuous); total cholesterol (continuous); diabetes (binary); and current smoking (binary).
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

	Study identification	**Rutten 2010[48]**
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Unclear. Of the 7983 participants recruited in 1990–1993, 3930 participants had blood sampled at the third examination cycle during 1997–1999. Furthermore, we included 2568 of 3011 participants, additionally recruited and examined in 2000–2001, fulfilling the same inclusion criteria as the original cohort.
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	No
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes. Note: included heart failure.
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	Conventional risk factors
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes Note: Missing data, <2% for all of the covariates, were handled using multiple imputation

	Study identification	Blankenberg 2010[7]
1.1	The study sample represents the population of interest with regard to key characteristics, sufficient to limit potential bias to the results	Yes. Models were refined in the FINRISK study, and validated in the PRIME study
1.2	Loss to follow-up is unrelated to key characteristics (that is, the study data adequately represent the sample), sufficient to limit potential bias	NR
1.3	The prognostic factor of interest is adequately measured in study participants, sufficient to limit potential bias	Yes
1.4	The outcome of interest is adequately measured in study participants, sufficient to limit bias	Yes
1.5	Important potential confounders are appropriately accounted for, limiting potential bias with respect to the prognostic factor of interest	NA
1.6	The statistical analysis is appropriate for the design of the study, limiting potential for the presentation of invalid results	Yes

APPENDIX 2. DATA EXTRACTION TABLES FOR ECONOMIC STUDIES

1	Reference (including all authors)	**Choudhry NK**, Patrick AR, Glynn RJ and Avorn J; The cost-effectiveness of C-reactive protein testing and Rosuvastatin treatment for patients with normal cholesterol levels Journal of the American College of Cardiologists **2011**; 57(7):784-91
2	Conflict of interest and/or study funding	One of the authors received salary from the industry (Astra Zeneca) for his involvement in the JUPITER trial
3	Country	US
4	Study question	Is testing of C-reactive protein in patients with normal cholesterol levels a cost-effective approach to primary prevention of cardiovascular disease?
5	Type of analysis (analytic technique)	Cost-utility analysis
6	Design	Decision tree and Markov model
7	Population	Men ≥ 50 and women ≥ 60 years of age with LDL cholesterol levels <130mg/dl and not known cardiovascular disease
8	Intervention	Testing hs-CRP levels. If these are ≥2,0mg/l, patients assumed to start treatment with 20mg/daily of rosuvastatin
9	Comparator	"usual care": patients only initiated on rosuvastatin if they had a myocardial infarction, unstable angina hospitalization, stroke or diabetes onset
10	Time horizon	Lifetime horizon
11	Discount rate	3% for both costs and effects
12	Perspective	Health care
13	Costs	
	• Cost items included	Hs-CRP testing Drug costs (rosuvastatin) Acute hospitalization Physician services Post-acute care

		Cholesterol testing not included
	• Measurement of resource use	Probabilities for events taken primarily from JUPITER study and multiplied by the resources required for treating such an event. The later derived from the literature.
	• Valuation of resource use	Cost of clinical events calculated by adding the costs of acute hospitalizations, physicians' services, drug use and post-acute care costs for every single possible health outcome/state.
	• Data sources	Medicare payment rate for hs-CRP costs Drug prices (rosuvastatin) from a major online pharmacy Cost of physician services from payment rates in the Medicare Physician Fee Schedule Cost of rehab from the published literature Informal care costs from the published literature
	• Currency and cost year	US$ of 2009
14	Outcomes	
	• Endpoints taken into account and/or health states	QALYs Utility scores applied to potential cardiovascular related events or death
	• Valuation of health states	Values were calculated by multiplying utilities values for specific events by age-specific utilities for healthy individuals.
	• Treatment effect and Extrapolation	Probabilities for key cardiovascular events taken from the JUPITER trial and assumptions made regarding their development over time (see assumptions heading for details)
	• Utility assessment (Quality of Life)	Utilities values taken from published literature QALYs over a lifetime for hs-CRP testing + treatment = 10,61 QALYs over a lifetime for no testing + no treatment = 10,29
	• Data sources for outcomes	Published and unpublished trial data obtained from JUPITER investigators, supplemented with data from the published literature.
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One-way sensitivity analysis with a particular focus on: statin treatment effectiveness, cardiovascular risk and drug costs Two-way sensitivity test simultaneously varying treatment efficacy and drug costs, treatment

