



LABletter

PATHOLOGY & LABORATORY MEDICINE NEWSLETTER

FEATURE ARTICLE HIGHLIGHTS>>

Cardiac Risk Assured

James Faix, M.D.

Director of Clinical Chemistry and Immunology

- Cardiac Risk Estimation – Now “Assured” by More Sensitive Test for Atherogenic Lipids & More Specific Marker of Atherosclerosis Inflammation:
 - Small Dense LDL Cholesterol
 - Lp-PLA2
- All Plaques Are Not Created Equal
- Lp-PLA2 is a Marker of Unstable Plaque
- New Ordering Options:
 - Lipid panel
 - Lipid Plus panel
 - Lipid Plus – Assured panel
 - Calculated Cardiac Risk only

Laboratory Update:

Changes to Random Urine Chemistry Testing

Q&A:

Read My Lipids: New Ways to Determine Cardiac Risk

James Faix, M.D.

Director of Clinical Chemistry and Immunology



James Faix, M.D.

Director of Clinical Chemistry and Immunology at Stanford Clinical Laboratory and

Associate Professor of Pathology at Stanford University School of Medicine. Dr. Faix directs the allergy, automated chemistry, blood gas, endocrinology, immunology, lipid, toxicology and tumor marker sections of the diagnostic laboratory.

CARDIAC RISK ASSURED

Cardiac Risk Estimation – Now “Assured” by New Tests

Cardiovascular disease continues to represent a significant problem. We do not know what causes the changes associated with atherosclerosis, but a number of risk factors have been identified. Much of the focus has been on inflammation and elevated serum lipids, and the most commonly employed schemes for risk calculation combine these two types of markers.

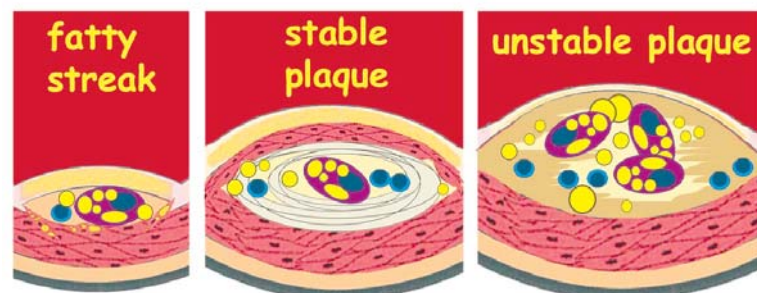
The major lipid marker is the cholesterol found in low-density lipoprotein (LDL) particles. Currently, the most commonly used marker of vascular inflammation is C-reactive protein (CRP). But many patients who experience an acute coronary event do not have elevated LDL cholesterol. Also, CRP may be elevated in many types of inflammatory disorders.

So, there has been a search for more sensitive markers of atherogenic lipids as well as more atherosclerosis-specific markers of inflammation. Stanford Clinical Laboratory will be adding two of these to our menu of cardiovascular tests in the fall of 2006:

>> Small Dense LDL - More sensitive test for atherogenic lipid.

LDL particles are not homogeneous; they range in size and in density. Smaller, more dense LDL particles have been shown to be more atherogenic. In the past Stanford has referred small dense LDL cholesterol to our reference labs. We will be bringing this test in-house in October 2006. *For more information about this test, see the “Q&A” section of this newsletter.*

Figure 1.



Progression of Atherosclerosis:

The initial lesion (left) is the fatty streak containing lipid, foam cells and some small lymphocytes. This may progress to a stable plaque (center) if inflammation (and lipid deposition) is limited. When there is excessive lipid deposition, macrophages produce enzymes that thin the collagen cap. They also die, forming a very thrombogenic material that may easily escape (and cause an occlusive thrombus) if the cap is torn. These cells are the source of Lp-PLA2.

“Lp-PLA₂ is specific for vascular inflammation because it is made by the macrophages in the plaque, in contrast to other markers of inflammation like CRP which are made in the liver.”

>> Lp-PLA₂ - More specific marker of vascular inflammation.

A new marker of inflammation, lipoprotein-associated phospholipase-A₂ (Lp-PLA₂) can help “assure” you that the cardiac risk determination is correct because, unlike CRP, Lp-PLA₂ seems to be a specific marker of *vascular* inflammation. The rest of this article discusses this new test.

