Objective. Beta-2-glycoprotein I (β2GPI) constitutes the major autoantigen in the antiphospholipid syndrome (APS), a common acquired cause of arterial and venous thrombosis. We recently described the novel observation that β2GPI may exist in healthy individuals in a free thiol (biochemically reduced) form. The present study was undertaken to quantify the levels of total, reduced, and posttranslationally modified oxidized β2GPI in APS patients compared to various control groups.

Methods. In a retrospective multicenter analysis, the proportion of β2GPI with free thiols in serum from healthy volunteers was quantified. Assays for measurement of reduced as well as total circulating β2GPI were developed and tested in the following groups: APS (with thrombosis) (n = 139), autoimmune disease with or without persistent antiphospholipid antibodies (aPL) but without APS (n = 188), vascular thrombosis without APS or aPL (n = 38), and healthy volunteers (n = 91).

Results. Total β2GPI was significantly elevated in patients with APS (median 216.2 μg/ml [interquartile range 173.3–263.8]) as compared to healthy subjects (median 178.4 μg/ml [interquartile range 149.4–227.5] [P < 0.0002]) or control patients with autoimmune disease or vascular thrombosis (both P < 0.0001). The proportion of total β2GPI in an oxidized form (i.e., lacking free thiols) was significantly greater in the APS group than in each of the 3 control groups (all P < 0.0001).

Conclusion. This large retrospective multicenter
study shows that posttranslational modification of β₂GPI via thiol-exchange reactions is a highly specific phenomenon in the setting of APS thrombosis. Quantification of posttranslational modifications of β₂GPI in conjunction with standard laboratory tests for APS may offer the potential to more accurately predict the risk of occurrence of a thrombotic event in the setting of APS.

The antiphospholipid syndrome (APS) is an autoimmune condition characterized by vascular thrombosis of the arterial and/or venous systems as well as recurrent miscarriages (1). Beta-2-glycoprotein I (β₂GPI) is the major autoantigen in APS (2). A number of studies have provided robust evidence that autoantibodies to β₂GPI are a significant risk factor for arterial thrombosis in young adults (3,4). In vivo and ex vivo studies by multiple groups have shown anti-β₂GPI autoantibodies to be directly thrombogenic (5).

At present it is not possible to stratify the risk for development of thrombosis in antiphospholipid antibody (aPL)–positive patients based on clinical features or use of currently available laboratory assays (6). The development of novel assays that could be used to stratify future thrombosis risk in patients with APS would hold immense clinical utility in informing the decision as to whether initiation of prophylactic therapy or intensification of therapy is warranted.

Beta-2-glycoprotein I is an evolutionarily conserved 50-kd protein circulating in the blood in relative abundance (~4 μM) (7). The physiologic role of β₂GPI is pleiotropic, with functional studies implicating a role in processes relating to coagulation (8), angiogenesis (9), and clearance of apoptotic cells (10). The crystal structure of β₂GPI, which has been ascertained based on the purified native protein, reveals that it does not possess free thiols (11,12). We have recently shown, however, that in vivo β₂GPI circulates in a free thiol form and that this free thiol form of β₂GPI is involved in the protection of endothelial cells against oxidative stress–induced cell injury (13). Beta-2-glycoprotein I can also participate in redox thiol-exchange reactions by acting as a substrate for oxidoreductase enzymes such as thioredoxin I (14). However, the proportion of β₂GPI circulating in the reduced state is unknown. Also unknown is whether the redox state of this autoantigen differs in patients with pathogenic anti-β₂GPI antibodies and a history of thrombosis.

In the present study we demonstrated that, in serum/plasma derived from healthy subjects, β₂GPI exists in a reduced biochemical state as the dominant molecular phenotype. Detailed in vitro quantitative as-

ELEVATED LEVELS OF OXIDIZED β₂GPI IN ANTIPHOSPHOLIPID SYNDROME

PATIENTS AND METHODS
Patient samples. Samples were collected through an international collaborative multicenter effort involving 5 centers (University of New South Wales [Sydney, Australia], University of Athens [Athens, Greece], University College London [London, UK], Tianjin Medical University [Tianjin, China], and Hokkaido University School of Medicine [Sapporo, Japan]). An APS group, 2 disease control groups, and 1 healthy control group were studied. The disease control groups consisted of an autoimmune disease group (with or without aPL, but with no clinical features of APS) and a clinical event control group (clinical features of APS, but no aPL or autoimmune disease).