		efficacy and cardiovascular risk and drug costs and cardiovascular risks. Probabilistic sensitivity analyses performed.
16	Assumptions	Constant treatment effects of rosuvastatin assumed for the first 5 years. Basecase scenario: Treatment effects persisted for 15 years at the level observed during the Jupiter trial (median follow-up in this trial 1,9 years). Then tapered off to no effect after 25 years. The cost of rosuvastatin assumed to go down to that of generic simvastatin after patent expiration (year 7 in the model).
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	ICER of hs-CRP testing versus no test of US$25 198 per QALY gained.
	• Sensitivity analysis	94% of all probabilistic simulations resulted in hs-CRP testing being cost-effective at a threshold of US$50 000/QALY
	• Other aspects	NA
18	Conclusions	A strategy of use of statins in patients with low cholesterol levels but elevated hs-CRP levels appear to be cost-effective at generally accepted thresholds (US$50 000)
19	Remarks	Sensitivity test undertaken and results relatively robust at a threshold of US$50 000 Model based study relying in multiple assumptions Main assumptions coming from one single, optimistic clinical trial (the JUPITER trial). Median length of follow-up for the trial on which the model was based was 1,9 years, since the study was stopped earlier than originally planned (originally designed to include a follow-up period of 4 years). Assumptions had to be made as to the maintenance or not over time of treatment results. Results rely on the maintenance of the positive treatment effect with statins for which there is uncertainty (no real evidence up to date). Only direct costs included and productivity costs not considered. Detailed explanation of costs and assumptions included in an annex.
1	Reference (including all authors)	**Lakić D**, Bogavac-Stanojević N, Jelic-Ivanovic Z et al; A multimarker approach for the prediction of coronary artery disease: cost-effectiveness analysis; Value in Health **2010**;13(6):770-777

	Conflict of interest and/or study funding	No conflict of interest reported
3	Country	Serbia
4	Study question	What markers are the most effective at predicting coronary artery disease?
5	Type of analysis (analytic technique)	Cost-effectiveness analysis
6	Design	Case control study
7	Population	188 CAD patients and 197 asymptomatic, CAD-free patients attending a regular annual medical check-up
8	Intervention	Alternative models consisting of the baseline model (FRS and LDL-c) with consecutive addition of other markers to assess their power in predicting CAD
9	Comparator	FRS and LDL-c as predictors of CAD
10	Time horizon	NA
11	Discount rate	NA
12	Perspective	Health care
13	Costs	
	• Cost items included	Total costs for laboratory markers based on 100 analyses: total cholesterol, triglycerides, High density lipoprotein cholesterol, Apolipoprotein A1; Apolipoprotein B; hs-CRP; Lipoprotein (a); Apolipoprotein (a) isoform; Malondialdehyde; superoxide anion; superoxide dismutase; sulfhydryl
	• Measurement of resource use	Total costs calculated by adding up the costs of all direct analytical and labor resources consumed.
	• Valuation of resource use	Service fees Microcosting used to calculate all other costs
	• Data sources	Costs for reimbursable markers derived from the health insurance price list
	• Currency and cost year	€ of 2008
14	Outcomes	
	• Endpoints taken into account and/or health states	Accuracy in predicting CAD

	• Valuation of health states	Logistic regression analysis studying the association between laboratory tests (different combinations) and the presence of CAD (dependent variable)
	• Treatment effect and Extrapolation	Measured by calculating the area under the curve (AUC) for the different models
	• Utility assessment (Quality of Life)	NA
	• Data sources for outcomes	Results from actual tests captured during the study
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One way sensitivity analysis: all costs varied within a ±30% interval, effectiveness within the 95% CI Two-way sensitivity analysis were performed varying simultaneously costs and effectiveness within the same intervals.
16	Assumptions	No specific assumptions made explicit
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	Only two strategies were not dominated (directly or indirectly) by others. These were FRS + SOD and FRS + SOD + O2. ICER of best predictive model (FRS+SOD+O2) versus baseline model (FRS)= €2 873
	• Scenario analysis	NA
	• Sensitivity analysis	Results showed to be robust during the sensitivity analyses performed
18	Conclusions	The introduction of oxidative stress/antioxidative defense markers in the laboratory will be convenient and cost-effective
19	Remarks	Only direct costs included Predictive regression model No other similar study performed so validation or comparison of results not possible Limited sample size for a regression model Consequences (necessary treatment and cost) linked to the additional CAD diagnoses, no considered. Costs of lifetime treatment for patients diagnosed and not diagnosed via the different models not

		included Similarly long-term (life-time) outcomes not modeled. (not the objective of the study)
1	Reference (including all authors)	**Lee K K**, Cipriano L E, Owens D K et al; Cost-effectiveness of using high-sensitive C-reactive protein to identify intermediate and low cardiovascular risk individuals for statin therapy Circulation **2010**; 122:1478-1487
2	Conflict of interest and/or study funding	Only 2 out of the 5 authors reported no conflict of interest
3	Country	USA
4	Study question	Is hs-CRP cost-effective at identifying intermediate and low cardiovascular risk patients suitable for statin therapy?
5	Type of analysis (analytic technique)	cost-utility analysis
6	Design	Markov model
7	Population	Individuals of 40+, with normal lipid levels and no clinical evidence of coronary artery disease, peripheral arterial disease or diabetes.
8	Intervention	hs-CRP screening added to basic cardiovascular risk assessment in patients over 40 without an indication for statin treatment according to ATP-III guidelines. Patients with hsCRP levels ≥2mg/L would undergo statin therapy. Those under that threshold would be tested (for hs-CRP) annually
9	Comparator	1. Treatment with statins only for patients with a 10-year Framingham risk of >20% or with diabetes 2. Framingham risk-based treatment without hs-CRS testing: statin treatment for all individuals at or above different predicted risk thresholds without hs-CRP (not necessarily the currently applied threshold)
10	Time horizon	Lifetime
11	Discount rate	3% for both costs and effects
12	Perspective	Health care
13	Costs	
	• Cost items included	General health costs for the healthy

