

READER RESPONSE

READER PRODUCT FEATURE

Q We are running the AspirinWorks Test in our laboratory, and our doctors have a couple of questions about the test:

1) How soon should a patient be tested after beginning aspirin therapy?

Answer: An aspirin effect can be detected within 12 hours post-ingestion, however it takes up to 5 days of low-dose aspirin therapy to achieve a maximum reduction in urinary 11 dehydro thromboxane B2 (11dhTXB2) levels.

2) How does the platelet count of an individual on aspirin therapy affect 11dhTXB2 levels?

Answer: A recent study showed that a high platelet count is associated with higher thromboxane production as measured by urinary 11dhTXB2 levels. However, the effect of aspirin on platelet is irreversible and lasts for the lifetime of the platelet. Unless a patient has a high rate of platelet turnover, a daily regimen of aspirin should eventually affect all of the platelets in individuals sensitive to the effects of aspirin.

UPCOMING CONFERENCES

July 29 – 31, 2008: The Annual Meeting of the American Association of Clinical Chemistry (AACC) will be held at the Washington Convention Center in Washington, DC. We invite you to stop by the Corgenix display at booth #3529 during the Clinical Laboratory Exposition for information about our products.

August 13 – 15, 2008: The Mayo Clinic is holding its 12th Coagulation Conference, Bleeding and Thrombosing Diseases: 2008 Mayo Update, at the Kahler Grand Hotel in Rochester, Minnesota. The Conference will include a Wet Workshop on Tuesday, August 12, in addition to the Coagulation Conference on August 13-15. We look forward to meeting you at booth #23 in the exhibit area, where Corgenix representatives will be available to answer your questions on our newest products, the Aspirin-Works™ Test, and the IgG Anti-AtherOx® Test, as well as our other Hemostasis products.

September 10 – 14, 2008: The 16th International Congress on Autoimmunity will convene at the Centro de Congressos e Exposições in Portro, Portugal. Corgenix will co-sponsor and have an active presence at this year's meeting. Please visit our display in the exhibit area to discuss our complete line of antiphospholipid and autoimmune ELISA products. More information about the meeting is available on the website: www.kenes.com/autoimmunity.

REAADS IgG Anti-AtherOx® Test Kit For *In Vitro* Diagnostic Use

| | |
|-------------------------|---|
| Assay format - | 96-well microtiter plate (8 x 12 strips) with breakaway wells |
| Sample matrix - | Human serum |
| Sample dilution - | 1:100 |
| Capture antigen- | oxLDL-β ₂ GPI complex |
| Detection antibody- | Horseradish peroxidase conjugated goat anti-human IgG antibody |
| Chromogenic substrate - | TMB (single component) |
| Stopping solution - | 0.36N sulfuric acid |
| Assay incubations | |
| Sample - | 60 min @ room temperature |
| Conjugate - | 60 min @ room temperature |
| Substrate - | 30 min @ room temperature |
| Wavelength - | 450 nm |
| Assay calibration - | single point or multipoint curve prepared from serum calibrators included in kit. |
| Clinical specificity - | 85% |
| Clinical sensitivity - | 30% in unselected SLE 30% in selected SLE w/ CVD 42.6% in APS 81% in secondary APS |
| Product number - | 11854 |

September 18 – 20: The AAFP (American Academy of Family Physicians) 2008 Scientific Assembly, an annual CME event, will convene in San Diego. Corgenix will feature the AspirinWorks™ Test at booth #4109 during the Exposition, where our representatives will be available to discuss the latest research on aspirin resistance.

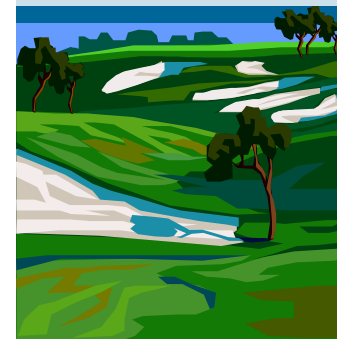
September 24-25, 2008: The Nichols Institute for Coagulation and Quest Diagnostics is sponsoring the Symposium on Bleeding and Thrombosis, a case-oriented seminar for healthcare professionals involved in diagnosis and management of hemostatic and thrombotic disorders. The Symposium will be held at the Hyatt Regency in Reston, VA. We look forward to seeing you at the Exhibitor showcase.

