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Preamble

National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Emerging Biomarkers for Primary Prevention of Cardiovascular Disease

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BACKGROUND: Heart disease and stroke continue to be the leading causes of death in the US. As a result, investigators continue to look for new and emerging biomarkers of disease risk. Because many of these emerging biomarkers are not as well documented as those of conventional lipid and lipoprotein risk factors, their value in clinical practice needs to be critically appraised and appropriate guidelines developed for their proposed use.

CONTENT: The National Academy of Clinical Biochemistry (NACB) convened a multidisciplinary expert panel to develop laboratory medicine practice guidelines for a selected subset of these emerging risk factors as applied in a primary prevention setting of heart disease and stroke. The NACB expert panel selected lipoprotein subclasses and particle concentration, lipoprotein(a), apolipoproteins A-I and B, high sensitivity C-reactive protein (hsCRP), fibrinogen, white blood cell count, homocysteine, B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP), and markers of renal function as biomarkers that fell within the scope of these guidelines.

CONCLUSIONS: Based on a thorough review of the published literature, only hsCRP met all of the stated criteria required for acceptance as a biomarker for risk assessment in primary prevention.

The purpose of this document is to present an Executive Summary of the National Academy of Clinical Biochemistry (NACB)[9] Laboratory Medicine Practice Guidelines (LMPG) for utilization of emerging laboratory biomarkers of cardiovascular and stroke risk in a primary prevention setting. The NACB is the scientific academy of the AACC. An important activity of the NACB is to develop laboratory medicine practice guidelines to assist clinical and laboratory practice decisions concerning patients at increased risk for specific diseases.

BACKGROUND

In recent years, the number of new candidate risk factors proposed as significant predictors of cardiovascular disease (CVD) and its complications has grown considerably (Table 1). These biomarkers are termed "emerging risk factors" because they are associated with an increased risk for CVD, but their causative, independent, and quantitative contributions to CVD are not as well documented as dyslipidemia, high blood pressure, and smoking—the major, longest established risk factors *(1)*. An emerging marker may not be necessarily a newly discovered marker, but may be an existing one for which evidence is only now available establishing it as effective for independently identifying risk or for monitoring treatment.

BIOMARKER ASSESSMENT

Several general principles are useful to bear in mind when evaluating the utility of biomarker measure-

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[9] Nonstandard abbreviations: NACB, National Academy of Clinical Biochemistry; LMPG, Laboratory Medicine Practice Guidelines; CVD, cardiovascular disease; hsCRP, high-sensitivity C-reactive protein; WBC, white cell blood count; Lp, lipoprotein; apo, apolipoprotein; Hcy, homocysteine; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal proBNP; CKD, chronic kidney disease; GFR, glomerular filtration rate; NKDEP, National Kidney Disease Education Program; eGFR, estimated GFR.

Table 1. Emerging risk factors for cardiovascular disease.

C-reactive protein	Interleukins (e.g., IL-6)
Serum amyloid A	Vascular and cellular adhesion molecules
Soluble CD40 ligand	Leukocyte count
Fibrinogen	Plasminogen activator inhibitor 1
D-Dimer	Tissue plasminogen activator
Factors V, VII, VIII	Small dense LDL
Lipoprotein(a)	Apolipoproteins A1 and B
LDL and HDL subtypes	Oxidized LDL
Homocysteine	Lipoprotein-associated phospholipase A_2
Microalbuminuria	Creatinine (glomerular filtration rate)
Cystatin C	Infectious agents
ApoE genotype	Fibrinopeptide A
Remnant lipoproteins	von Willebrand factor antigen

ments in advancing science, guiding risk screening strategies, and affecting clinical care.

The first step in the evaluation of a biomarker is an assessment of whether its concentration is different in persons affected by disease in comparison to those who are not. Initial studies have typically used a case-control design, and reporting of the results has focused on the risks related specifically to the biomarker, with secondary consideration of statistical adjustments. Published reports at this phase may show relatively strong estimates of relative risk for persons with concentrations of the new biomarker that are significantly different in cases compared with controls.