		Laboratory testing Medications Cost of adverse reactions to statins Treatment and interventions for acute MI Post-MI treatment costs Treatment and interventions for stroke Post-stroke treatment End-of-life costs
	• Measurement of resource use	Age specific baseline costs applied to the risk data for the different individuals in order to calculate the cost per QALY for the three strategies analysed
	• Valuation of resource use	Reimbursement rates
	• Data sources	Medicare reimbursement rates Literature Drug information handbook for drug costs
	• Currency and cost year	2008 US $
14	Outcomes	
	• Endpoints taken into account and/or health states	MI, stroke and death
	• Valuation of health states	Values for model parameters (probabilities of events) from published literature
	• Treatment effect and Extrapolation	Modeled using estimates from the literature
	• Utility assessment (Quality of Life)	Age specific QoL values from the medical Expenditure Panel Survey, adjusted by weights for CV health states
	• Data sources for outcomes	Non-cardiovascular mortality from US life tables 2004 Morbidity and mortality from CV events from Medicare data and published community-based studies
15	Uncertainty	
	• Scenario analysis	NA

	• Sensitivity analysis	One-way, two-way and probabilistic sensitivity tests performed
16	Assumptions	Screening for hs-CRP added to basic cardiovascular risk assessment which often starts at the age of 40 Individuals who originally showed CRP levels < 2mg/L would go through annual tests ever after. Lipid levels in all patients assumed to be the median values found in the JUPITER trial The cost of statins assumed to be that of generic simvastatin.
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	For the equal effect scenario: Both hs-CRP and risk-based statin treatment without hs-CRP screening appeared to be cost-effective when compared to ATP III guidelines, although the latter appeared to offer more QALYs but at a higher cost. For the differential scenario: Hs-CRP screening would the optimal strategy at nearly all risk levels.
	• Scenario analysis	Two scenarios were explored: the base case scenario to check results if statins worked independently from hs-CRP or Framingham scores (equal effects scenario) and another scenario in which individuals with low hs-CRP would experience no reduction in risk due to statin therapy (differential scenario). A further scenario was explored in which individuals undergoing hs-CRP screening and presenting high levels would increase their compliance to statin therapy .
	• Sensitivity analysis	Results were highly sensitive to potential adverse event from statins. Results were also sensitive to the cost of statins with current ATP-III guidelines being the most cost-effective strategy at a US$50 000 threshold when prices of statins are $3.85/day or above. Hs-CRP would be the optimal strategy at the same threshold when the price of statins is between US$2 and US$3/day and risk-based treatment without hs-CRP testing would be the preferred option at prices below US$2/day. Probabilistic sensitivity tests found high uncertainty amongst individuals at lower risk of CV events.
18	Conclusions	Risk based statin treatment without hs-CRP testing appears to be cost-effective at WTP thresholds of US$50 000, for as long as statins prove to have good long-term safety and provide benefits among low-risk people with normal hs-CRP levels.

19	Remarks	Model-based analysis relying in multiple assumptions, although these were backed up whenever possible with literature. Results highly sensitive to the rate and severity of adverse events experienced when following statin treatment (a small QoL decrease of 1-2% among statin users would already make hs-CRP screening preferable at a threshold of US$ 50 000.) Results highly sensitive to assumptions with regard to effects on risk reduction from statin treatment in patients with normal hs-CRP levels. Some sensitivity to changes in the cost of statins. Non-robust results make generalization risky.

1	Reference (including all authors)	**Bogavac-Stanojević N**, Petrova GI, Jelic-Ivanovic Z et al; Cost-effectiveness analysis in diagnosis of coronary artery disease: choice of laboratory markers; Clinical Biochemistry **2007** 40:1180-1187
2	Conflict of interest and/or study funding	No conflict of interest reported – funded by the MoH in Serbia
3	Country	Serbia
4	Study question	Are laboratory markers added to the current Framingham scoring system cost-effective in coronary artery disease diagnosis?
5	Type of analysis (analytic technique)	Cost-effectiveness analysis
6	Design	Case control study
7	Population	510 individuals (289 healthy individuals with normal electrocardiograms and exercise test results, and 221 individuals with coronary heart disease – proven by coronary angiography)
8	Intervention	Addition of the following markers (as second line) to the Framingham scores: ApoA-I < 1,2g/L ApoB>1,2g/L Apo (a)<22K IV Lp(a)>300mg/L Hs-CRP>3mg/L
9	Comparator	Framingham scores for 10-year CHD risk:

		High:>20% Intermediate: 10-20% Low <10%
10	Time horizon	NA
11	Discount rate	NA
12	Perspective	Health care
13	Costs	
	• Cost items included	Prices of diagnostic consumables and tests Labor costs for diagnostic procedures
	• Measurement of resource use	Through actual tests performed during the study – all expenses were added and multiplied by the amounts consumed
	• Valuation of resource use	Market prices for consumables and tests Average monthly salaries for laboratory staff
	• Data sources	Publicly available sources for prices (laboratory prices) and salaries
	• Currency and cost year	€ (year not specified)
14	Outcomes	
	• Endpoints taken into account and/or health states	Number needed to diagnose (NND)
	• Valuation of health states	
	• Treatment effect and Extrapolation	No extrapolation performed
	• Utility assessment (Quality of Life)	NA
	• Data sources for outcomes	Lab tests
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One-way sensitivity test performed varying ±30% each cost input, and effectiveness within the

		95% CI values. Two-way sensitivity testing performed by varying simultaneously the total costs and effectiveness within the same intervals.
16	Assumptions	No assumptions made explicit
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	Two preferred options: addition of either Apo-I or Hs-CRP as additional classification tool. Framingham (low risk) + Apo-I: lowest cost per additional successfully diagnosed patient (2,63 versus 24,47€ for the Apo-I and the Hs-CRP groups respectively). Framingham (intermediate risk) + Apo-I: lowest cost per additional successfully diagnosed patient (2,96 versus 122,85€ for the Apo-I and the Hs-CRP groups respectively Framingham (high risk) + Apo-I: -9,14 € versus the hs-CRP option
	• Scenario analysis	NA
	• Sensitivity analysis	Sensitivity tests were performed for dominating scenarios. The cost per NND showed no sensitivity to changes in costs or NDD for any of the three risk categories – Robust results.
18	Conclusions	Cost-effectiveness analysis of different diagnostic markers results in improved identification of at-risk patients at a lower health care costs.
19	Remarks	Study limited to look at the cost of testing per additional successfully diagnosed patient. No consideration of long-term costs or consequences derived from treatment with statins for those patients requiring it according to the new classification.

1	Reference (including all authors)	**Blake G J**, Ridker P M, Kuntz K M Potential cost-effectiveness of C-reactive protein screening followed by targeted statin therapy for primary prevention of cardiovascular disease among patients without overt hyperlipidemia The American Journal of medicine **2003**; 114:485-494
2	Conflict of interest and/or study funding	One of the authors co-inventor of a pending patent application on the use of markers of inflammation in coronary artery disease
3	Country	US

4	Study question	Is testing of C-reactive protein in patients with normal cholesterol levels a cost-effective approach to primary prevention of cardiovascular disease?
5	Type of analysis (analytic technique)	Cost-utility/cost-effectiveness study
6	Design	Decision tree and Markov model
7	Population	Men ≥ 50 and women ≥ 60 years of age with no overt hyperlipidemia (LDL<149mg/dL)
8	Intervention	C-reactive protein screening followed by targeted statin therapy for patient with C-reactive protein levels ≥0,16mg/dL
9	Comparator	1. No C-reactive protein screening and no statin therapy (“usual care”) 2. Statin therapy for all patients
10	Time horizon	Lifetime horizon
11	Discount rate	3% for both costs and effects
12	Perspective	Health care
13	Costs	
	• Cost items included	Costs of myocardial infarction Acute and chronic (post-event) costs for stroke Cost of statin therapy Cost of C-reactive protein test GP office visits
	• Measurement of resource use	Modeled based on published literature
	• Valuation of resource use	Costs (as opposed to charges) used
	• Data sources	Lifetime costs for myocardial infarction and stroke based on published data and adjusted for age. Estimates for the cost of statin therapy from the Red Book 2000 Cost for C-reactive protein test from personal communication. Cost of office visits and liver function tests (required for those on statin therapy) from the National Physician Fee Schedule Relative Value File and the Clinical Diagnostic Laboratory Fee Schedule (publicly available online).