APS group. A total of 139 samples from patients with APS were collected and analyzed (24 from Sydney, 38 from Athens, 22 from London, and 55 from Sapporo). Every APS patient fulfilled the revised consensus classification criteria for vascular thrombosis–associated APS (1). All serologic tests for aPL were performed using standard commercially available kits and in accordance with the revised classification criteria. A venous thrombotic event was diagnosed based on a combination of clinical assessment and appropriate imaging with either Doppler ultrasonography or venography to confirm deep venous thrombosis, or isotope ventilation/perfusion scanning or computed tomography (CT) (with or without angiography) to confirm pulmonary embolism. An arterial event was diagnosed based on clinical findings along with one or more of the following: electrocardiographic evidence of myocardial ischemia or infarction, confirmation of infarction by brain CT or magnetic resonance imaging, or confirmation of peripheral vascular disease or arterial thrombosis by Doppler ultrasonography or angiography.

Autoimmune disease control group. Of the 189 autoimmune disease controls, samples from 188 were analyzed (42 from Sydney, 43 from Athens, 29 from London, and 74 from Sapporo). One sample (from a patient with systemic lupus erythematosus [SLE] and no aPL) was found to be deficient in β₂GPI and was withdrawn from the study. Among the autoimmune disease controls, 74 had persistently positive serologic findings for aPL satisfying the serologic component of the APS classification criteria (1), but did not have APS given the lack of a clinical event. All patients with SLE fulfilled the American College of Rheumatology revised classification criteria (15), and those with Sjögren’s syndrome fulfilled the revised European classification criteria (16).

Clinical event control group. Thirty-eight samples from aPL-negative patients with a clinical event were collected and analyzed (26 from Sydney and 12 from Tianjin). Clinical events were diagnosed as described above for the APS group.
Healthy control group. Samples from 93 healthy controls were collected, 92 of which were analyzed (28 from Sydney, 35 from Athens, and 29 from Sapporo). One healthy control sample was found to be deficient in H2GPI by standard enzyme-linked immunosorbent assay (ELISA) and was withdrawn from the study.

Demographic and clinical details of the study groups are summarized in Table 1. Institutional ethics approval for patient sampling was attained from each center participating in the study, and informed consent was obtained from all subjects prior to venipuncture. Assays were performed under blinded conditions with regard to the underlying diagnosis.

Chemicals and reagents. HEPES and streptavidin beads were purchased from Sigma. N-(3-maleimidylpropionyl) biocytin (MPB) was purchased from Invitrogen. All other chemicals were of reagent grade.

Proteins. Bovine serum albumin (BSA), alkaline phosphatase (AP)–conjugated anti-mouse IgG, AP-conjugated antirabbit IgG, and AP-conjugated anti-human IgG were from Sigma. Purified native human β2GPI was from Haematologic Technologies and also sourced as a kind gift from Dr. Inger Schousboe (University of Copenhagen, Denmark). Affinity-purified murine IgG2 anti-β2GPI monoclonal antibody (mAb) 4B2E7 (previously designated “mAb number 16”) and affinity-purified rabbit anti-β2GPI polyclonal antibody were produced as previously described (17,18). Isotype control rabbit polyclonal IgG was purchased from BD PharMingen.

**Assay for quantifying the absolute proportion of serum β2GPI that can be labeled with MPB.** With the demonstration that β2GPI exists in vivo in a reduced state with free thiols (13), it was then pertinent to determine the absolute proportion of total β2GPI that circulates in this reduced state. This was done in experiments with a sample of pooled serum derived from 10 healthy volunteers. The sex and age distribution of the pooled serum sample was chosen to match the APS disease group.

MPB-labeled and non–MPB-labeled serum samples were acetone precipitated to remove free MPB as described previously (13). The protein pellets were then dissolved in phosphate buffered saline (PBS)–0.1% Tween to a final dilution of 4,000-fold (total volume 1,400 μl), and streptavidin beads (50 μl) were added. After incubation with streptavidin beads (1 hour at 4°C), the beads were removed by centrifugation for 2 minutes at 3,000 g and the supernatants assayed for β2GPI. The proportion of β2GPI that was labeled with MPB was calculated as (optical density at 405 nm [OD405] of the biotin-depleted MPB-labeled sample/OD 405 of the biotin-depleted non–MPB-labeled sample) \( \times 100 \). Validation of this method is described in full in the supplementary information (available in the online version of this article at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

