Published in final edited form as:

J Reprod Immunol. 2010 June ; 85(2): 180–185. doi:10.1016/j.jri.2010.03.007.

Antiphospholipid antibodies and pregnancy outcomes in women heterozygous for Factor V Leiden

Tracy Manuck, M.D.^a, D. Ware Branch, MD^a, Yinglei Lai, Ph.D.^b, Baha Sibai, M.D.^c, Catherine Y. Spong, M.D.^d, George Wendel Jr., M.D.^e, Katharine Wenstrom, M.D.^f, Philip Samuels, M.D.^g, Steve N. Caritis, M.D.^h, Yoram Sorokin, M.D.ⁱ, Menachem Miodovnik, M.D.^j, Mary J. O'Sullivan, M.D.^k, Deborah Conway, M.D.^l, and Ronald J. Wapner, M.D.^m for the *Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network*

^aDepartment of Obstetrics and Gynecology at the University of Utah, 30N 1900E room 2B200, Salt Lake City, UT 84132

^bThe George Washington University Biostatistics Center, 6110 Executive Blvd, Suite 750, Rockville MD 20852

^cDepartment of Obstetrics and Gynecology at the University of Cincinnati, 231 Albert Sabin Way, Cincinnati, OH 45267

^dEunice Kennedy Shriver National Institute of Child Health and Human Development, 6100 Executive Blvd, MSC 7510, Rockville, MD 20852

^eDepartment of Obstetrics and Gynecology at the University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75235

^fDepartment of Obstetrics and Gynecology at the University of Alabama at Birmingham, 619 19th St. S, OHB 458, Birmingham, AL 35249

^gDepartment of Obstetrics and Gynecology at the The Ohio State University, 395 West 12th Ave, room 516, Columbus, OH 43210

^hDepartment of Obstetrics and Gynecology at the University of Pittsburgh, 300 Halket Street room 15213, Pittsburgh, PA 15213

ⁱDepartment of Obstetrics and Gynecology at the Wayne State University, 3990 John R., 7 Brush North, Mail Stop 163, Detroit, MI 48201

^jDepartment of Obstetrics and Gynecology at the University of Tennessee, 853 Jefferson Ave, Room E102, Memphis, TN 38103

^kDepartment of Obstetrics and Gynecology at the University of Miami, Dominion Tower, 10th floor, 1400 NW 10th Ave, Miami, FL 33136

© 2010 Elsevier Ireland Ltd. All rights reserved.

Correspondence & Reprints: Tracy Manuck, M.D., University of Utah Health Sciences Center, Department of Obstetrics & Gynecology, 30 North 1900 East, Room 2B200, Salt Lake City, UT 84132, Work phone: 801.581.7260, FAX: 801.585.2594, tracy.manuck@hsc.utah.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

This work was presented in part at the 29th Annual Society for Maternal-Fetal Medicine Meeting, San Diego, CA, January 26–31, 2009.

^lDepartment of Obstetrics and Gynecology at the University of Texas at San Antonio, MSC 7836, 7703 Floyd Curl Drive, San Antonio, TX 78229

^mDepartment of Obstetrics and Gynecology at the Thomas Jefferson University, Philadelphia, PA

Abstract

Antiphospholipid antibodies are associated with a spectrum of pregnancy complications, including preeclampsia and small for gestational age (SGA) fetuses. We sought to assess anticardiolipin and anti- β_2 -glycoprotein I (anti- β_2 -GPI) IgG and IgM antibody prevalence and the relationship of these antibodies to pregnancy complications in women with the Factor V Leiden (FVL) mutation. The study comprised a secondary analysis of a multicenter, prospective observational study of FVL prevalence among 5,188 asymptomatic pregnant women. A subset of 362 women (117 FVL heterozygotes, 245 matched controls) had serum collected at the time of the original study and underwent serum analysis for anticardiolipin and anti- β_2 -GPI IgG and IgM as a part of this analysis. The primary outcome was preeclampsia and/or SGA (<10%). The overall prevalence of anticardiolipin and anti- β_2 -GPI IgG and IgM antibodies was low and did not vary with FVL status. Forty-seven women (13.0%) developed preeclampsia and/or SGA. There were no differences in primary outcome rates between women with and without aPL antibodies, regardless of FVL mutation status. Among FVL carriers, the presence of antiphospholipid antibodies does not appear to contribute to adverse pregnancy outcome.

Keywords

Antiphospholipid antibodies; Factor V Leiden; preeclampsia; small for gestational age

Introduction¹

Antiphospholipid (aPL) antibodies have been previously associated with a spectrum of pregnancy complications including recurrent spontaneous miscarriage, placental insufficiency, venous thromboembolism, preeclampsia, small for gestational age (SGA), and fetal demise (Branch 2004, Lim et al. 2006, Lynch et al. 1999). These complications are common among gravidas with aPL antibodies, but they do not occur in all women.

