

hs-CRP Lp-PLA₂ hs-CRP
 Lp-PLA₂ hs-CRP
 hs-CRP Lp-PLA₂ hs-CRP
 Lp-PLA₂

Biomarkers for cardiovascular risk assessment

By Robert L. Wolfert, PhD

Research into inflammatory biomarkers has opened up a new era in the assessment of risk in patients with cardiovascular disease (CVD). Of the dozens of candidate biomarkers, there are two that have accumulated sufficient published evidence to support their utility in clinical practice: high-sensitivity C-reactive protein (hs-CRP) and lipoprotein-associated phospholipase A₂ (Lp-PLA₂).

Clinical review of Lp-PLA₂

Lp-PLA₂ is produced predominantly by macrophages and is strongly associated with rupture-prone plaque. Because it is produced by macrophages in atherosclerotic lesions in the arterial intima, it is a more vascular-specific marker than hs-CRP or other acute phase reactant inflammatory markers, many of which are produced in the liver.¹ Lp-PLA₂ is potentially linked to the causal pathway of plaque inflammation, instability, and eventual rupture; found at high levels in thin fibrous cap atheroma; and can be lowered by lipid-modifying medications (statins, fibrates, niacin, ezetimibe, and omega-3 fish oil).

Lipid-lowering therapies, including statins, are proven to reduce cardiovascular events, regardless of baseline LDL-C levels.

An elevated Lp-PLA₂ result may indicate a need for more aggressive therapy, including treatment to lower low-density lipoprotein cholesterol (LDL-C) goals. Lipid-lowering therapies, including statins, are proven to reduce cardiovascular events, regardless of baseline LDL-C levels.

CE CONTINUING EDUCATION

To earn CEUs, see test on page 22, or at www.mlo-online.com under the CE Tests tab. The May 2009 test covers both articles in this section.

LEARNING OBJECTIVES

Upon completion of this article, the reader will be able to:

1. Identify inflammatory biomarkers related to cardiovascular disease.
2. Correlate Lp-PLA₂ with atherosclerotic plaque.
3. Identify laboratory methods for measuring Lp-PLA₂.
4. Describe genetic changes of cells resulting in tumors.
5. Identify biomarker factors useful to guide cancer treatment.

sponsored by



In multiple clinical studies, Lp-PLA₂ has been shown to be a predictor of unstable plaque, myocardial infarction (MI), and ischemic stroke.² Since low-density lipoprotein has proven *not* to be a reliable predictor of stroke, measuring levels of Lp-PLA₂ addresses this unmet clinical need.

Lp-PLA₂ resides mainly on and travels with LDL particles in plasma via apolipoprotein B binding, although it is also associated with high-density lipoprotein, or HDL, particles, lipoprotein (a), and remnant lipoproteins. Lp-PLA₂ is highly upregulated in atherosclerotic plaque; and through hydrolysis of oxidized LDL, this enzyme generates two pro-inflammatory mediators, lysophosphatidylcholine and non-esterified oxidized fatty acid. In pre-clinical animal studies, inhibition of the enzyme attenuates the inflammatory process and slows atherosclerotic-disease progression. A Phase II study sponsored by GlaxoSmithKline showed that a direct Lp-PLA₂ inhibitor (darapladib), in addition to standard-of-care treatment, prevented expansion of the necrotic core, a region within coronary plaque associated with a high risk of rupture.³

A substantial body of evidence supports Lp-PLA₂ as a cardiovascular risk marker that provides new information, over and above traditional risk factors, to help identify individuals at increased risk of suffering a heart attack or stroke.