A second step in the evaluation of a new biomarker is the development of a body of evidence from case-control and prospective studies that have evaluated the new test. The prospective studies can include nested case-control studies and full cohort investigations. Evaluation is aided at this phase if the biomarker can be measured by using previously collected and stored samples, provided that measurements on aliquots yield accurate and precise data. The storage of samples at temperatures ≤ -70 °C has greatly facilitated such investigations. A nested case-control study with adequate statistical power to address the question is an appropriate cost-effective strategy. An ancillary issue at this phase is to rigorously test whether the new biomarker effects are present in multivariate statistical analyses after appropriate corrections are performed for variables including age, sex, ethnicity, underlying diseases, and the type and severity of CVD.

A third step in the evaluation of a new biomarker is whether the measurement improves our ability to assess risk above and beyond the current approaches. In other words, can the new test improve the ability to discriminate between future cases and noncases? Of course, accurate assessment of the new marker value can only be determined after correction for appropriate confounders, and the marker can only be deemed useful if it offers additional value over that provided by existing risk algorithms (such as the Framingham Risk Score *(2)* in the context of CVD). Unfortunately, many investigations lack such information, which can make it difficult to adequately evaluate the value of a new biomarker.

Finally, reliable analytical methods must be available for the measurement of the intended biomarker.

GUIDELINE DEVELOPMENT

The NACB convened a multidisciplinary panel of experts to develop recommendations regarding the laboratory measurement and clinical utility of a selected number of emerging risk markers for use in primary prevention of heart disease and stroke. The selection of risk markers for evaluation and inclusion in these guidelines was based on consensus of the expert panel after systematically reviewing available evidence and evaluating criteria of clinical usefulness, consistency of epidemiologic data, improved predictive value, independence from other factors, and available analytical methods *(3)*. Specific recommendations in these NACB guidelines are based, whenever possible, on relevant published information from prospective observational studies of initially healthy populations published through February 2005. We did not consider retrospective studies or studies of populations with existing vascular diseases, except in the case of evaluating the use of biomarkers in secondary prevention (because fewer data are available in primary prevention settings). The strength of scientific data supporting each recommendation was characterized using the scoring criteria adopted from the American Heart Association/American College of Cardiology, as summarized in Table 2. For each recommendation, the designations I, IIa, IIb, and III describe the indications, and the uppercase letters A, B, and C describe the weight of evidence.

The NACB expert panel defined the following risk markers as within the scope of these guidelines: high-sensitivity C-reactive protein (hsCRP), fibrinogen, white blood cell count (WBC), lipoprotein (Lp) subclasses and particle concentration, lipoprotein(a), apolipoprotein A-I (apoA-I) and apoB, homocysteine (Hcy), B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP), and markers of renal function.

Table 2. American Heart Association/American College of Cardiology classification summary of indications.

I	Conditions for which there is evidence and/or general agreement that a given procedure or treatment is useful and effective
II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a procedure or treatment
IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
IIb	Usefulness/efficacy is less well established by evidence/opinion
III	Conditions for which there is evidence and/or general agreement that the procedure/treatment is not useful/effective and in some cases may be harmful
Weight of evidence	
A	Data derived from multiple randomized clinical trials that involved large numbers of patients
B	Data derived from a limited number of randomized trials that involved small numbers of patients or from careful analyses of nonrandomized studies or observational registries
C	Expert consensus as the primary basis for the recommendation

The draft guidelines were posted on the NACB website in September 2006 for public comment from individuals, organizations, and other interested parties. The guidelines were also presented at the 27th Arnold O. Beckman Conference in Baltimore, Maryland, in October 2006. Public comments received through these channels were carefully reviewed by the committee and actions were taken to address them. These responses are summarized in a supplemental document at http://www.aacc.org/members/nacb/LMPG/Pages/default.aspx.

Based on a thorough review of the published literature as detailed in the full guideline document (available at http://www.aacc.org/members/nacb/LMPG/Pages/default.aspx), only hsCRP met all of the stated criteria required for acceptance as a biomarker for risk assessment in primary prevention.

As a result, the following recommendations for assessing risk for CVD in primary prevention are made.

