

National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: Emerging Biomarkers of Cardiovascular Disease and Stroke

Draft Guidelines to be discussed at the 2006 Beckman Conference

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Overview

For more than 20 years, coronary heart disease and stroke have been the first and third leading causes of death and major causes of disability in the United States and other developed countries (1). These are estimated to be the first and second leading causes of death in the world today and are expected to remain so by the year 2020 (2). Despite significant reduction in all standardized mortality from cardiovascular disease (CVD) over the past 20 years, CVD remains the number one cause of death in the United States, out ranking all cancers by more than 60% (3). More than 70.1 million Americans have some form of cardiovascular disease (3). Public health priorities for prevention of cardiovascular events and stroke as addressed in Healthy People 2010 are prevention of risk, detection and treatment of risk factors, early identification and treatment of heart attacks and stroke, and prevention of recurrent cardiovascular events (4,5). Thus the search for biomarkers that will better detect coronary patients with disease who could potentially benefit from intensive primary prevention efforts is critically important.

The American Heart Association (6) and the National Cholesterol Education Program's (NCEP) Adult Treatment Panel III (ATP III) (7) have each issued recommendations designed to identify more people who are asymptomatic and clinically free of coronary heart disease, but at sufficiently high risk for a future coronary event in order to justify more intensive risk reduction efforts. Within these recommendations are specific risk factors, including total cholesterol, LDL and HDL cholesterol, that are typically used in risk prediction algorithms, such as the Framingham Risk Score, to estimate a global risk

assessment for CVD. However, these predictive models based on conventional risk factors are underutilized and have a lower than desired accuracy, thus providing a stimulus to search for new tools to refine CVD risk prediction (8). In recent years the number of new candidate risk factors that have been proposed as significant predictors of cardiovascular disease 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 those of dyslipdemia, high blood pressure, and smoking – the major, longest established risk factors (9).

Table 1. Emerging Risk Factors for Cardiovascular Disease

C-Reactive protein	Interlukins (eg, IL-6)
Serum amyloid A	Vascular and cellular adhesion molecules
Soluble CD-40 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
Apo E genotype	Fibrinopeptide A
Remnant lipoproteins	von Willebrand factor antigen

While the guidelines issued by the NCEP's ATP III for global risk assessment using the traditional risk factors are based on strong evidence supporting their role in the pathogenesis of CVD, the role for the emerging risk factors is not as clear. Debate has taken place on whether a risk marker must be causally related to disease, or whether clinical utility can be advocated for a marker that might not be causal, but might indicate a different course of therapy than would otherwise be considered. Additional guidance is needed to help clarify and define the contribution that these emerging risk factors may have in identifying persons at risk for CVD.

The National Academy for Clinical Biochemistry (NACB) is the American Association for Clinical Chemistry's scientific academy. 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. The NACB has convened a panel of experts to develop recommendations for the laboratory measurement and clinical utility of a selective number of these emerging risk factors for CVD. The NACB panel of experts selected the following risk factors to include in this guideline: lipoprotein subclasses and particle concentration, lipoprotein (a), apolipoproteins A-I and B, C-reactive protein, fibrinogen, white blood cell count, homocysteine, BNP, and markers of renal impairment. These risk factors were selected for inclusion based on

expert consensus as to which factors were closest in the available information evaluated against criteria of clinical usefulness, consistency of epidemiologic data, improved predictive value, independence from other factors, and available analytical methods.

The current guidelines for these emerging risk factors have been developed based on the published evidence for their use in primary prevention to predict CVD and stroke risk in non-diseased populations compared to the ATP III recommendations based on the measurement of total cholesterol, LDL and HDL cholesterol. The first premise is that the first step in primary risk prediction is calculation of the 10-year predicted risk based on Framingham Risk Score or other classification which incorporates a lipid profile (Total cholesterol, HDL-C, triglycerides, calculated LDL-C and non-HDL-C).

Specific recommendations in this NACB guideline are based whenever possible on relevant published information. The strength of scientific data supporting each recommendation is 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 upper case letters A through C describe the weight of evidence.

Table 2: American Heart Association/American College of Cardiology Classifications 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 was the primary basis for the recommendation

This document is an executive listing of the proposed recommendations only. The complete draft guidelines with supporting discussion are available at http://www.aacc.org/NR/rdonlyres/5A4F3427-3626-4D1E-91D2-25FCBA3B1C82/0/NACB_full_guidelines_draft_091906.pdf

The NACB needs your feedback on The Laboratory Medicine Practice Guidelines on Emerging Risk Factors for Cardiovascular Disease and Stroke. All comments are welcome and all feedback will be carefully examined by the committee. We hope to

interact with you at the 2006 Beckman Conference in Baltimore, October 20-21. For those who are unable to attend, please examine the following recommendations and e-mail your comments and feedback to nacberf@aacc.org.