The Lp-PLA₂ difference

Numerous peer-reviewed publications have confirmed that elevated plasma levels of Lp-PLA₂ are independently associated with risk of coronary heart disease (CHD) and ischemic stroke. The Atherosclerosis Risk in Communities, or ARIC, study showed that in individuals with normal LDL, elevated Lp-PLA₂ levels were strongly associated with heart disease and ischemic stroke, independent of traditional risk factors and hs-CRP.^{4,5} Elevated levels of both inflammatory markers conferred an even higher risk of MI and stroke. Individuals with elevated Lp-PLA₂ and hs-CRP levels had greater than a fourfold increase in risk for heart attacks, and more than an elevenfold increase in risk for ischemic stroke. Additionally, increased levels of Lp-PLA₂ doubled the risk of ischemic stroke at every level of systolic blood pressure, while individuals with the highest levels of Lp-PLA₂ and elevated blood pressure had nearly a sevenfold increase in risk of suffering an ischemic stroke.⁶ In the KAROLA study, high-risk patients followed for four to six years showed a significantly lower incidence of cardiovascular events if their Lp-PLA₂ levels were <223 ng/mL.⁷

Lab measurement of Lp-PLA₂

Testing for Lp-PLA₂ in the laboratory is available in an ELISA test format or as an automated format. Two highly specific monoclonal antibodies are used in the assay, and it is calibrated to a well-characterized recombinant Lp-PLA₂ standard to increase the accuracy of the test. The automated assay employs immunoturbidimetric technology and can



DxS Mutation Test Kits

Validated biomarkers for the detection of key mutations in oncogenes

Research Kits available for K-RAS, EGFR, B-RAF, PI3K and BCR-ABL T315I genes

Every DxS Mutation Test Kit:

- Is based on class-leading Scorpions® and ARMS® real-time PCR technology
- Can be performed in-house with a simple work flow and time to result < 3 hours
- Detects mutations frequently missed by sequencing methods
- Is compatible with a range of DNA sample types

Each highly sensitive and selective assay can detect 1% of mutant in a background of wild-type genomic DNA.

For further information visit www.dxsdiagnostics.com

be run on the Hitachi, Roche Modular P and Olympus analyzers. Additional applications are in development. The Lp-PLA₂ protein in serum is generally stable (i.e., the protein itself does not degrade), but it is highly recommended that the serum and plasma samples be collected and stored according to the Recommended Specimen Collection and Storage procedures.

Acknowledging the limitations of traditional risk factors to precisely assess cardiovascular risk across the general population, the National Cholesterol Education Program Adult Treatment Panel, or NCEP ATP III, report recognized the potential of inflammatory markers to help refine cardiovascular risk assessment. As Lp-PLA₂ evaluates vascular inflammation specifically, persons with elevated levels of Lp-PLA₂ could potentially be classified into a higher risk category, prompting the need to further intensify lifestyle and medication therapy in direct proportion to the degree of determined risk.⁸⁻¹¹

An elevated Lp-PLA₂ result may indicate a need for more aggressive therapy, including treatment to lower low-density lipoprotein cholesterol (LDL-C) goals.

The current literature has reported that the central 90th percentile of Lp-PLA₂ levels range from 120 to 342 ng/mL for women and 131 to 376 ng/mL for men.¹² Recently, using data from all currently published Lp-PLA₂ studies, an independent consensus panel of cardiologists, neurologists and laboratorians endorsed a cut point of >200 ng/mL to identify patients at higher risk for CHD/CVD.¹³

The same consensus panel recommended, consistent with the ATP III guidelines, that Lp-PLA₂ should be used as an adjunct to traditional risk-factor assessment. They suggested that elevated Lp-PLA₂ levels would justify more aggressive risk-reducing strategies, including treatment to lower LDL-C goals.

In summary, a substantial body of evidence supports Lp-PLA₂ as a cardiovascular risk marker that provides new information, over and above traditional risk factors, to help identify individuals at increased risk of suffering a heart attack or stroke. The level of the enzyme in the bloodstream is related to the progression of instability of the atherosclerotic plaque, and the likelihood for plaque rupture and a resulting thrombotic event. As such, Lp-PLA₂ should be used as an adjunct in persons assessed to be at moderate or high cardiovascular risk by traditional risk factor assessment, to help refine absolute risk status and identify the individuals who would most benefit from intensification of lifestyle modification and lipid lowering therapies. □

Robert L. Wolfert, PhD, is executive vice president and chief scientific officer at diaDexus Inc. in South San Francisco. For more information, please visit www.diadexus.com.