Inflammation Biomarkers and CVD Risk

CLINICAL SCIENCE

Recommendation 1

(*a*) After standard global risk assessment, if the 10-year predicted risk is <5%, hsCRP should not be measured.

(Classification of recommendation: I; Level of evidence: A)

(*b*) If the 10-year risk is 5% to <10%, it is expected that 10% might be reclassified to a higher risk group with the test. More information is needed on clinical application, particularly in relation to longer-term lifetime risk prediction and selection of an appropriate intervention (lifestyle/medical).

(Classification recommendation: II; Level of evidence: B)

(*c*) If risk is intermediate (10%–20%) and uncertainty remains as to the use of preventive therapies such as statins or aspirin, then hsCRP measurement might be useful for further stratification into a higher or lower risk category.

(Classification of recommendation: I; Level of evidence: A)

Recommendation 2

Therapies prescribed based on hsCRP concentrations should be based on clinical judgment of the physician because benefits of such treatment are uncertain.

(Classification of recommendation: IIb; Level of evidence: B)

Recommendation 3

There are insufficient data that therapeutic monitoring using hsCRP over time is useful to evaluate effects of treatments in primary prevention.

(Classification of recommendation: III (against use); Level of evidence: C)

Recommendation 4

The utility of hsCRP concentrations to motivate patients to improve lifestyle behaviors has not been demonstrated.

(Classification of recommendation: IIb; Level of evidence: C)

Recommendation 5

Evidence is inadequate to support concurrent measurement of other inflammatory markers in addition to hsCRP for coronary risk assessment.

(Classification of recommendation: IIb; Level of evidence: C)

POPULATION SCIENCE

1. The preponderance of evidence supports that higher hsCRP, fibrinogen, and WBC are associated with increased risk of cardiovascular events after adjustment for other known risk factors.

CLINICAL SCIENCE/LABORATORY TESTING

Recommendation 1

Measurement of hsCRP should be done, in the fasting or nonfasting state, in metabolically stable patients free of infection or acute illness. If the hsCRP concentration is <3 mg/L, it does not need to be repeated. If the value is >3 mg/L, repeat the measurement at least 2 weeks later in metabolically stable state, free of infection or acute illness. The lower of the 2 results should be considered the patient's value. If hsCRP is ≥10 mg/L this might relate to CVD risk. Other conditions such as active infection or inflammation or inflammatory disorders might be responsible. Extensive evaluations with imaging tests or other testing for these patients is not recommended unless pertinent history and physical exam findings are present, or if pursuing normal practice for age-appropriate population screening.

(Classification of recommendation: IIa; Level of evidence: A)

LABORATORY TESTING

Recommendation 1

Of the examined inflammatory markers for assessing CVD risk, hsCRP has the analyte and assay characteristics most appropriate for use in clinical practice.

(Classification of recommendation: I; Level of evidence: A)

Recommendation 2

There are sufficient data that fibrinogen is an independent marker of CVD risk; however, because of analytical concerns, insufficient assay standardization, and uncertainty in identifying treatment strategies, measurement is not recommended for this application.

(Classification of recommendation: III; Level of evidence: A)

Recommendation 3

There are sufficient data that WBC is an independent marker of CVD risk; however, because utility in reclassifying risk level and identifying treatment strategies is not known, measurement is not recommended for this application.

(Classification of recommendation: III; Level of evidence: C)

Recommendation 4

hsCRP results, regardless of the method used, should be expressed as mg/L.

(Classification of recommendation: I; Level of evidence: C)

Recommendation 5

hsCRP using standardized assays categorizes patients as follows:

(*a*) Low risk <1.0 mg/L

(*b*) Average risk 1.0–3.0 mg/L

(*c*) High risk <3.0 mg/L

(*d*) Very high risk ≥10.0 mg/L

(Classification of recommendation: IIa; Level of evidence: A)

Recommendation 6

Manufacturers of diagnostic assays for hsCRP should follow approved value transfer protocols to ensure that standardized assays are used for vascular risk assessment.

(Classification of recommendation: I; Level of evidence: C)

Recommendation 7

Caution is recommended in application of the hsCRP categorization in recommendation 5 for risk prediction in certain populations such as nonwhites and the elderly, as the clinical utility is less established.