	• Currency and cost year	US$ of 2000
14	Outcomes	
	• Endpoints taken into account and/or health states	QALYs
	• Valuation of health states	Utility values for specific events taken from the published literature. Utility values for those not experiencing any cardiovascular event taken as age-specific utilities from the literature
	• Treatment effect and Extrapolation	Done via a Markov model over the lifetime of a patient
	• Utility assessment (Quality of Life)	Patients without CV events: assigned QoL weights based on age and sex-adjusted data. Utilities post stroke and utilities post MI: adapted from the literature For post MI and stroke: since no data available, utilities assumed to be the product of the utilities of the individual states (post MI and post stroke).
	• Data sources for outcomes	Utility weights: Published cost-effectiveness studies for stroke and myocardial patients Probabilities of myocardial infarction (MI): from the AFCAPS/TexCAPS prevention trial published in 1998. Probabilities were further adjusted for different age groups Risk of stroke: (by age and sex) obtained from published population-based studies. Increased risk of stroke after MI or increased risk of MI after stroke from published trial data. Mortality rates from in-hospital reported rates (1993 and 1995)
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One-way and three –way sensitivity analyses performed
16	Assumptions	• All patients received dietary counseling • 54% of coronary heart disease events are myocardial infarctions • For all patients, elevated C-reactive protein levels • would increase the risk of myocardial infarction by 2.2 • compared with patients with low levels. • Statin therapy reduced the risk of myocardial infarction • by 45% among patients with LDL cholesterol levels<149 • mg/dL and elevated C-reactive protein levels. • The risk of stroke is independent of LDL levels.

		• 10% benefit from statin therapy for stroke prevention • applied to all patients. • All patients receive statin treatment after a MI. • Statin therapy reduced the risk of stroke after • myocardial infarction by 22%. • 25% of non-fatal strokes were moderate to severe. • 33% of non fatal strokes left no residual deficit. • The utility for the post–MI and post-stroke state was the product of the utilities for these two states. • Patient time costs considered negligible – not included in the analysis. • Adherence rate unchanged after 5 years.
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	ICER of hs-CRP testing versus no test of US$48 100/QALY gained over a life time of a 58-year old men Treating all patients with statins resulted on an ICER of US$ 506 100/QALY over the lifetime of a 58-year old men. Screening was most effective in 65-year-old men (US$ 42 600/QALY) and lest cost-effective in 35-year-old women (US$ 207 300/QALY)
	• Sensitivity analysis	Results most sensitive to the relative risk of MI, the cost of statin therapy and the efficacy of statin in preventing MI in individuals with high CRP levels. Results moderately sensitive to utilities associated to MI, discount rates, cost of CV events or efficacy of statins for stroke prevention. Results insensitive to all other parameters.
18	Conclusions	A strategy of use of statins in middle-aged patients with no overt hyperlipidemia but elevated hs-CRP levels could be relatively cost-effective and in some cases cost-saving.
19	Remarks	Structure of the model not explained in enough detail. Model based study relying in multiple assumptions, not always well -backed up by literature. Model assumptions and sources used for costs and outcomes well explained. Costs and outcomes discounted. Only direct costs included and productivity costs or costs linked to patient's time not considered. Results sensitive to changes in the inputs with regards to cost of statin therapy and efficacy of statins in preventing MI.

1	Reference (including all authors)	**Ess SM**, Szucs TD Economical aspects of high sensitivity C-reactive protein as predictor of coronary heart disease. An analysis in France, Spain and Switzerland Heart Drug **2002**; 2:61-68
2	Conflict of interest and/or study funding	Financial support from Dade Behring, Marburg, Germany (biotech company)
3	Country	Switzerland (but calculations done for France, Spain and Switzerland)
4	Study question	Is CRP determination a cost-effective tool when compared to standard lipid screening in primary prevention of coronary heart disease?
5	Type of analysis (analytic technique)	Decision-analytic model (not explained in detail).
6	Design	Cost-effectiveness analysis.
7	Population	Hypothetical cohort of 300 000, apparently healthy men, divided in three age groups (35-44, 45-54 and 55-64 years of age).
8	Intervention	Two different strategies in which CRP levels are taken into consideration for patient stratification and the decision on whether or not statins or aspirin should be prescribed. Strategy 1: CRP test performed. Men with CRP levels >3mg/l and borderline lipid levels assumed to be on statins. Men with CRP >3mg/l but desirable lipid levels assumed to receive aspirin Strategy 2: Only individuals who could qualify for lipid-lowering therapy under current risk assessment methods assumed to be treated with statins. Individuals with CRP levels >3mg/l, who would not qualify for lipid-lowering treatment under current risk assessment methods, put on aspirin.
9	Comparator	Current risk assessment method (based on cholesterol levels and presence or not of other risk factors) for putting patients on cholesterol lowering drug therapy (statins). No CRP testing performed.
10	Time horizon	5 years
11	Discount rate	No discounting performed
12	Perspective	Health care
13	Costs	