**Assay for quantifying total human β2GPI.** A sandwich ELISA for quantifying total β2GPI levels within serum/plasma samples was performed based on a previously published method (19), with modifications. Briefly, a high-binding 96-
well plate was coated overnight at 4°C with rabbit polyclonal anti-human β2GPI (10 nM/well). Plates were washed 4 times with PBS–0.1% Tween and then blocked with 2% BSA/PBS–0.1% Tween for 1 hour at room temperature. Following washing, 100 μl of anti-human β2GPI mouse mAb (clone 4B2E7) was added (10 nM/well, diluted in 0.25% BSA/PBS–0.1% Tween) and then 100 μl of the patient sample diluted 4,000-fold in PBS–0.1% Tween was incubated for 1 hour at room temperature. After washing 4 times with PBS–0.1% Tween, AP-conjugated goat anti-mouse IgG (1:1,500 dilution) and incubated for 1 hour at room temperature, and samples read at OD405 after addition of chromogenic substrate. An in-house standard, consisting of pooled serum from 10 healthy controls, was used to construct a standard curve for every ELISA. The level of β2GPI in the pooled-serum in-house standard was determined initially using a β2GPI in-house standard curve and then validated with a calibrator from a commercially available β2GPI quantification kit (Hyphen BioMed). Each new batch of the pooled-serum in-house standard was recalibrated against the commercial calibrator. Samples were assayed in duplicate.

Within-plate coefficients of variation (CVs) for this ELISA were calculated by running 10 duplicates of the same patient sample on a single plate. Between-plate CVs were calculated by taking 10 independent assays performed consecutively on separate days and calculating the CV based on the variation of the number obtained by dividing the OD of the standard at 4,000-fold dilution by the OD of the standard at 8,000-fold dilution for each plate.

**Assay for measuring the relative amount of β2GPI with free thiols within patient samples as compared to a pooled-serum in-house standard sample.** The amount of β2GPI with free thiols in patient samples relative to the standard sample was assayed as previously described (13), with minor modifications. Measurement of the amount of β2GPI that is reduced is based on labeling of free thiols of β2GPI with the biotin-conjugated selective free thiol binding reagent MPB, capturing biotin-labeled proteins on a streptavidin plate, and detecting the presence of MPB-labeled β2GPI with a specific anti-β2GPI mAb. The mean ± SD within-plate CV for this ELISA is 5.08 ± 3.09%, and the between-plate CV is 6.25% (13).

MPB (4 mM) was added to 50 μl of patient plasma or serum and incubated for 30 minutes at room temperature in the dark with agitation, diluted 50-fold in 20 mM HEPES buffer (pH 7.4), and incubated for a further 10 minutes at room temperature in the dark. Unbound MPB was then removed by acetone precipitation. The protein pellet was resuspended in PBS–0.05% Tween (final dilution 100-fold). The samples were then diluted a further 40-fold (4,000 times final), added in duplicate to a streptavidin-coated 96-well plate (100 μl/well; Nunc), and incubated for 90 minutes at room temperature. Prior to addition of MPB-labeled serum samples, streptavidin-coated plates were washed 3 times with PBS–0.1% Tween and blocked with 2% BSA/PBS–0.1% Tween. After washing 3 times with PBS–0.1% Tween, the murine anti-β2GPI mAb (clone 4B2E7) was added (25 nM) and incubated for 1 hour at room temperature. After 3 further washings with PBS–0.1% Tween, AP-conjugated goat anti-mouse IgG (1:1,500 dilution) was added for 1 hour at room temperature and samples read at 405 nm after addition of chromogenic substrate. For each experiment, the pooled in-house standard used for the above-described β2GPI quantification ELISA was MPB labeled, acetone precipitated, and used as an internal control and standard. The degree of MPB labeling in each patient sample was expressed as a percentage of that observed with the pooled in-house standard, after correction for the total amount of β2GPI. The proportion of non–MPB-labeled β2GPI represents the oxidized form of the molecule.

**Statistical analysis.** Box plots were created to depict the distributions of β2GPI across groups. Medians and interquartile ranges (IQRs) were calculated. For comparisons between individual samples, the Mann-Whitney U test was used. Odds-ratios (ORs) and 95% confidence intervals (95% CIs) of exposure or disease incidence were computed using logistic regression. Adjustment for age and sex was carried out to remove potential confounders linked to these predictors.