Note: This article is followed by another article, "Cancer biomarkers — a good start," that is also part of the Continuing Education test.

References

1. McConnell JP, Hoefner DA. Lipoprotein-associated phospholipase A₂. *J Clin Lab Med*. 2006;26:679-697.
2. Garza CA, et al. Association between lipoprotein-associated phospholipase A₂ and cardiovascular disease: a systemic review. *Mayo Clin Proc*. 2007;82(2):159-165.
3. Serruys PW, et al. Darapladib: effects of the direct lipoprotein-associated phospholipase A₂ inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation*. 2008;118:1172-1182.
4. Ballantyne CM, Hoogeveen RC, Band H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A₂, high-sensitivity C-reactive protein, and risk for incident heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation*. 2004;109:837-842.
5. Ballantyne CM, et al. Lipoprotein-associated phospholipase A₂, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arch Intern Med*. 2005;165:2479-2484.
6. Gorelick PB. Lipoprotein-associated phospholipase A₂ and risk of stroke. *Am J Cardiol*. 2008; 101[suppl]:34F-40F.
7. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A₂, predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function and hemodynamic stress (KAROLA). *Arterioscler Thromb Vasc Biol*. 2006;26:1586-1593.
8. 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.
9. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). 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) final report. *Circulation*. 2002;106:3143-3421 II-30-II-31.
10. Pearson TA, Mensah GA, Alexander RW, Anderson JL, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*. 2003;107:499-511.
11. Smith SC, Allen J., Blair SN, Bonow RO, Brass LM, Fonarow GC, et al. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update. *Circulation*. 2006;113:2363-2372.
12. Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A₂ levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. *Eur Heart J*. 2005;26:137-144.
13. Davidson MH, Corson MA, Alberts MJ, et al. Consensus Panel Recommendation for Incorporating Lipoprotein-Associated Phospholipase A₂ Testing Into Cardiovascular Disease Risk Assessment Guidelines. *Am J Cardiol*. 2008; 101 [suppl]:51F-57F.

DxS Mutation Test Kits

Research Kits are available for K-RAS, EGFR, B-RAF, PI3K and BCR-ABL T315I genes.

Visit www.dxsdiagnostics.com for more information about DxS products and class-leading technology.

Cancer biomarkers — a good start

By Stephen Little, PhD

Cancer researchers and the pharmaceutical industry have invested trillions of dollars and millions of man years in the search for drugs that will cure cancer; but despite this gargantuan effort, success to date has been patchy. In 2009, there is a sense of “could do better” rather than the conclusive triumph over cancer that everyone would hope for. Why is this, and how can the situation be improved?

Perhaps part of the problem is the remarkable power of cancer cells to evolve in response to their environment. The pharmaceutical industry is incredibly good at developing new drugs with the ability to kill cancer cells; but, often, when these are administered to a patient, there is an initially positive response until the cancer mutates and evolves in an effort to establish a way to overcome the effects of the therapy.

The plasticity of the cancer genome means that it is not sufficient to kill a cancer as it exists at one point in time — there is a need for a dynamic approach that follows the cancer as it twists and turns to escape the effect of the therapy, and to hunt it down until every last cell is destroyed. This, of course, is easier said than done.

There are, however, grounds for optimism. The Human Genome Project is complete, the Cancer Genome Project is underway, and our understanding of how cancer works has never been better. At a basic level there are three types of genetic change responsible for a normal cell turning into a tumor:

- activation of oncogenes — genes which tell the new cancer to grow;
- loss of tumor suppressors — genes which would have told the cancer to stop growing; and
- loss of DNA repair genes — genes normally work to maintain the integrity of the genome; without them, the other changes are much more likely to occur.