	• Cost items included	CRP testing Statin treatment Aspirin treatment MI costs Other CV event costs
	• Measurement of resource use	Costs for each cohort over the 5-year period added up.
	• Valuation of resource use	Market prices in the two countries analysed (Germany and Italy).
	• Data sources	CRP testing - from manufacturer Stating treatment – not clear Aspirin treatment – not clear MI costs – from published data Other CV event costs – from published data.
	• Currency and cost year	€s (year not mentioned).
14	Outcomes	
	• Endpoints taken into account and/or health states	Cardiovascular deaths, MIs, other cardiovascular events.
	• Valuation of health states	Life years saved (LYS)
	• Treatment effect and Extrapolation	Number of deaths averted (over the study period) multiplied by the life expectancy for the mean age of each cohort.
	• Utility assessment (Quality of Life)	NA
	• Data sources for outcomes	Literature. Event rates mainly from epidemiological studies and clinical trials.
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One way sensitivity tests performed on the price of the test and the cost of cardiovascular events.
16	Assumptions	Prices of statins and aspirin. When a CV event occurred the following probabilities were assumed: 0,15 for CHD death, 0,50

		for non-fatal MI and 0,35 for all other non-fatal CV events. CRP testing assumed to cost four times the selling price for the actual test.
17	Results	
	• Cost-effectiveness and/or cost-utility (base case)	Strategy 1 versus comparator: 35-44 years of age: 39430€/LYS in FR, 36620/LYS in SP and 39390€/LYS in SW 45-54 years of age: 25134€/LYS in FR, 22915€/LYS in SP and 18590€/LYS in SW 55-64 years of age: 18413€/LYS in FR, 13790€/LYS in SP and 13327€/LYS in SW Strategy 2 versus comparator: 35-44 years of age: 9260€/LYS in FR, 11370€/LYS in SP and 9170€/LYS in SW 45-54 years of age: 513€/LYS in FR, 1950€/LYS in SP and 270€/LYS in SW 55-64 years of age: dominant in all three countries.
	• Sensitivity analysis	Sensitivity analysis showed results to be robust.
	• Other aspects	NA
18	Conclusions	Hs-CRP may be a cost-effective marker to predict increased risk of cardiovascular disease, especially in individuals aged 45+.
19	Remarks	Cost estimations for CV events or deaths not clearly explained. Costs for statins/aspirin not clearly described. Assumptions not always well backed-up by literature (eg prevalence of high levels of hs-CRP in apparently healthy Europeans, or effectiveness of aspirin versus statins). Only one way sensitivity test performed. Industry sponsored study. Limited scope: study only in males at working ages. Limited study period (5 years) for a cost-effectiveness analysis in this specific area where a life time approach would be preferred. No discounting.

1	Reference (including all authors)	**Ess SM**, Szucs TD Medical-economical aspects of high sensitivity C-reactive protein assay for the prediction of coronary heart disease. An analysis in Germany and Italy

		Ital Heart J **2001**; 2(3):181-188
2	Conflict of interest and/or study funding	Financial support from Dade Behring, Marburg, Germany (biotech company)
3	Country	Switzerland (but calculations performed for Germany and Italy)
4	Study question	Is CRP determination a cost-effective tool in primary and secondary prevention of coronary heart disease?
5	Type of analysis (analytic technique)	Decision-analytic model (not well explained).
6	Design	Cost-effectiveness analysis.
7	Population	In primary prevention: hypothetical cohort of 300 000, apparently healthy men, divided in three age groups (35-44, 45-54 and 55-64 years of age).
8	Intervention	Primary prevention: Strategy 1 and 2: CRP test performed every two years. Strategy 1: men with CRP levels >3mg/l , borderline lipid levels and less than 2 other risks factors were considered to be put on statins. Those CRP positive men with LDL titers <4,11 mmol/l or total cholesterol levels <5,11 mmol/l were considered to be on aspirin. Strategy 2: all CRP positive individuals considered to be put on aspirin.
9	Comparator	Current risk assessment method (based on cholesterol levels and presence or not of other risk factors) for putting patients on statins. No CRP testing performed.
10	Time horizon	5 years.
11	Discount rate	No discounting performed.
12	Perspective	Health care.
13	Costs	
	• Cost items included	CRP testing Stating treatment Aspirin treatment MI costs Other CV event costs Cardiovascular death costs.

	• Measurement of resource use	Costs for each cohort over the 5 year period added up.
	• Valuation of resource use	Market prices in the two countries analysed (Germany and Italy).
	• Data sources	CRP testing - from manufacturers Stating treatment – public prices Aspirin treatment – public prices MI costs - unclear Other CV event costs - unclear Cardiovascular death costs – unclear.
	• Currency and cost year	€s (year not mentioned).
14	Outcomes	
	• Endpoints taken into account and/or health states	Cardiovascular deaths, MIs, other cardiovascular events.
	• Valuation of health states	In life years saved (LYS).
	• Treatment effect and Extrapolation	Number of deaths averted (over the study period) multiplied by the life expectancy at the middle age of each cohort.
	• Utility assessment (Quality of Life)	NA
	• Data sources for outcomes	Literature and expert opinion (when literature not available). Event rates mainly from epidemiological studies and clinical trials.
15	Uncertainty	
	• Scenario analysis	NA
	• Sensitivity analysis	One way sensitivity tests performed on the price of the test and the cost of cardiovascular events.
16	Assumptions	Prices of statins and aspirin. When a CV event occurred the following probabilities were assumed: 0,15 for CHD death, 0,50 for non-fatal MI and 0,35 for all other non-fatal CV events. CRP testing assumed to cost four times the selling price for the actual test.
17	Results	