**RESULTS**

A significant proportion of β2GPI in vivo in healthy volunteers circulates in the reduced form. We have recently demonstrated that β2GPI circulates in vivo in a reduced form (13), and we therefore wished to determine the absolute proportion of β2GPI that is in this biochemically reduced state. This was investigated using a sample of human serum pooled from 10 healthy volunteers. Figure 1 shows that a mean of 45.6% of β2GPI in pooled serum from healthy subjects was labeled with the biotin-conjugated free thiol binding reagent MPB. Validation of this method is demonstrated in detail in Supplementary Figure 1, available in the online version of this article at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131.

Total β2GPI levels are elevated in APS and are associated with thrombogenic pathogenicity in aPL-positive patients. Given that biochemically reduced β2GPI was found to represent a large proportion of circulating β2GPI in healthy subjects, it was then relevant to ascertain whether this level was altered in patients with APS as compared to both disease control and healthy control groups. Serum or plasma levels of total β2GPI were quantified in each individual patient sample so that a relative proportion of reduced and oxidized β2GPI could be calculated for each sample.

The assay used for detecting total levels of β2GPI in patient serum and plasma was optimized for use with in-house anti-β2GPI antibodies, as shown in Supplementary Figure 2 (http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131). The within-plate CV for this assay was 5.8% and the between-plate CV was 3.3%, indicating good reproducibility.

The median level of total β2GPI in the healthy control group was 178.4 μg/ml (IQR 149.4–227.5) (n = 91). In addition to healthy controls, an autoimmune
disease control group (autoimmune disease with or without aPL but without APS) and a clinical event control group (thrombosis without aPL) were included, as described above. As shown in Figure 2A, the concentration of total $\beta_2$GPI was significantly higher in the APS group (median 216.2 ng/ml [IQR 173.3–263.8]) ($n = 139$) as compared to the healthy control group ($P < 0.0002$), the autoimmune disease control group ($P < 0.0001$), and the clinical event control group ($P < 0.0001$). Compared to healthy controls, cases were twice as likely to have an elevated $\beta_2$GPI level (defined as plasma levels ≥200 ng/ml). The effect remained after adjustment for age and sex (OR 2.2 [95% CI 1.2–3.9]). Given that the odds ratios of disease and of exposure can be considered the same, this translates to a 2-fold increase in thrombosis for patients with elevated $\beta_2$GPI.

Figure 1. Beta-2-glycoprotein I ($\beta_2$GPI) with free thiols represents a large proportion of total circulating $\beta_2$GPI in vivo. Pooled serum from 10 healthy volunteers was labeled with N-(3-maleimidylpropionyl) biocytin (MPB) (4 mM) or treated with control buffer alone, after which the MPB-labeled proteins were depleted by incubation with streptavidin beads. Both samples were then centrifuged at 3,000g for 10 minutes to remove the beads, and an enzyme-linked immunosorbent assay for total $\beta_2$GPI was performed on the supernatant of both MPB-labeled and non–MPB-labeled samples post-streptavidin incubation. The relative reduction (in optical density) of the MPB-labeled sample as compared to the non–MPB-labeled sample indicates the relative amount of $\beta_2$GPI with free thiols labeled with MPB. Values are the mean ± SD.

Figure 2. Elevated levels of $\beta_2$-glycoprotein I ($\beta_2$GPI) in patients with the antiphospholipid syndrome (APS). A, Total $\beta_2$GPI in the serum of patients with thrombosis-associated APS and in the serum of patients in the 3 control groups, i.e., healthy controls, patients with autoimmune disease (AID) with or without antiphospholipid antibodies (aPL) but without APS, and patients with clinical thrombotic events without APS. B, Total $\beta_2$GPI in the serum of patients in the APS group who had an autoimmune disease compared to patients in the autoimmune disease control group who were positive for aPL and patients in the autoimmune disease control group who were negative for aPL. Elevated levels of $\beta_2$GPI were demonstrated only when aPL positivity was combined with a thrombotic clinical event. Data are presented as box plots, where the boxes represent the 25th to 75th percentiles, the lines within the boxes represent the median, and the lines outside the boxes represent the 10th and 90th percentiles. Circles indicate outliers.
levels, in the absence of further confounding effects. The association was stronger when the comparison was with the control group consisting of patients with autoimmune disease with or without aPL (OR 4.6 [95% CI 2.9–7.5]). It is also possible to treat total β2GPI as a continuous variable in the model. When this was done, the results were consistent with the other findings (i.e., there was a strong positive association between total β2GPI level and thrombosis risk).