A common analogy is to liken the cell to an automobile. The accelerator pedal represents the oncogenes, which, when activated, is like locking the pedal in the “fully on” position. The brake pedal is likened to the tumor suppressors; the loss of these means that the car cannot stop. Perhaps it is stretching the analogy a little, but an incompetent car mechanic would represent the loss of DNA repair genes like that having an inept individual working on the car would cause an increase in the likelihood of brake or accelerator problems.

It follows then, that to truly wipe out cancer cells within the body, it is not enough to have effective drugs that target some of the cancer-growth pathways — it is also essential to have a way of monitoring the cancer itself, so the drug therapy can be adjusted to match the tumor as it evolves. In this way, it might be possible to use a sequence of treatments to allow a better outcome for the patient.

The tools to allow this are now emerging in the form of cancer biomarkers. The word “biomarker” has a very broad definition and is essentially anything associated with drug

response that can be measured. This includes predictive biomarkers — which can be used to select patients — and response biomarkers which (as their name suggests) indicates whether a drug is working or not. There are many classes of biological molecules which can be tested as biomarkers. The most fundamental for cancer are genetic biomarkers, because cancer is essentially a genetic disease in that it is caused by somatic gene changes. Somatic gene changes are the underlying alterations which can predict how an individual tumor will respond to treatment and include mutations, methylation changes, gene rearrangements, and gene-expression changes. All of these genetic changes cause a plethora of other variations to the cell, its immediate environment, and to the whole body; so, as a result, other biomarkers include proteins, peptides, carbohydrates, and metabolites.

It follows then, that to truly wipe out cancer cells within the body, it is not enough to have effective drugs that target some of the cancer-growth pathways ...

For a biomarker to be successful in guiding future drug treatments, there are two key requirements. The first is obviously that the marker, whatever it is, must be associated with drug response. In the language of diagnostics, it must show clinical utility; or, in common parlance, it must answer the “so what” question. If there is no clearly defined treatment decision based on the use of the biomarker, then it was probably not worth testing for in the first place.

The second requirement has much more to do with the practicality of implementing a biomarker-driven drug selection strategy. Normally at the start of treatment, there will be a tumor biopsy available — this is excellent material for the measurement of many types of biomarkers and is probably the ideal sample. There is, however, a major problem with the tumor biopsy. Earlier in this article, it was pointed out that it would be essential to have a way of monitoring the tumor as it evolved. Unfortunately, for most cancers, there is no practical and safe way to take repeated primary biopsies, so it becomes essential to be able to use a different source of tumor material or even a surrogate for the tumor itself.

There is currently a great deal of interest in the detection of circulating tumor cells¹ or circulating nucleic acid which has been shed from the cancer. These methods can be technically demanding and suffer from a lack of sensitivity, but they do have the huge advantage that regular sampling is both feasible and practical. If these methods can be honed to a workable level, the door can be opened to biomarker monitoring and therapy adjustment.

There is also the possibility of using a surrogate biomarker. For example, hair follicles have the same epithelial origins as many cancers and can be used to indicate whether or not a drug is having the expected effect within the body.

The use of biomarkers to guide therapy is still in its infancy; and although there have been a number of notable recent successes such as the use of KRAS mutation status² to guide the use of the colorectal-cancer drugs Erbitux (cetuximab)^{3,4,5,6} and Vectibix (panitumumab),⁷ and the association between EGFR mutations and response to Iressa (gefitinib)^{8,9,10} and Tarceva (erlotinib)¹¹,

there is still a long way to go before biomarkers become part of the complete cancer-treatment regime. A greater use of cancer biomarkers is not the only innovation needed to improve outcomes with cancer drugs; but, perhaps, with their increasing adoption, we will soon be able to report that cancer treatment has improved from "could do better" to "a good start." □

Stephen Little, PhD, is the CEO of DxS Limited (www.dxsdiagnostics.com), Manchester, UK.