All Plaques Are Not Created Equal: The earliest lesion of atherosclerosis is the fatty streak. Probably all Americans have some fatty streaks by the time that they are in their 20s. Whatever the primary endothelial injury was (and there are a number of theories), monocytes have investigated the scene and become macrophages. Although macrophages do not have receptors for LDL cholesterol, they do have so-called “scavenger” receptors. Lipid deposited in the site is oxidized and this is eagerly taken up by macrophages, which become foam cells. Inflammation also provokes some migration of smooth muscle cells from the media.

What happens next is important. If there is relatively little continued lipid deposition, “healing” occurs. Although foam cells persist, there is significant collagen formation by the migrating smooth muscle cells and a dense cap covers the injured area. This is called a “stable plaque”.

However, if the patient has high levels of lipids (or lipids that either easily enter the vessel wall or are easily oxidized), the activated macrophages dominate the process. As more foam cells appear, there is less “healing”. Engorged macrophages start to die, exploding and releasing their lipid contents into the center of the plaque. The fibrous cap is attenuated (and thinned by enzymes released by the macrophages). This is an “unstable plaque”.

Any breach of the thinned cap will send the soupy mess into the vessel lumen, causing an occluding thrombus (*Figure 1*).

Lp-PLA₂ is a Marker of Unstable Plaque: Small elevations of CRP (measured by “high-sensitivity” immunoassays) can detect the systemic inflammation that is part of atherosclerosis. Levels greater than 3 mg/L are especially associated with increased risk of a cardiovascular event. But elevated CRP levels may be due to some other problem. CRP has been used for many years to monitor a variety of infectious and inflammatory disorders, and even mild viral infections may elevate it. On our test report, we add a comment when result of a CRP test ordered to evaluate cardiac risk (hs-CRP) is elevated, warning that the result may not represent the patient’s baseline value (and suggesting that the test be repeated in several weeks).

Lp-PLA₂ is one of a number of enzymes that hydrolyze phospholipids. Lp-PLA₂ is somewhat unique for two reasons: one is that it is associated with lipoprotein particles; the other is that it is the enzyme produced by macrophages in response to the presence of oxidized lipid (*Figure 2*). Historically, there was debate as to whether or not this enzyme helps (by degrading dangerous oxidized lipids) or hurts (by producing pro-inflammatory mediators downstream). However, it now seems clear that circulating Lp-PLA₂ is a marker of unstable plaques (containing increased oxidized lipid and activated macrophages) as opposed to stable ones.

Not surprisingly, several recent epidemiological studies strongly suggest that elevated serum Lp-PLA₂ levels identify patients at increased risk of cardiovascular events (as well as stroke).

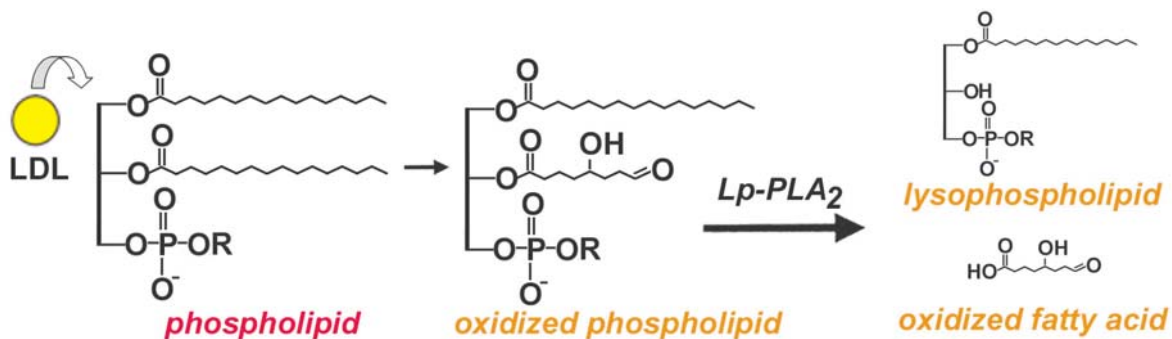


Figure 2
Action of Lp-PLA₂: Phospholipids that leach out of LDL particles deposited in the atherosclerotic plaque are oxidized in the site of inflammation. These are the substrate for Lp-PLA₂ produced by lipid-laden macrophages and the resulting lysophospholipids and oxidized fatty acids may play a role in amplifying the inflammation and the damage. (Revised from Zalewski A and Macphee C, *Arterioscler Thromb Vasc Biol* 25:923-931, 2005.)

Its predictive power is modest compared with the combination of LDL cholesterol and hs-CRP, but consistent, even when adjusted for other risk factors. Most importantly, Lp-PLA₂ is specific for vascular inflammation because it is made by the macrophages in the plaque, in contrast to other markers of inflammation like CRP, which are made in the liver.

