

## Advances in the Early Detection of Rupture-prone Plaque

a report by  
**diaDexus**

diaDexus is a private biotechnology company focused on the discovery, development, and commercialization of novel, patent-protected diagnostic and therapeutic products with a high clinical value. The company's lead product, the PLAC<sup>®</sup> test is to aid in predicting the risk of coronary heart disease (CHD). It is the first blood test, passed by the US Food and Drug Administration (FDA), to aid in determining an individual's risk of ischemic stroke associated with atherosclerosis. The PLAC test is an enzyme-linked immunoassay (ELISA) for the quantitative measurement of lipoprotein-associated phospholipase A2 (Lp-PLA2) in blood. Numerous published or presented studies, involving more than 9,000 subjects, highlight Lp-PLA2 as an important risk factor for cardiovascular disease.

Stroke is the third leading cause of death in the US, and a leading cause of severe, long-term disability. Approximately 700,000 Americans will suffer from a stroke this year and 500,000 of those will be first attacks. Nearly 90% strokes are ischemic and of atherosclerotic origin. However, predictive models used in cardiovascular disease, such as the Framingham Risk Score and national prevention guidelines such as the Adult Treatment Panel III (ATP III) have determined all patient risk estimates and treatment recommendations entirely on CHD outcomes and do not include stroke. Unlike CHD event rates that are strongly associated with increasing cholesterol levels, no such association has been made for stroke events and cholesterol levels. The lack of association between stroke and cholesterol levels makes the identification of patients at high risk for stroke challenging, however, numerous pharmacologic agents have been shown to be beneficial in reducing the incidence of primary stroke.

Findings from the National Heart, Lung and Blood Institute's (NHLBI's) Atherosclerosis Risk in Communities (ARIC) study demonstrate a strong, independent correlation between elevated Lp-PLA2 levels and incidence ischemic stroke. A total of 223 stroke events occurred in this group over approximately a six-year follow-up period and, of this, 194 (87%) were ischemic. This proportion of ischemic

stroke is consistent with the percentage found in the general population. The research demonstrated that, unlike cholesterol levels, which did not vary between cases and controls, individuals with high Lp-PLA2 had twice the risk of suffering a stroke compared with individuals with the lowest levels of Lp-PLA2. Further analyses were performed to determine whether Lp-PLA2 was predictive across the complete range of systolic blood pressure values in the population. Findings suggest that Lp-PLA2 and blood pressure are additive in their ability to predict risk for ischemic stroke and that those with the highest levels of Lp-PLA2 and SBP above 130mmHg were at a greater than six-fold increased risk of suffering a stroke.

No consistent correlation exists between cholesterol levels and stroke incidence, however, statin therapy is associated with a significant reduction in stroke events across all cholesterol levels. Reduction in stroke events observed in various statin trials may be a direct result of atherosclerotic disease progression, plaque stabilization, endothelial dysfunction, or some other unexplained mechanism. Levels of Lp-PLA2 are significantly reduced by statin therapy and could be a possible mechanism by which statins reduce stroke incidence.

Research has demonstrated a linkage between inflammation of the arterial plaque and atherosclerotic disease. The mechanism is understood to be a cascade of biochemical events leading to this inflammation process. Lp-PLA2 plays a key role in this process, releasing chemoattractants from the oxidized low density lipoprotein (LDL)-cholesterol into the arterial intima causing attraction of monocytes, which are subsequently converted to macrophage 'foam cells'. This process leads to the creation of vulnerable plaque – also known as high-risk plaque or inflamed plaque – that can expand along the entire vascular bed and upon rupture leads to thrombus generation, blockage, and many of the acute coronary events including myocardial infarction, as well as the cause of most ischemic strokes. Lp-PLA2 circulates in the blood stream bound to LDL, and is readily detected in plasma samples by the PLAC test. Measuring levels of Lp-PLA2 with the PLAC test can help physicians identify this disease process in patients and determine



whether they should be targeted with more aggressive treatment programs to help minimize the risk of plaque rupture.

Clinical studies suggest that the Lp-PLA2 enzyme is an independent risk factor that can predict clinical coronary events. Plasma Lp-PLA2 mass was found to be significantly elevated in patients with angiographically established coronary artery disease when compared with age-matched controls, even though LDL cholesterol was matched between groups. Results from the West of Scotland Coronary Prevention Study (WOSCOPS) demonstrated that plasma Lp-PLA2 was a strong and independent predictor of clinical events not influenced by traditional risk factors or other markers of inflammation. This increase in risk with increased levels of Lp-PLA2 was as strong in a pravastatin-treated arm as it was in the patients who received placebo, suggesting that the risk associated with increased Lp-PLA2 is separate from that associated with elevated levels of LDL. In WOSCOPS, Lp-PLA2 remained a strong independent predictor of events even upon multivariate analysis, in contrast to other inflammatory markers, such as C-reactive protein (CRP) and fibrinogen. These results suggest that Lp-PLA2 operates at a unique position in the pathologic sequence of atherosclerosis as compared with other markers of inflammation.

In a case-cohort analysis from ARIC study in CHD, it was found that patients who had LDL levels below the median (less than 130mg/dl), but had Lp-PLA2 levels greater than 310ng/ml, were roughly twice as likely to suffer a coronary event compared with those in the lowest tertile of Lp-PLA2. These results were maintained even after adjustment for all other risk factors, as in WOSCOPS.

In a sub-study the Monitoring Trends and Determinants in Cardiovascular Diseases (MONICA) examined men with moderately elevated cholesterol levels and found that elevated Lp-PLA2 significantly increased an individual's chances of having a heart attack, regardless of other risk factors. These results combined demonstrate that Lp-PLA2 is an independent risk factor for cardiovascular disease that can detect risk even in those patients with normal to moderate LDL levels.

Lp-PLA2 has been shown to play a pivotal role in the atherosclerotic disease process. Research demonstrates that this enzyme is subject to modification and that reduction of its activity may impact disease progression. Additionally, a growing body of evidence suggests that Lp-PLA2 is an independent marker of cardiovascular disease. The PLAC test, first cleared by the FDA in 2003, was passed a second time by the FDA for the identification of persons at risk for ischemic stroke associated with atherosclerosis in June 2005. The figures of total cholesterol and LDL cholesterol do not aid in the prediction of strokes, and so the PLAC test provides critical information to help physicians better identify those patients at risk for a stroke before it occurs. Stroke, like CHD, is a preventable condition provided that rupture-prone plaque can be identified early. An elevated Lp-PLA2 as reported by the PLAC test should prompt consideration of aggressive therapeutic lifestyle intervention, blood pressure management and lipid lowering treatments—to prevent both heart attack and stroke. The PLAC test is currently available through multiple clinical reference laboratories, including Quest Diagnostics Incorporated®, LabCorp® of America, Mayo Medical Laboratories™ (MML), ARUP® Laboratories, and Berkeley HeartLab, Inc. (BHL). ■