Antiphospholipid antibodies include lupus anticoagulant, anticardiolipin, and anti- β_2 glycoprotein I (β_2 GPI) antibodies. The prevalence of aPL antibodies among women of childbearing age in the United States is estimated to be between 0.3–9.1% (Lockwood et al. 1989, Tsapanos et al. 2000, Vila et al. 1994). However, among women with pregnancy complications, particularly adverse outcomes that may be associated with placental insufficiency, the incidence may be even higher. For example, anticardiolipin antibodies have been found in as many as 30% of pregnancies complicated by preeclampsia, though not all studies are in agreement (Branch et al. 1989, Lee et al. 2003).

The mechanisms by which some women have adverse pregnancy outcomes in the presence of these antibodies, while others do not, is unknown. One possibility is that there is an

ABBREVIATIONS used in manuscript (alphabetical order):

anticardiolipin = Anticardiolipin antibodies

aPL = Antiphospholipid

β_2 GPI = β_2 Glycoprotein I

FVL = Factor V Leiden

LAC = Lupus anti-coagulant

preeclampsia = pre-eclampsia

SGA = small for gestational age

interaction between aPL antibodies and other predisposing factors and the combination may increase the overall risk. One such predisposition might be the Factor V Leiden mutation (FVL), a factor known to be associated with venous thrombosis (Crowther and Kelton 2003, Simini et al. 2006) that is carried by approximately 2% of the general United States population (Dizon-Townson et al. 2005).

Pregnancy outcomes in the setting of both aPL antibodies (anticardiolipin IgG and IgM & anti- β_2 GPI IgG and IgM) and the FVL mutation have not previously been examined. Thus, the aims of this study were: (1) to determine the frequency of anticardiolipin and anti- β_2 GPI antibodies among a group of asymptomatic pregnant women with and without the FVL mutation, (2) to determine if rates are higher among women heterozygous for the FVL mutation, (3) to identify the proportion of women who experienced preeclampsia and/or SGA based on anticardiolipin and anti- β_2 GPI antibody status, and (4) to quantify whether there is increased risk of obstetric complications among women with both anticardiolipin or anti- β_2 GPI IgG and IgM antibodies and the FVL mutation. We hypothesize that adverse pregnancy outcomes, particularly those associated with placental insufficiency (preeclampsia and/or SGA), occur at a higher rate in women with multiple factors known to be associated with defects in coagulation – the FVL mutation and anticardiolipin and anti- β_2 GPI IgG and IgM antibodies.

Materials & Methods

This is a secondary analysis of a subset of 5,188 women enrolled from April 2000 to August 2001 in a prospective, observational, multicenter study conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Maternal-Fetal Medicine Units (MFMU) Network as previously described (Dizon-Townson et al. 2005). Briefly, the purpose of the original study was to determine the rate of thromboembolic events among a group of gravidas with no previous history of thromboembolism, and to relate these complications to carriage of the FVL mutation. Women with a singleton pregnancy less than or equal to 14 weeks gestation by best obstetrical estimate were offered enrollment. Patients receiving (or planning to receive) anticoagulation therapy, those with a diagnosis of antiphospholipid syndrome, and those with known FVL status were excluded from the original study.

Institutional Review Board (IRB) approval and subject consent for the original study, as well as future analyses such as this study, were obtained at each of the 13 participating Network sites by trained research nurses as previously described (Dizon-Townson et al. 2005). After local IRB review, this analysis was determined to be exempt from IRB approval procedures secondary to de-identification of data and study samples.

As a part of the original study, 4,885 women had a venous blood sample collected and submitted to a central laboratory (DNA Diagnostic Laboratory, University of Utah), where analysis for the presence of the FVL mutation was performed as previously described (Dizon-Townson et al. 2005). One-hundred-thirty-four of 4,885 women (2.7%) were identified as FVL carriers; 122 of these women subsequently had an additional serum sample collected at the time of the original study. For purposes of comparison, 258 control women who were FVL mutation negative [matched 2:1 with cases for maternal age ($\pm$ 5 years), clinical center, and race/ethnicity] also provided an additional serum sample during the original study. All specimens were collected during pregnancy.

Enrollment in the current study was limited to the subset of case and control women with a stored serum sample from the original trial. This included 117 FVL heterozygotes and 245 FVL-negative controls. Serum samples were analyzed at the Branch Perinatal Laboratory

(Salt Lake City, UT). Samples were originally labeled with unique, de-identified study barcodes and were frozen at -20 degrees Celsius prior to this assay in July 2008. Commercially available kits were used for analysis, including QUANTA Lite™ β_2 GPI IgG and QUANTA Lite™ β_2 GPI IgM for anti- β_2 GPI IgG and IgM respectively, and QUANTA Lite™ ACA IgG III and QUANTA Lite™ ACA IgM III for anticardiolipin IgG and IgM respectively (all INOVA Diagnostics, Inc., San Diego, CA).