Lipid Plus – Assured

Stanford Clinical Laboratory offers several ways to evaluate cardiac risk but, for ordering convenience (especially for new patients), all of the major markers are included in the Lipid Plus™ panel. Beginning in October 2006, we will introduce a new version – Lipid Plus – Assured™, with the addition of both small dense LDL cholesterol and Lp-PLA₂. Like all of the cardiac risk markers, you may also order either of these new tests individually.

New Ordering Options

- >> Lipid panel
- >> Lipid Plus panel
- >> Lipid Plus – Assured panel
- >> Calculated Cardiac Risk only

We will still calculate cardiac risk using “non-HDL cholesterol” (an estimate of LDL cholesterol plus remnants) and hs-CRP, because this is still the commonly accepted approach. However, if you order Lipid Plus – Assured™ and the hs-CRP level is elevated, an interpretative comment will refer to the Lp-PLA₂ result. It will emphasize that, when this marker is also elevated, you should definitely consider the patient at increased risk (and probably do not need to repeat the hs-CRP determination). Patients with increased relative cardiac risk should be re-evaluated, even if their LDL cholesterol is not elevated.

If the Lp-PLA₂ level is not elevated, the hs-CRP elevation may have less significance in terms of cardiac risk. Although it may still be prudent to repeat the test at some point in the near future, you may reassure your patient that it is likely that this result reflects some other source of inflammation.

Please see the Q&A section of this newsletter for more information about lipids, including small dense LDL. If you have any additional questions about this new approach to cardiac risk determination, please call Customer Service at 1-877-717-3733 and ask to speak to Dr. Faix, the Chemistry Medical Director.

** For more information: See the Stanford Lab Test Directory at www.stanfordlab.com or contact your Stanford Account Representative*

Laboratory Update: **Changes To Random Urine Chemistry Testing**

It is often necessary to determine how much of a particular substance found in the blood is being excreted in the urine. This information may be needed to evaluate whether renal loss is contributing to a low plasma level (e.g., hypokalemia or hypocalcemia) or whether there is renal dysfunction (e.g., proteinuria).

Although a timed urine collection is ideal (especially if collected over a 24-hour period), this is often not practical. Measuring the urine concentration of an analyte in a random (or “spot”) urine sample is easier, but this may produce an erroneous result. If the specimen is unusually concentrated or dilute, the concentration of the analyte will be over- or under-estimated.

A useful maneuver is to simultaneously measure creatinine in the random urine specimen. Creatinine is the breakdown product of creatine, found in the muscles. Every day, there is a constant production of creatinine at a regular rate. It is freely filtered by the renal glomerulus and not reabsorbed. Although it is secreted by the renal tubules to a small extent, this is not significant unless the patient’s plasma creatinine level is elevated. Consequently, the amount of creatinine in any random urine specimen should be similar. Dividing the analyte concentration by the creatinine concentration (producing an analyte/creatinine ratio) normalizes the result and removes any error due to the amount of water, which may be in the sample.

We have routinely measured creatinine (and calculated the analyte/creatinine ratio) for any analyte ordered on a random urine specimen. We have not billed for the creatinine, because we had no way to ensure that only one creatinine determination would be billed if tests for multiple analytes were ordered on the same random urine specimen. (We only perform one creatinine measurement in such a case.) However, recently, we have discovered a way to do this.

Beginning in October, random urine specimens submitted for any analyte will be billed for one creatinine result as well. Only one creatinine will be billed, even if multiple analytes (including creatinine itself) are ordered. The names of these tests will be changed to clearly indicate that creatinine is also being requested and the creatinine result will appear, along with the analyte/creatinine ratio.

If you wish to only order the analyte on a random urine specimen, a new test will be available for each analyte without creatinine. No reference ranges for these results will be provided, however, as we have no way of correcting for the amount of water which may be in the sample. The only exception to this rule is urine albumin (“microalbumin”). Current practice requires that creatinine be measured and the albumin/creatinine ratio be reported for this important marker of early glomerular disease.

Q&A: Submit Q&A topics of interest to: labmarketing@stanfordmed.org

An opportunity for the Bay Area Medical Community to request specific Q&A topics to our medical directors that are relevant to patient's clinical needs.



James Faix, M.D.

Read My Lipids: New Ways to Determine Cardiac Risk

Q&A with Jim Faix, M.D., Director of Clinical Chemistry and Immunology at Stanford Clinical Laboratory and Associate Professor of Pathology at Stanford University School of Medicine.