	• Cost-effectiveness and/or cost-utility (base case)	Strategy 1 versus current risk assessment methods: 35-44 years of age: 44630€/LYS in DE and 36270€/LYS in IT 45-54 years of age: 10217€/LYS in DE and 16950€ in IT 55-64 years of age: 7760€/LYS in DE and 9905€/LYS in IT Strategy 2 versus current strategy current risk assessment methods: 35-44 years of age: 5318€/LYS in DE and 11203€/LYS in IT 45-54 years of age: dominant both in DE and IT 55-64 years of age: dominant in both DE and IT.
	• Sensitivity analysis	Sensitivity analysis showed results to be robust.
	• Other aspects	NA
18	Conclusions	Hs-CRP testing in primary prevention can improve patient outcomes and result in a more cost-effective treatment strategy.
19	Remarks	Cost estimations for CV events or deaths not clearly described/explained Assumptions not always well backed-up by literature Only one way sensitivity test performed Industry sponsored study Limited scope: analysis only in male on their working age (35-64) Limited study period (5 years) for a cost-effectiveness analysis in this area where life time costs would have been more appropriate. No discount applied.

APPENDIX 3. SEARCH STRATEGY - ECONOMIC STUDIES

Project number	2011-21
Project name	Predicting primary cardiovascular disease: added-value information from new serum biomarkers
Search question(s)	Are novel serum biomarkers cost effective at predicting primary cardiovascular disease when compared to currently used patient stratification systems?
Structured search question(s) (PICO, SPICE, ECLIPSE, ..)	
P (patient)	General adult population
I (Intervention)	Novel serum biomarkers
C (comparison)	Other patient risk scoring systems
O (outcome)	QALYs, LYGs

Date	January 28, 2013
Database	Medline OVID
Search Strategy	1 biomarkers.mp. or exp Biological Markers/ (575418) 2 (apolipoprotein adj (A1 or B100)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (1308) 3 (Phospholipase adj A2).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (12818) 4 Paraoxonase-1.mp. or (paraoxonase.mp. adj (biomarkers.mp. or exp Biological Markers/)) [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (865) 5 ((C-reactive adj protein) or hsCRP or CRP).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (43975) 6 (fibrinogen or interleukin-1B or (interleukin adj 1B)).mp. [mp=title, abstract, original title, name of substance word,

	subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (48447)	
	7 (interleukin-6 or (interleukin adj (fibrinogen or interleukin-1B or (interleukin adj 1B)))).mp. (49213)	
	8 interleukin-1.mp. or (interleukin.mp. adj (biomarkers.mp. or exp Biological Markers/)) [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (45195)	
	9 interleukin-1.mp. or (interleukin.mp. adj (biomarkers.mp. or exp Biological Markers/)) [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (45195)	
	10 Neopterin.mp. or exp Neopterin/	(2717)
	11 exp Peroxisome Proliferator-Activated Receptors/	(9917)
	12 "serum amyloid A".mp.	(3569)
	13 exp Tumor Necrosis Factor-alpha/	(87003)
	14 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13	(788301)
	15 exp Cardiovascular Diseases/ or cardiovascular disease*.mp.	(1772017)
	16 14 and 15	(88746)
	17 exp "Costs and Cost Analysis"/	(168037)
	18 Economics, Pharmaceutical/ or Economics, Medical/ or Economics, Hospital/ or Economics, Nursing/ (24077)	
	19 exp Quality-Adjusted Life Years/	(5890)
	20 exp "Value of Life"/	(5249)
	21 (budget* or expenditure or expenses).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (49672)	
	22 17 or 18 or 19 or 20 or 21	(229225)
	23 16 and 22	(331)
	24 "systematic coronary risk evaluation".mp.	(98)
	25 PROCAM*.mp.	(956)
	26 Framingham*.mp.	(4933)
	27 FRS*.mp.	(1692)
	28 Reynolds*.mp.	(2195)

	29 assign*.mp.	(169262)
	30 QRISK1*.mp.	(2)
	31 QRISK2*.mp.	(15)
	32 "adult treatment panel III".mp.	(1552)
	33 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32	(180027)
	34 (screen* or stratificat* or risk or predict* or classifi* or reclassifi*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]	(2472289)
	35 23 and 34	(223)
	36 23 and 33	(18)
	37 34 and 36	(13)