Figure 2B shows that elevated β2GPI levels were observed only when persistent aPL positivity was combined with a thrombotic event, thus fulfilling classification criteria for APS. Levels of β2GPI in the autoimmune disease controls (without thrombotic events) with persistent aPL did not differ from levels in autoimmune disease controls without aPL, and also were not different from levels in healthy controls.

Subgroup analysis of the total level of β2GPI within the APS group revealed no differences between those with and those without an additional autoimmune disease. Furthermore, there was no difference between those with arterial thrombosis and those with venous thrombosis (Supplementary Figure 3, http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131).

**APS is associated with a greater proportion of β2GPI being in an oxidized state.** Each patient sample was labeled with MPB, and the amount of β2GPI in the reduced form was compared and expressed as a percentage of that observed in a pooled standard (derived from 10 healthy volunteers who were matched for age and sex with the APS group), after correction for the total amount of β2GPI. The same in-house pooled standard was used for every MPB labeling experiment and assay. The sensitivity for detecting reduced β2GPI with this assay extends to a dilution of 1:128,000-fold, indicating marked sensitivity (Figure 3). The linear range was found to be between dilutions of 400- and 128,000-fold. The dilution found to yield ~50% of maximum OD was found to be 1:4,000, and hence this dilution was used to screen all patient samples for reduced β2GPI. This assay has previously been shown to yield identical results when serum and plasma sampled from the same patient are tested in parallel (13).

Figure 4 shows that the relative proportion of β2GPI in the reduced form, expressed as a percentage of that observed with the in-house standard, was significantly less in APS patients presenting with vascular thrombosis as compared to healthy controls, autoimmune disease controls, and clinical event controls (all $P < 0.0001$). Thus, β2GPI in APS patients presenting with thrombosis is in an oxidized state relative to each of the other 3 control groups. Similar to the findings in the analysis of total β2GPI, a lower level of the reduced β2GPI (proportion £50%) was associated with a greater risk of thrombosis. An OR of 4.1 (95% CI 1.9–8.8) in relation to healthy subjects was observed after adjustment for age and sex. A similar but somewhat smaller effect (OR 2.0 [95% CI 1.2–3.4]) was also obtained when the reference group was patients with autoimmune disease with or without aPL but without thrombosis.

Patient positivity for lupus anticoagulant (LAC) activity has been reported to be a strong predictor of thrombosis compared to anti-β2GPI or anticardiolipin antibodies without LAC activity, particularly with regard to arterial thrombosis and the development of stroke (4,20). Subgroup analysis of the various aPL subtypes within the APS group revealed that the proportion of β2GPI circulating in the reduced state was significantly lower in the APS patients who were positive for both anti-β2GPI and LAC as compared to those positive for anti-β2GPI but not LAC (median 53.58% [IQR 39.18–73.56] [n = 45] versus 74.80% [IQR 60.69–84.51] [n =
Interestingly, levels of $\beta_2$GPI were also lower in APS patients presenting with arterial thrombosis only (median 53.81% [IQR 39.38–74.62] [n = 67]) versus those presenting with venous thrombosis only (62.09% [IQR 49.64–83.11] [n = 59]) ($P < 0.045$), as shown in Supplementary Figure 4, http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1529-0131.

DISCUSSION

This is, to our knowledge, the first reported demonstration that the redox state of the autoantigen $\beta_2$GPI, in conjunction with plasma concentration levels, is different in APS patients compared to healthy or disease control subjects. Our study is the first to definitively confirm that $\beta_2$GPI levels are elevated in APS patients—both those with and those without an additional autoimmune disease—as compared to healthy and disease control groups. The finding of elevated levels of $\beta_2$GPI was observed by our group previously, albeit utilizing far lower numbers of patients (19). In addition, it is reported herein that levels of oxidized $\beta_2$GPI are elevated in APS patients compared to healthy and disease controls. A novel assay to measure relative amounts of reduced $\beta_2$GPI, as well as the ELISA for total $\beta_2$GPI, had good reproducibility and demonstrated strong associations with the APS disease phenotype. The robust nature of these findings is highlighted by the large numbers of well-characterized patients (>450) screened through this large international collaborative multicenter effort coupled with the use of both healthy and 2 distinct disease control groups. Such assays that precisely quantify the amount of posttranslationally modified autoantigen are unique in the field of APS, and even autoimmunity.