Recommendations for Inflammation Markers:

Twenty-four analytes were discussed that have some supportive data from observational studies. These were rated by an Inflammation Work Group according to their practicality for clinical use, availability of a commercial assay that could be standardized, and whether the observational data were sufficient. From this rating process hs-C-reactive protein, fibrinogen and white blood cell count were selected for evaluation.

Clinical Science

Recommendation 1:

a. After standard global risk assessment, if the 10-year predicted risk is <5%, hs-CRP should not be measured.

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

b. If the 10-year risk is 5-<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

(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 hs-CRP measurement might be useful for further stratification into a higher or lower risk category

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

Recommendation 2:

The benefits of therapies prescribed based on hs-CRP levels are uncertain

(Classification of recommendation: IIa, Level of evidence: B).

Recommendation 3:

There is insufficient data that therapeutic monitoring using hs-CRP over time is useful to evaluate effects of treatments in primary prevention

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

Recommendation 4:

hs-CRP levels may be useful in motivating patients to improve lifestyle behaviors. The benefits of this strategy remain unstudied

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

Recommendation 5:

Evidence is inadequate to support concurrent measurement of other inflammatory markers in addition to hs-CRP for coronary risk assessment
(Classification of recommendation: IIb; Level of evidence: C).

Population Science

1. The preponderance of evidence supports that higher hs-CRP, fibrinogen, and white blood cell count are associated with increased risk of cardiovascular events after adjustment for other known risk factors.

Laboratory testing

Recommendation 1:

Of the current inflammatory markers for assessing CV risk, hs-CRP has the analyte and assay characteristics most appropriate for use in clinical practice
(Classification of recommendation: I; Level of Evidence: A).

Recommendation 2:

There is sufficient data that fibrinogen is an independent marker of cardiovascular risk.
(Classification of recommendation: I; Level of Evidence: A.)

Recommendation 3:

There is insufficient assay standardization for reliable measurement of fibrinogen for cardiovascular risk assessment in clinical practice
(Classification of recommendation: I; Level of Evidence: C)

Recommendation 4:

There is sufficient data that WBC is an independent marker of cardiovascular risk.
(Classification of recommendation: I; Level of Evidence: A.)

Recommendation 5:

The utility of measuring fibrinogen or WBC for identifying treatment strategies is not clear
(Classification of recommendation: I; Level of evidence: A)

Recommendation 6:

hs-CRP results should be expressed as mg/L

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

Recommendation 7:

CRP using standardized assays categorizes patients as follows:

- a. Low level < 1 mg/L
- b. Average level 1-3 mg/L
- c. High level >3.0 mg/L
- d. Very high level \geq 10 mg/L

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

Recommendation 8:

Measurement of CRP should be done in the fasting or nonfasting state in metabolically stable patients free of infection or acute illness. If the hs-CRP level is < 3 mg/L it does not need to be repeated. If the level is >3 mg/L, repeat the measurement in the fasting or nonfasting state at least 2 weeks later in metabolically stable, free of infection or acute illness. The lower of the two results should be considered the patient's value. If hs-CRP is >10 mg/L this might relate to CV risk. Other conditions such as obesity, inflammatory disorders or cancer might be considered. 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).

Recommendation 8:

Caution is recommended in application of the hs-CRP categorization in #7 for risk prediction in certain populations such as non-Caucasians and the elderly, as the clinical utility is less established

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

Recommendations for Lipid-Related Markers

LDL subclasses and particle size

Recommendation 1:

Lipid 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.

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

Recommendation 2:

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

(Classification of recommendation: IIb; 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)

Lp(a)

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:

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 3:

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 4:

The benefits of therapies based on *Lp(a)* levels are uncertain. If both *Lp(a)* and LDL cholesterol are highly elevated, an attempt can be made at the physician's discretion to lower *Lp(a)* level by lowering the elevated LDL cholesterol.

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

Recommendation 5:

There is insufficient evidence to support therapeutic monitoring of Lp(a) levels 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)

Apolipoproteins B and AI

Recommendation 1:

Although apoB measures atherogenic lipoproteins and is a good predictor of CHD risk equal at least to LDL cholesterol and non-HDL cholesterol, it is only marginally better than that provided by the lipid profile and should not be routinely measured for use in global risk assessment.