(Classification of recommendation: IIa; Level of evidence: C)

LIPOPROTEIN SUBCLASSES AND PARTICLE CONCENTRATION AND CVD RISK

Recommendation 1

Lipoprotein subclasses, especially the number or concentration of small dense LDL particles, have been shown to be related to the development of initial coronary heart disease events, but the data analyses of existing studies are generally not adequate to show added benefit over standard risk assessment for primary prevention.

(Classification of recommendation: III (lipoprotein subclass determination is not recommended); Level of Evidence: A)

Recommendation 2

There are insufficient data that measurement of lipoprotein subclasses over time is useful to evaluate the effects of treatments.

(Classification of recommendation: III; Level of evidence: C)

Recommendation 3

Several methods are available to assess lipoprotein subclasses. Standardization is needed for this technology.

(Classification of recommendation: IIa; Level of evidence: C)

LIPOPROTEIN(a) AND CVD RISK

Recommendation 1
Lp(a) screening is not warranted for primary prevention and assessment of cardiovascular risk.

(Classification of recommendation: III (against measurement); Level of evidence: A)

Recommendation 2
If risk is intermediate (10%–20%) and uncertainty remains as to the use of preventive therapies such as statins or aspirin, then Lp(a) measurement may be done at the physician's discretion.

(Classification of recommendations: IIb; Level of evidence: C)

Recommendation 3
After global risk assessment, Lp(a) measurements in patients with a strong family history of premature cardiovascular disease may be useful for identifying individuals having a genetic predisposition of cardiovascular disease.

(Classification of recommendation: IIb; Level of evidence: C)

Recommendation 4
The benefits of therapies based on Lp(a) concentrations are uncertain. If both Lp(a) and LDL cholesterol are highly increased, an attempt can be made at the physician's discretion to lower Lp(a) value by lowering the increased LDL cholesterol.

(Classification of recommendation: IIb; Level of evidence: C)

Recommendation 5
There is insufficient evidence to support therapeutic monitoring of Lp(a) concentrations for evaluating the effects of treatment.

(Classification of recommendation: III (against measurement); Level of evidence: C)

Recommendation 6
Population routine testing for small size apolipoprotein(a) is not warranted.

(Classification of recommendation: IIb; Level of evidence: C)

apoA-I, apoB, AND CVD RISK

Recommendation 1
The first step to monitor efficacy of lipid-lowering therapies is to measure LDL cholesterol (and non-HDL cholesterol in patients with increased triglycerides).

(Classification of recommendation: I; Level of evidence: A)

Recommendation 2
Although apoB measures atherogenic lipoproteins and is a good predictor of CVD risk (equal at least to LDL cholesterol and non-HDL cholesterol), it is only a marginally better predictor than the current lipid profile and should not be routinely measured at this time for use in global risk assessment.

(Classification of recommendation: IIa (against measurement); Level of evidence: B)

Recommendation 3
Measurement of apoB can be used to monitor efficacy of lipid-lowering therapies as an alternative to non-HDL cholesterol.

(Classification of recommendation: IIb; Level of evidence: B)

Recommendation 4
The apoB/apoA-I ratio can be used as an alternative to the usual total cholesterol/HDL cholesterol ratio to determine lipoprotein-related CVD risk.

(Classification of recommendation: IIa; Level of evidence: A)

Recommendation 5
Manufacturers of apoB and apoAI assays should establish traceability to accepted standards to assure reliable and comparable results.

(Classification of recommendation: IIa; Level of evidence: C)

MARKERS OF RENAL FUNCTION AND CVD RISK

Recommendation 1
Chronic kidney disease (CKD) testing is not routinely recommended if the 10-year predicted risk is $<5\%$ without specific CKD or CVD risk factors, either for CKD detection or CVD risk assessment.