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THE READER

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Anti-IgG OxLDL/β₂GPI (Anti-AtherOx®) Antibodies, a Thrombosis Risk Factor in Autoimmune Patients

The premature development of atherosclerosis in patients with systemic autoimmune diseases such as systemic lupus erythematosus (SLE) has been recognized and well documented over the last 20 years. While the traditional Framingham risk factors, (hypertension, hypercholesterolemia, diabetes mellitus, obesity, smoking, family history and inactive lifestyles), in combination with steroid therapy are contributing factors, they do not fully explain the accelerated atherosclerotic changes in this group. In fact, studies have shown that more SLE patients now die from cardiovascular disease than from their underlying disease or complications such as infections. The incidence of heart attacks in SLE patients is five times higher than in the general population. Symptomatic coronary heart disease is present in 6 to 15% of SLE patients, while 43% of asymptomatic SLE patients showed abnormal myocardial perfusion by Tc99m emission tomography and 33% had increased carotid intima medial thickness (IMT) due to atherosclerotic plaques by B-mode ultrasound. These findings have led researchers to investigate the potential role of autoimmunity in the development of atherosclerosis and cardiovascular disease.

Antiphospholipid syndrome (APS) is an antibody-mediated pro-thrombotic state characterized by the presence of antiphospholipid antibodies, frequently found as a secondary condition in patients with SLE or other systemic autoimmune disease. APS is characterized by the presence of antiphospholipid antibodies such as anti-Cardiolipin (aCL), Lupus Anticoagulant (LA), anti-Phosphatidylserine (aPS), anti-Beta2 glycoprotein I (aβ₂GPI), and anti-Prothrombin (aPT) antibodies, and by thromboembolic complications in the arterial or venous vasculatures or pregnancy morbidity (miscarriage and fetal loss). While venous thrombosis is the most common clinical presentation, arterial manifestations of APS include coronary heart disease, stroke, carotid stenosis and peripheral vascular disease, all with underlying features of atherosclerosis and thrombus formation.

Antiphospholipid antibodies are a heterogeneous family of immunoglobulins. Most of these antibodies do not directly recognize phospholipids but instead recognize phospholipid binding proteins such as β₂GPI and prothrombin. β₂GPI is the most relevant antigenic target for antiphospholipid antibodies clinically associated with thrombosis. It has been shown that anti-β₂GPI antibodies are more specific for arterial thrombosis than aCL antibodies.¹ In addition, recent prospective studies have shown that β₂GPI-dependent aCL or anti-β₂GPI antibodies are strong predictors of myocardial infarction and stroke in men.²

Oxidative stress and oxLDL formation are common in patients with systemic autoimmune disease. It has been reported that antiphospholipid antibody-positive SLE patients have higher urinary isoprostane F_{2a}, a marker of *in vivo* lipid oxidation, and higher plasma levels of prothrombin fragment F₁₊₂, compared to antiphospholipid antibody-negative SLE patients.³ These findings suggest an important relationship between lipid peroxidation and clotting activation (hypercoagulability) in APS patients. Some aCL antibodies cross react with oxidized low-density lipoprotein (oxLDL), indicating that antiphospholipid antibodies may also participate in atherothrombosis. In addition, β₂GPI, oxLDL and immunoglobulins have been localized in atherosclerotic lesions,⁴ suggesting that β₂GPI, oxLDL and anti-β₂GPI antibodies play a pathogenic role in the development of thrombosis, particularly in arterial thrombosis (atherothrombosis).