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

Recommendation 2:

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

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

Recommendation 3:

Measurement of apo-B used to monitor efficacy of lipid lowering therapies as an alternative to non-HDL assessment in patients with elevated triglycerides.

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

Recommendation 4:

The apo B/apo A-I ratio used as an alternative to the usual TC/HDL cholesterol ratio to determine lipoprotein-related risk for CVD.

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

Other Risk Factors

Renal Markers

Recommendation 1:

CKD testing, including microalbuminuria and serum creatinine for GFR estimation, should be performed for all individuals with hypertension, diabetes, family history of CKD, and those with CVD or at increased risk of CVD. In addition, measurement of serum creatinine for GFR estimation should be performed in all individuals >65 years old. Individual decision making is recommended for individuals with other CKD risk factors.

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

Recommendation 2:

CKD testing is not routinely recommended for otherwise healthy individuals 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 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 calculation based on creatinine. Research should be conducted to examine if interventions based on cystatin C measurements for further risk stratification in individuals with diminished estimated GFR will provide added clinical benefit.

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

Recommendation 5:

Properly designed studies focusing on the role of kidney disease markers (microalbumin, creatinine, eGFR and cystatin C) should be conducted to evaluate clinical utility along with global cardiovascular risk assessment in the primary prevention population.

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

Homocysteine

Recommendation 1:

Homocysteine measurement for primary prevention and assessment of cardiovascular risk is not warranted.

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

Recommendation 2:

Testing of homocysteine in high risk patients may have marginal ability to predict future cardiovascular events in patients with multiple risk factors such as renal insufficiency, hypertension, or metabolic syndrome.

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

Recommendation 3:

Measuring homocysteine concentrations for monitoring therapeutic treatment aimed at lowering cardiovascular risk is not warranted.

(Classification of recommendation: III (against monitoring); Level of Evidence C)

Recommendation 4:

Report results in micromole/L ($\mu\text{mol/L}$) and interpret Hcy reported results in terms of the following reference values ($\mu\text{mol/L}$)

Desirable ≤ 10

Intermediate (low high) >10 to <15

High ≥ 15 to <30

Very high ≥ 30

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

Recommendation 5:

Goal of analytical performance acceptable for clinical usefulness for routine measurement of Hcy should be $<10\%$ bias and $<5\%$ CV, using primary standards, stable in-house quality control materials that are traceable to the NIST SRM for Hcy #1955.

(Classification of recommendation: IIb; Level of Evidence A)

Natriuretic Peptide (BNP)

Recommendation 1:

Elevated B-type natriuretic peptide (BNP) or the N-terminal proBNP (NT-proBNP) measurements are associated with increased mortality in the next 2 to 7 years in community-based populations. However the benefits of therapy based on these measurements is uncertain. Measurement for primary prevention and assessment of cardiovascular risk 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 can be utilized for 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 the B-type natriuretic peptide (BNP) or N-terminal proBNP (NT-proBNP) should be in compliance with current specifications developed by government and professional organizations.

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

Recommendation 4:

Laboratorians, clinicians and manufacturers involved in utilizing and, or producing natriuretic peptide must work together to assure that all stakeholders are properly educated regarding preanalytical (e.g. biological variation, specimen stability), analytical (the impact of various proBNP forms on assays, methodological variation of BNP results), and post analytical (appropriate reference intervals and confounding clinical conditions) issues.

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

References

1. World Health Organization. The World Health Report 2002. Available at: <http://www.who.int/whr/en>. Accessible February, 2005.
2. Murray CJ, Lopez AD, Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet 1997;349:1269-1276.
3. American Heart Association, 2006 Heart and Stroke Statistics – 2006 Update. Dallas, TX: American Heart Association; 2006
4. US Department of Health and Human Services. Healthy People 2010: Understanding and Improving Health and Objectives for Improving Health. 2nd ed. Vol 1. Washington, DC: US Government Printing Office; November 2000.
5. US Department of Health and Human Services. A Public Health Action Plan to Prevent Heart Disease and Stroke. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention; 2003.
6. Smith SC, Greenland P, Grundy SM. AHA Conference Proceedings: Prevention Conference V: beyond secondary prevention: identifying the high-risk patient for

primary prevention: executive summary: American Heart Association. *Circulation* 2000;101:111-116.

7. Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486-2497.
8. Wierzbicki AS, Reynolds TM, Gill K, et al. A comparison of algorithms for initiation of lipid lowering therapy in primary prevention of coronary heart disease. *J Cardiovasc Risk*. 2000;7:63-71.
9. Hackman DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: A critical review of the evidence. *JAMA* 2003;290:932-940.