An extensive number of in vitro and in vivo studies suggest that anti-$\beta_2$GPI autoantibodies in complex with $\beta_2$GPI directly contribute to the APS clinical phenotype of thrombosis (5). In the present study, we...
have demonstrated that patients who are persistently positive for aPL and have the clinical features of APS have higher levels of total and oxidized β2GPI compared to controls. It is reasonable to hypothesize that clinical states associated with an increased oxidative stress load, such as pregnancy and infection (21), may lead to further increases in the levels of oxidized β2GPI in the plasma, potentially elevating the risk of pathologic thrombosis in patients who are positive for anti-β2GPI antibodies. This is based on the premise that an increased plasma load of oxidized β2GPI may lower the threshold for provoking an anti-β2GPI autoantibody-mediated dysregulated prothrombotic response. A recent study demonstrated that oxidative stress may drive β2GPI production in vivo through activator protein 1 and NF-kB–mediated up-regulation of β2GPI gene promoter activity (22). Hence, an enhanced oxidative stress load may increase antigenic load, potentially driving anti-β2GPI production in autoimmunity-prone subjects and lowering the threshold for a clinical event. This hypothesis supports a rationale as to why SLE in particular is associated with anti-β2GPI antibodies, given that this condition is characterized by a propensity toward autoreactivity, B cell hyperactivity, and oxidative stress (23,24).

It was recently shown that β2GPI with free thiols protects endothelial cells against oxidative stress–induced cell injury, whereas oxidized β2GPI (which lacks free thiols) has no such protective effect (13). Given the present finding that a significant proportion of circulating β2GPI is in this protective reduced form in healthy individuals, it may be reasonable to hypothesize that the relative abundance of oxidized β2GPI in APS lowers the threshold for development of vascular thrombosis. If this hypothesis is correct, then one would expect elevated levels of oxidized β2GPI to represent an independent risk factor for thrombosis. Analysis of posttranslational modifications of β2GPI on patient samples collected prospectively and subsequent determination of the presence or absence of a thrombotic event would allow for predictive calculations that could be used to test such a hypothesis.

With the development of novel assays to detect and quantify plasma β2GPI–related redox changes, it is expected that stratification of anti-β2GPI antibody–positive individuals for thrombotic risk according to the levels of total, reduced, and oxidized β2GPI may be possible, with the attendant potential opportunity for implementing medical prophylactic measures during these periods of elevated risk. Prospective longitudinal studies aimed at validating the predictive and diagnostic role of such an approach are needed.

ACKNOWLEDGMENTS

We would like to thank Dr. Inger Schousboe (University of Copenhagen, Copenhagen, Denmark) for kindly donating purified native β2GPI. Also thanks to Professor Marissa Lassere (St. George Hospital, University of New South Wales) for initial advice regarding statistical analyses.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Krilis had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Ioannou, Zhang, Lau, Vlachoyiannopoulos, Atsumi, Giannakopoulos, Krilis.

Acquisition of data. Ioannou, Zhang, M. Qi, Gao, J. C. Qi, Lau, Sturgess, Vlachoyiannopoulos, Moutsopoulos, Rahman, Pericleous, Atsumi, Giannakopoulos, Krilis.

Analysis and interpretation of data. Ioannou, Zhang, M. Qi, Gao, J. C. Qi, Yu, Lau, Vlachoyiannopoulos, Moutsopoulos, Rahman, Atsumi, Koike, Heritier, Giannakopoulos, Krilis.

REFERENCES

1. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, et al. International consensus statement on the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006;4:295–306.

2. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci U S A 1990;87:4120–4.

3. Meroni PL, Peyvandi F, Foco L, Bernardinelli L, Fettiveau R, Mannucci PM, et al. Anti-β2-glycoprotein I antibodies and the risk of myocardial infarction in young premenopausal women. J Thromb Haemost 2007;5:2421–8.

4. Urbanus RT, Siegerink B, Roest M, Rosendaal FR, de Groot PG, Algra A. Antiphospholipid antibodies and risk of myocardial infarction and ischaemic stroke in young women in the RATIO study: a case-control study. Lancet Neurol 2009;8:998–1005.

5. Giannakopoulos B, Passam F, Rahgozar S, Krilis SA. Current concepts on the pathogenesis of the antiphospholipid syndrome. Blood 2007;109:22–30.