(continued on page 2)

β 2GPI specifically binds to oxLDL (but not unmodified LDL). OxLDL/ β 2GPI complexes have been characterized, demonstrated in patients with APS and SLE, and implicated as pro-atherothrombotic autoantigens.⁵ It has been hypothesized that under normal conditions, the interaction between oxLDL and β 2GPI may promote oxLDL clearance from circulation. In addition, oxLDL/ β 2GPI complexes are immunogenic; autoantibodies to these complexes are seen in patients with SLE, systemic sclerosis (SSc) and APS.

The physiologic relevance of IgG antibodies to oxLDL/ β 2GPI complexes was demonstrated *in vitro* by the enhanced macrophage uptake of IgG immune complexes containing oxLDL/ β 2GPI. The role of macrophage Fc γ receptors in the uptake of these complexes seems to be particularly important in the development of foam cells, atherosclerotic plaques and arterial thrombosis.⁵⁻⁷ IgG anti-oxLDL/ β 2GPI antibodies in autoimmune patients may further accelerate the development of atherothrombosis.

Corgenix recently received FDA clearance for the IgG Anti-AtherOx® Test Kit, an indirect ELISA for measuring IgG anti-oxLDL/ β 2GPI antibodies. The intended use of the test is to determine IgG antibodies to complexes formed by the interaction of oxidized low-density lipoprotein (oxLDL) with β 2-glycoprotein I (β 2GPI) in human serum.

With the IgG Anti-AtherOx® Test, diluted serum samples, calibrator(s), and controls are incubated in microwells coated with the oxLDL- β 2GPI complex. During incubation, the IgG anti-oxLDL/ β 2GPI antibody present in samples binds to the immobilized antigen complex. The wells are washed to remove unbound serum proteins, and anti-human IgG antibody - horseradish peroxidase (HRP) conjugate is added. The conjugate forms complexes with the bound IgG anti-oxLDL/ β 2GPI antibody. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a chromogenic substrate containing tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂). Color develops in the wells at an intensity proportional to the concentration of IgG anti-oxLDL/ β 2GPI antibody present in the sample. The absorbance of each well is read in a spectrophotometer. Results are calculated against a single calibrator or a reference curve prepared using calibrator sera provided in the kit.

When IgG anti-oxLDL/ β 2GPI antibodies were measured with the IgG Anti-AtherOx™ kit in patients with SLE, systemic sclerosis (SSc), rheumatoid arthritis (RA), and healthy controls, significantly higher levels were seen in SLE and SSc than in healthy controls. RA patients showed higher antibody levels than the controls, but this difference was not statistically significant. IgG anti-oxLDL/ β 2GPI antibodies were significantly higher in SLE patients with APS compared to SLE controls without APS. When evaluated for their association with the major clinical manifestations of APS, a stronger association was seen with arterial thrombosis compared to venous thrombosis or pregnancy morbidity.⁸ Thus, the presence of circulating IgG anti-oxLDL/ β 2GPI antibodies seem to be etiologically important and may be a useful serologic marker for venous and arterial (atherothrombotic) risk in autoimmune patients.

Anti-OxLDL/ β 2GPI References:

1. Lopez LR, Dier KJ, Lopez D, et al. Anti- β 2-glycoprotein I and antiphosphatidylserine antibodies are predictors of arterial thrombosis in patients with antiphospholipid syndrome. *Am J Clin Pathol* 2004;121:42.
2. Vaarala O. Antiphospholipid antibodies and myocardial infarction. *Lupus* 1998;7 Suppl 2: S132-4.

(Refs. continued on pg. 3)

An APS Consensus Conference was held in Milan, Italy, May 24-25, 2008, to discuss recent controversies related to the laboratory and clinical classification criteria for the Antiphospholipid Syndrome (APS) with researchers and representatives from the diagnostic industry. The conference was organized by a group of international experts on APS and hosted by Prof PL Meroni of the Istituto Auxologico Italiano. The participants previously discussed these issues in recent meetings of the European aPL Forum (Ljubjana, November 2007) and the 12th International Antiphospholipid Symposium (Florence, April 2007). A letter to the editor inviting participants to a debate on the serological criteria that define the antiphospholipid syndrome published by M Galli, G Reber, P de Moerloose and PG De Groot (*J Thromb Haemost* 2008; 6:399-401) sparked off the APS Consensus Conference.