Date	February 7, 2013	
Database	Embase	
Search Strategy	#38. 14 and 15 and 31 and 37	(40)
	#37. 32 or 33 or 34 or 35 or 36	(3,567,138)
	#36. predict* AND [embase]/lim	(1,103,106)
	#35. 'risk'/exp OR risk AND [embase]/lim	(1,755,283)
	#34. classifi* OR reclassifi* AND [embase]/lim	(883,898)
	#33. stratificat* AND [embase]/lim	(33,638)
	#32. screen* AND [embase]/lim	(579,968)
	#31. 24 or 25 or 26 or 27 or 28 or 29 or 30	(29,781)
	#30. assign* AND risk AND [embase]/lim	(25,899)
	#29. 'adult treatment panel III' AND [embase]/lim	(1,939)
	#28. 'qrisk score'/exp OR 'qrisk score' AND [embase]/lim	(24)
	#27. 'reynolds risk score'/exp OR 'reynolds risk score' AND [embase]/lim	(80)
	#26. 'framingham risk score'/exp OR 'framingham risk	(1,868)

score' AND [embase]/lim	
#25. 'procam score'/exp OR 'procam score' AND [embase]/lim	(57)
#24. 'systematic coronary risk evaluation' AND [embase]/lim	(166)
#23. 16 or 17 or 18 or 19 or 20 or 21 or 22	(296,101)
#22. 'quality adjusted life year'/exp OR 'quality adjusted life year' AND [embase]/lim	(9,413)
#21. 'health care cost'/exp OR 'health care cost' AND [embase]/lim	(151,881)
#20.'pharmacoeconomics'/exp OR 'pharmacoeconomics' AND [embase]/lim	(108,922)
#19. 'economic evaluation'/exp OR 'economic evaluation' AND [embase]/lim	(149,762)
#18. 'cost effectiveness analysis'/exp OR 'cost effectiveness analysis' AND [embase]/lim	(86,591)
#17. 'cost utility analysis'/exp OR 'cost utility analysis' AND [embase]/lim	(4,909)
#16. 'cost benefit analysis'/exp OR 'cost benefit analysis' AND [embase]/lim	(41,355)
#15. 'cardiovascular disease'/exp OR 'cardiovascular disease' AND [embase]/lim	(2,122,248)
#14. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13	(446,029)
#13. 'tumor necrosis factor alpha'/exp OR 'tumor necrosis factor alpha' AND [embase]/lim	(131,710)
#12. 'serum amyloid a'/exp OR 'serum amyloid a' AND [embase]/lim	(3,462)
#11. 'peroxisome proliferator activated receptor'/exp OR 'peroxisome proliferator activated receptor' AND [embase]/lim	(28,176)
#10. 'neopterin'/exp OR 'neopterin' AND [embase]/lim	(3,476)
#9. 'interleukin 6'/exp OR 'interleukin 6' AND [embase]/lim	(102,653)
#8. 'interleukin 1beta'/exp OR 'interleukin 1beta' AND [embase]/lim	(52,169)
#7. 'fibrinogen'/exp OR 'fibrinogen' AND [embase]/lim	(67,438)
#6. 'c reactive protein'/exp OR 'c reactive protein' AND [embase]/lim	(67 ,907)
#5.'aryldialkylphosphatase 1'/exp OR 'aryldialkylphosphatase 1' AND [embase]/lim	

	(1,602)	
	#4. 'phospholipase a2'/exp OR 'phospholipase a2' AND [embase]/lim	(15,144)
	#3. 'apolipoprotein b100'/exp OR 'apolipoprotein b100' AND [embase]/lim	(2,370)
	#2. 'apolipoprotein a1'/exp OR 'apolipoprotein a1' AND [embase]/lim	(11,761)
	#1. 'biological marker'/exp OR 'biological marker' AND [embase]/lim	(74,118)

Date	January 21, 2013	
Database	Econlit	
Search Strategy	1 (biomarker* or biological markers).mp. [mp=heading words, abstract, title, country as subject] (56)	
	2 ((cardiovascular adj disease) or CVD or cardi*).mp. [mp=heading words, abstract, title, country as subject] (1035)	
	3 1 and 2	(1)

Date	January 21, 2013	
Database	Centre for reviews and dissemination (CRD) NHS HTA	
Search Strategy	1. biological AND marker*	(300)
	2. biomarker*	(34)
	3. #1 OR #2	(314)
	4. cardiovascular AND disease	(141)
	5. CVD	(17)
	6. #4 OR #5	(142)
	7. #3 AND #6	(15)

Date	January 21, 2013	
Database	Centre for reviews and dissemination (CRD) NHS EDD	
Search Strategy	1. biological AND marker*	(104)
	2. biomarker*	(31)
	3. #1 OR #2	(124)
	4. cardiovascular AND disease	(543)
	5. CVD	(49)
	6. #4 OR #5	(545)
	7. #3 AND #6	(11)

Date	January 21, 2013	
Database	Cochrane Database of Systematic reviews (CDSR) Economic Evaluation	
Search Strategy	1. biomarker*	(2973)
	2. cardiovascular ADJ disease	(516)
	3. CVD	(1044)
	4. #2 OR #3	(1476)
	5. #1 AND #4	(73)
	6. Limit: econ eval database	(0)

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