6. Cervera R, Khamashia MA, Shoenfeld Y, Camps MT, Jacobsen S, Kiss E, et al. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicentre prospective study of 1000 patients. Ann Rheum Dis 2009;68:1428–32.

7. Miyakis S, Giannakopoulos B, Krilis SA, β2 glycoprotein I—function in health and disease. Thromb Res 2004;114:335–46.

8. Shi T, Iverson GM, Qi JC, Cockerill KA, Linnik MD, Koncny P, et al. β2-glycoprotein I binds factor XI and inhibits its activation by thrombin and factor XIIa: loss of inhibition by clipped β2-glycoprotein I. Proc Natl Acad Sci U S A 2004;101:3939–44.

9. Yu P, Passam FH, Yu DM, Denyer G, Krilis S. β2-glycoprotein I inhibits vascular endothelial growth factor and basic fibroblast growth factor induced angiogenesis through its amino terminal domain. J Thromb Haemost 2008;6:1215–23.

10. Maiti SN, Balasubramanian K, Ramoth JA, Schroit AJ. β2-glycoprotein I-dependent macrophage uptake of apoptotic cells: binding to lipoprotein receptor-related protein receptor family members. J Biol Chem 2008;283:3761–6.

11. Bouma B, de Groot PG, van den Elsen JM, Ravelli RB, Schouten...
A, Simmelink MJ, et al. Adhesion mechanism of human β2-glycoprotein I to phospholipids based on its crystal structure. EMBO J 1999;18:5166–74.

12. Schwarzenbacher R, Zeth K, Diederichs K, Gries A, Kostner GM, Laggner P, et al. Crystal structure of human β2-glycoprotein I: implications for phospholipid binding and the antiphospholipid syndrome. EMBO J 1999;18:6228–39.

13. Ioannou Y, Zhang JY, Passam FH, Rahgozar S, Qi JC, Giannakopoulos B, et al. Naturally occurring free thiols within β2-glycoprotein I in vivo: nitrosylation, redox modification by endothelial cells and regulation of oxidative stress induced cell injury. Blood 2010;116:1961–70.

14. Passam FH, Rahgozar S, Qi M, Raftery MJ, Wong J, Tanaka K, et al. β2 glycoprotein I is a substrate of thiol oxidoreductases. Blood 2010;116:1995–7.

15. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

16. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carson SE, et al, and the European Study Group on Classification criteria for Sjogren’s Syndrome. Classification criteria for Sjogren’s syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554–8.

17. Sheng Y, Hanly JG, Reddel SW, Kouts S, Guerin J, Koike T, et al. Detection of ‘antiphospholipid’ antibodies: a single chromogenic assay of thrombin generation sensitively detects lupus anticoagulants, anticardiolipin antibodies, plus antibodies binding β2-glycoprotein I and prothrombin. Clin Exp Immunol 2001;124:502–8.

18. Kouts S, Wang MX, Adelstein S, Krilis SA. Immunization of a rabbit with β2-glycoprotein I induces charge-dependent crossreactive antibodies that bind anionic phospholipids and have similar reactivity as autoimmune anti-phospholipid antibodies. J Immunol 1995;155:958–66.

19. Vlachoyiannopoulos PG, Krilis SA, Hunte JE, Manousakis MN, Moutsopoulos HM. Patients with anticardiolipin antibodies with and without antiphospholipid syndrome: their clinical features and β2-glycoprotein-I plasma levels. Eur J Clin Invest 1992;22:482–7.

20. De Laat HB, Derksen RH, Urbanus RT, Roest M, de Groot PG. β2-glycoprotein I-dependent lupus anticoagulant highly correlates with thrombosis in the antiphospholipid syndrome. Blood 2004;104:3598–602.

21. Morris JM, Gopaul NK, Endresen MJ, Knight M, Linton EA, Dhir S, et al. Circulating markers of oxidative stress are raised in normal pregnancy and pre-eclampsia. Br J Obstet Gynaecol 1998;105:1195–9.

22. Chiu WC, Chen CI, Lee TS, Chen ZJ, Ke PH, Chiang AN. Oxidative stress enhances AP-1 and NF-κB-mediated regulation of β2-glycoprotein I gene expression in hepatoma cells. J Cell Biochem 2010;111:988–98.

23. Rahman A, Isenberg DA. Systemic lupus erythematosus. N Engl J Med 2008;358:929–39.

24. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. Arthritis Rheum 2010;62:2064–72.