(Classification of recommendation: III (against routine measurement); Level of evidence: C)

Recommendation 2
CKD testing, including serum creatinine for glomerular filtration rate (GFR) estimation and microalbuminuria, for primary prevention should be performed for all individuals with hypertension, diabetes mellitus, family history of CKD, and those at intermediate risk (10%–20%) for CVD. In addition, measurement of serum creatinine for GFR estimation should be performed in all individuals >65 years old. Individual decisions are recommended in those with other CKD risk factors.

(Classification of recommendation: IIa; Level of evidence: B)

Recommendation 3
Manufacturers of creatinine assays should comply with the most recent recommendations for standardization and other performance characteristics recommended by the National Kidney Disease Education Program (NKDEP). Calculation of estimated GFR from creatinine values should be consistent with the most recent NKDEP recommendations.

(Classification of recommendation: I; Level of evidence: C)

Recommendation 4
Cystatin C may be a more powerful predictor of cardiovascular events than estimated GFR (eGFR) calculation based on creatinine. Research should be conducted to examine if interventions based on cystatin C measurements for risk stratification in individuals with diminished estimated GFR will provide added clinical benefit.

(Classification of recommendation: IIa; Level of evidence: C)

Recommendation 5
Properly designed studies focusing on the role of kidney disease markers (microalbumin, eGFR, and cystatin C) should be conducted to characterize the utility of these markers in the global assessment of CVD risk in the primary prevention setting.

(Classification of recommendation: I; Level of evidence: C)

HOMOCYSTEINE AND CVD RISK

The clinical application of homocysteine measurement for risk assessment of primary prevention of CVD is uncertain given that several trials investigating folic acid and vitamin B supplementation published after our literature review indicated no benefit or lowering of CVD risk. A more detailed review of the literature for homocysteine appears in the online version of this guideline.

Recommendation 1
Hcy concentrations (μmol/L) derived from standardized assays categorize patients as follows:

Desirable $\leq$10
Intermediate (low to high) $>$10 to $<$15
High $\geq$15 to $<$30
Very high $\geq$30

(Classification of recommendation: IIa; Level of evidence: C)

Recommendation 2
The analytical performance goal for clinical usefulness for measurement of Hcy should be $<$10% for bias, $<$5% for precision, and $<$18% for total error. Manufacturers of diagnostic assays for Hcy should follow approved value transfer protocols to ensure that standardized assays are used for vascular risk assessment.

(Classification of recommendation: IIa; Level of evidence: C)

NATRIURETIC PEPTIDES (BNP AND NT-proBNP) AND CVD RISK

Recommendation 1
Increased BNP or NT-proBNP concentrations are associated with increased mortality in the next 2–7 years in community-based populations. However, the benefits of therapy based on these measurements are uncertain. Measurement for CVD risk assessment in the primary prevention setting is unwarranted.

(Classification of recommendation: III (against measurement); Level of evidence: B)

Recommendation 2
More research should be performed to determine if BNP and NT-proBNP measurements are useful in identifying individuals who are at increased risk of developing heart failure and might benefit from therapies for prevention of heart failure and cardiovascular disease.

(Classification of recommendation: I; Level of evidence: C)

Recommendation 3
Manufacturers of reagents and kits for measurement of BNP or NT-proBNP should be in compliance with current specifications developed by government and professional organizations, such as the IFCC.

(Classification of recommendation: I; Level of evidence: C)

Recommendation 4
Laboratorians, clinicians, and manufacturers involved in using and/or producing natriuretic peptide assays must work together to ensure that all stakeholders are properly educated regarding preanalytical (e.g., biological variation, specimen stability), analytical (the impact of various proBNP-derived peptides forms on assays, methodological variation of BNP results), and postanalytical (appropriate reference intervals, decision limits, and confounding clinical conditions) issues.

(Classification of recommendation: I; Level of evidence: C)

Guideline Implementation

Adoption of these guidelines is voluntary. The literature continues to grow with published reports providing new information on these and other emerging biomarkers for heart disease and stroke. It is increasingly important that as these candidate biomarkers

emerge, their value for possible clinical application along with measurement issues be properly evaluated. As a result of this continuous expanding body of research, the current NACB guidelines will undoubtedly require continuous review and updating as knowledge and understanding of existing and new biomarkers for primary prevention of heart disease and stroke emerge.

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