Note per the author. Erbitux - trademark of Merck KGaA/Imclone Systems; Vectibix - trademark of Amgen Inc.; Iressa - trademark of AstraZeneca group of companies; and Tarceva - trademark of OSI Pharmaceuticals.

References

1. Horiike A, Kimura H, Nishio K, Ohyanagi F, et al. Detection of Epidermal Growth Factor Receptor Mutation in Transbronchial Needle Aspirates of Non-Small Cell Lung Cancer. *Chest*. 2007;131(6):1628-1634.
2. Jimeno A, Messersmith WA, Hirsch FR, Franklin WA, Eckhardt SG. KRAS Mutations and Sensitivity to Epidermal Growth Factor Receptor Inhibitors in Colorectal Cancer: Practical Application of Patient Selection. *J Clin Oncol*. 2009;27(7):1130-1136.
3. Bokemeyer C, et al. K-RAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: The OPUS experience. *J Clin Oncol*. 26:2008. (May 20 suppl; abstr 4000)
4. Van Cutsem E, et al. K-RAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: The CRYSTAL experience. *J Clin Oncol*. 26: 2008. (May 20 suppl; abstr 2)
5. Tol J, Koopman M, Rodenburg CJ, Punt CJ, et al. A randomised phase III study on capecitabine, oxaliplatin and bevacizumab with or without cetuximab in first-line advanced colorectal cancer, the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). *Annals of Oncology*. April 2008.
6. Tejpar S, et al., Relationship of efficacy with K-RAS status (wild type versus mutant) in patients with irinotecan-refractory metastatic colorectal cancer (mCRC), treated with irinotecan (q2w) and escalating doses of cetuximab (q1w): The EVEREST experience (preliminary data). *J Clin Oncol*. 26: 2008. (May 20 suppl; abstr 4001)
7. Amado RG, et al. Analysis of K-RAS mutations in patients with metastatic colorectal cancer receiving panitumumab monotherapy. Paper presented at: European Cancer Organization (ECCO), May 24-26, 2007, Limassol, Cyprus.
8. Kimura H, Kasahara K, Kawaiishi M, et al. Detection of Epidermal Growth Factor Receptor Mutations in Serum as a Predictor of the Response to Gefitinib in Patients with Non Small-Cell Lung Cancer. *Clin Cancer Res*. 2006;12(13).
9. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129-2139.
10. Guillermo Paez J, Pasi A, Jänne, Jeffrey C.Lee, et al. EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy. Originally published in *Science Express*, 1-10. 4 AD. *Science*. 2004;304(5676):1497-1500.
11. Mack P, Holland W, Burich R, Davies A, Gandara D, et al. Predictive value of EGFR and KRAS mutations detected in plasma from non-small cell lung cancer (NSCLC) patients treated with docetaxel and intermittent erlotinib. In: Proceedings from the American Society of Clinical Oncology; May 30-June 2, 2008; Chicago, IL. Abstract 8062.



Tired of waiting for samples to spin?

In just 3 minutes a StatSpin® Express can get you up and running!

Many labs are moving to "Lean" processing and single piece flow. Now you don't need to wait for samples to spin in a slow conventional centrifuge. The StatSpin® Express family of small high speed centrifuges is your answer to rapid sample processing.

The high speed horizontal StatSpin® Express 4 provides flat gel separation in only 3 minutes. Samples can be put directly on the analyzer. Both the StatSpin® Express 3 and StatSpin® Express 4 hold 8 tubes up to 10 mL. They have a rugged brushless motor for added reliability and the entire unit is backed by a 2-year warranty. Plus they are incredibly easy to use. Just push the button and walk away.

Anywhere a STAT sample is needed, the StatSpin® Express 2 provides Platelet Poor Plasma in just 2 minutes. The small footprint let's you put it anywhere in the lab.

Tired of waiting for samples to spin? Place any Express centrifuge next to your analyzer and get results fast!

To get up and running contact Iris Sample Processing or your Iris distributor .



A Division of IRIS International, Inc.

800-782-8774 • www.statspin.com

Iris
Sample Processing