The invitation to the conference cited “**whether or not we should continue to use the anticardiolipin solid-phase assay as diagnostic/classification criteria**” as a hot topic of discussion. Two of the issues at the heart of the controversy were: 1. wide acceptance of β 2GPI as one of the most relevant antigenic targets for antiphospholipid antibodies and the better specificity of anti- β 2GPI antibodies for thrombosis and APS versus aCL, and 2. the lack of standardization of aCL testing in spite of multiple past efforts. At the conference, clinical and technical presentations were followed by discussions, which clearly indicated a **majority consensus NOT to modify the current classification criteria for diagnosing APS** (Sapporo 1998/Sydney 2006). In other words, aCL testing should remain in the diagnostic criteria, along with lupus anticoagulant and anti- β 2-GPI testing. As several researchers pointed out, despite the lack of standardization of ANA and dsDNA testing, these assays still play a valuable role in the diagnosis of autoimmune disease.

In addition, by majority consensus it was also proposed **not** to limit the use of aPL antibodies to the diagnosis of APS but instead to also use them as risk/prognostic markers for thrombotic events and/or APS. **The consensus recommendations included the use of at least 3 tests: Lupus anticoagulant, aCL and anti-B2GPI.** It was felt that the antibody titer (higher = increased risk), isotype (IgG, IgM and IgA), and the presence of multiple antiphospholipid antibodies (associated with higher risk) also provides valuable clinical information for therapeutic and/or preventive management. These recommendations open the door to the use of other aPL antibodies (aPS, aPT, etc.), and promote the search for new and better markers. The analogy to the current use of a panel of tests and algorithms in Autoimmune and Coagulation testing for evaluating thrombotic events would also apply to aPL testing.

Finally, to improve the current state of aPL standardization, minimal requirements for ELISA protocols were issued: the use of human B2GPI instead of bovine; testing samples in duplicate; laboratories establishing their own cut-off; expressing the cut-off in percentiles (99thtile); establishing positive interpretive ranges; using external controls, etc. These recommendations and more detailed information on the discussions during the Milan APS Consensus Conference will be published soon for everyone's review.

Anti-OxLDL/ β 2GPI References (continued from pg. 2):

3. Lopez D, Kobayashi K, Merrill JT, et al. Autoantibodies against β 2-glycoprotein I complexed with a lipid ligand derived from oxidized low-density lipoprotein are associated with arterial thrombosis in antiphospholipid syndrome. *Clin Dev Immunol* 2003;10:203-11
4. George J, Harats D, Gilburd B, Afek A, Levy Y, Schneiderman J, et al. Immunolocalization of β 2-glycoprotein I (apolipoprotein H) to human atherosclerotic plaques: potential implications for lesion progression. *Circulation* 1999;99:2227-30.
5. Kobayashi K, Kishi M, Atsumi T, Bertolaccini ML, Makino H, Sakairi N, et al. Circulating oxidized LDL forms complexes with beta-2 glycoprotein I: implication as an atherogenic autoantigen. *J Lipid Res* 2003;44:716-26.
6. Hasunuma Y, Matsuura E, Makita Z, Kaatahira T, Nishi S, Loike T. Involvement of β 2-glycoprotein I and anticardiolipin antibodies in oxidatively modified low-density lipoprotein uptake by macrophages. *Clin Exp Immunol* 1997;107:569-73.
7. Liu Q, Kobayashi K, Furukawa J, et al. Omega-carboxyl variants of 7-ketocholesteryl esters are ligands for β 2-glycoprotein I and mediate antibody-dependent uptake of oxidized LDL by macrophages. *J Lipid Res* 2002;43:1486-95.
8. Lopez LR, Simpson DF, Hurley BL, Matsuura E. OxLDL/ β 2GPI Complexes and Autoantibodies in Patients with Systemic Lupus Erythematosus, Systemic Sclerosis, and Antiphospholipid Syndrome: Pathogenic Implications for Vascular Involvement. *Ann NY Acad Sci* 2005;1051:313-22.