Stanford Clinical Laboratory will offer a new version of our current Lipid Plus™ panel for evaluation of cardiac risk, Lipid Plus – Assured™, with the addition of both small dense LDL cholesterol and Lp-PLA₂. See the main article of this issue for more information about Lp-PLA₂.

Q1: What is the difference between the different “cholesterols”?

A1: Cholesterol is carried in the blood packaged inside balls of protein called lipoprotein particles. Two can be considered cholesterol-poor: chylomicrons and very-low density lipoprotein (VLDL); they deliver triglycerides (fatty acids) to the tissues (especially muscle) for energy. The other two can be considered cholesterol-rich: low density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL particles carry cholesterol to tissues (for membrane synthesis and other purposes). HDL particles are like garbage trucks, bringing excess cholesterol to the liver for disposal. This is why LDL is the “bad” cholesterol and HDL is the “good” cholesterol.

Q2: Should the patient always be fasting when cholesterol tests are drawn?

A2: Ideally, yes. But this is primarily to avoid underestimating the calculated LDL cholesterol because of elevated chylomicrons. Also, the fast should only be “overnight” or about 8 hours. If the patient is fasting for a longer period of time, the same error may occur because of elevated VLDL particles. If you must have the patient tested and cannot draw a fasting specimen, options include ordering the direct LDL cholesterol or looking at the non-HDL cholesterol (the total cholesterol minus the HDL cholesterol).

Q3: What is the current target for LDL cholesterol?

A3: This subject is somewhat controversial. In 2003, the National Cholesterol Education Program (NCEP) recommended <100 mg/dl as the optimal LDL cholesterol for everyone. Most physicians continued to target <130 mg/dl if the patient did not have coronary artery disease (CHD) but did have moderate risk, and reserved <100 mg/dl as the treatment goal for their patients with CHD (or diabetes). In 2004, the NCEP stressed that patients with moderate risk should try to reach <100 mg/dl and that patients with CHD should try to reach 70 mg/dl or lower. In our report, the reference range for LDL cholesterol is <130 mg/dl but a comment advises that “Patients with coronary heart disease or diabetes should target <100 mg/dl (or lower) as the desirable level.”

Q4: Can patients still be at risk even if their LDL cholesterol is not elevated?

A4: Yes. Some have estimated that almost 50% of patients who have an acute myocardial infarction do not have elevated LDL cholesterol. This is why other risk factors must be considered when evaluating a patient’s potential risk. These include clinical factors (history of smoking, hypertension, etc.), as well as other laboratory markers (such as the additional tests on our Lipid Plus™ panel). Small dense LDL cholesterol will be added (to a new panel) beginning this fall.

Q5: What is small dense LDL cholesterol?

A5: Even though LDL particles are (by definition) of “low density”, they are heterogeneous and range in both size and density. The smaller, more dense LDL particles appear to be more dangerous. This may be because they can get into damaged blood vessels more easily, are more easily oxidized, or perhaps because they circulate longer than larger LDL particles. Small dense LDL cholesterol is usually seen in conjunction with elevated triglycerides and low HDL cholesterol but, even if other lipid studies are normal, an elevated level of small dense LDL cholesterol may be an important risk factor. We will be adding this test this fall. Although physicians may continue to order it alone, it will also be part of the expanded cardiac risk panel (Lipid Plus – Assured™).

Q6: How will small dense LDL cholesterol be reported?

A6: There are a number of ways to measure (and report) small dense LDL cholesterol. Our reference laboratories currently use either gradient gel electrophoresis or ultracentrifugation; another uses nuclear magnetic resonance. We will be using polyacrylamide gel electrophoresis. In the report generated by the Stanford Clinical Laboratory (SHC) LIS, we will give the result of the test as either “Type A” (no increase in small dense LDL cholesterol) or “Type B” (increased small dense LDL cholesterol is present) along with an interpretative comment. A recent study (Clinical Chemistry 52:1722-1727, 2006) has shown that different methods for small dense LDL produce different results, both for the “phenotype” (Type A vs. Type B) as well as particle size. We hope to eventually summarize all of the cardiac risk markers in a separate, supplemental report for the ordering physician but, in the meantime, anyone with concerns about how this new method for small dense LDL compares with any previous results obtained should contact me (James Faix, M.D. Director Clinical Chemistry).



Stanford University Medical Center

1-877-717-3733

In association with:

Lucile Packard
Children's Hospital
AT STANFORD



www.stanfordlab.com