The anti- β_2 GPI IgG and IgM antibody kits had purified β_2 GPI antigen bound to the wells of a microtiter plate. Assays were performed according to the manufacturer's instructions. In short, pre-diluted control and subject samples (100 μ L each) were added to duplicate wells, allowing anti- β_2 GPI IgG antibodies to bind to the plated antigen. Unbound sample was washed away, and enzyme labeled anti-human IgG or anti-human IgM conjugate was added to each well. After incubation, unbound enzyme-labeled antibody was again washed away. The remaining enzyme activity was then measured by adding a chromogenic substrate to each well and measuring the intensity of the color spectrophotometrically. Color intensities were compared to a five point calibration curve; results were reported semi-quantitatively in standard anti- β_2 GPI units. Similar procedures were undertaken for anticardiolipin IgG and IgM assays using the appropriate reagents. All assays were performed by the same laboratory doctorate physician-researcher. FVL cases and matched controls were distributed randomly among the experimental samples on each assay plate.

For anti- β_2 GPI IgG and IgM, negative values are 0–20 standard IgG units or standard IgM units, respectively and positive results are greater than 20 standard IgG units or standard IgM units; for the purposes of this analysis, we also used these cutoffs to define “negative” and “positive” antibody status. The relative concordance of QUANTA Lite™ anti- β_2 GPI IgM (compared side by side with an IgM anticardiolipin test) is 83.3%. For QUANTA Lite™ anti- β_2 GPI IgG (compared side by side with an IgG anticardiolipin test), the relative concordance is 20.8% (INOVA Diagnostics, Inc., San Diego, CA).

For anticardiolipin IgG and IgM, results are reported out semi-quantitatively using international units (GPL or MPL); negative values are less than 20 units, low-to-medium positive values range from 20–80 units, and high positive values are greater than 80 units. Results are highly sensitive and specific; for QUANTA Lite™ ACA IgG, 96.6% and 98.7%, and for QUANTA Lite™ ACA IgM, 94.0% and 97.8%, respectively (INOVA Diagnostics). For the purposes of this analysis, we defined “negative” as less than 20 units, and “positive” as greater than or equal to 20 units.

Lupus anticoagulant assays were performed as a part of the original study (Dizon-Townson et al. 2005). Five patients of the 362 included in this secondary analysis were positive for lupus anticoagulant. Of these five, 4 were FVL negative and 1 was a FVL heterozygote. Clinical outcome data were previously abstracted from medical records at each institution. A diagnosis of preeclampsia was made if the diastolic blood pressure measured greater than or equal to 90 mmHg on 2 occasions 4 hours to 14 days apart, occurring within 4 hours to 14 days of significant proteinuria (greater than or equal to 300 mg protein/24 hours, urine protein:creatinine ratio greater than or equal to 0.35, at least 2+ proteinuria from a single dipstick evaluation or 1+ proteinuria from 2 or more measurements obtained 4 hours to 14 days apart) in previously normotensive, nonproteinuric patients. SGA was defined as birth weight less than the 10th percentile derived from gender and race specific growth curves (Battaglia and Lubchenco 1967, Jahn et al. 1998).

The primary outcome was a composite of preeclampsia and/or SGA. Women negative for anticardiolipin and anti- β_2 GPI antibodies, as defined above, were then compared with those

testing positive; patients were further stratified by FVL status. Relative risks of the composite adverse outcome were calculated.

Data were analyzed at the Biostatistics Center at George Washington University, Washington, D.C. using SAS (SAS Institute Inc., Cary, NC). Univariate analyses were performed using Chi-square and Fisher's exact test where appropriate. Statistical significance was set at $p < 0.05$. No adjustments were made for multiple comparisons.

Results

A total of 380 women in the initial MFMU Network study had serum samples collected. Of these, 362 were available with complete outcome data and successfully analyzed, including 245 patients negative for FVL and 117 FVL heterozygotes. There were no FVL homozygotes. Women with and without the FVL mutation did not vary by pre-pregnancy body mass index, age, race, incidence of diabetes or hypertension, or parity (including incidence of nulliparity).

Of the 362 patients analyzed, the majority (71.8%) were Caucasian; 9.7% were African-American, and 18.2% Hispanic. Furthermore, most patients were non-smokers (87.9%), did not have pre-gestational diabetes (97.5%), and were normotensive at study enrollment (99.5%). Forty-one women (11.3%) had a family history of thromboembolism. There was one antepartum stillbirth and one intrapartum stillbirth.

The incidence of positive anti- β_2 GPI and anticardiolipin antibodies among the entire cohort and stratified by FVL mutation is displayed in Table 1. No women tested positive for both anti- β_2 GPI IgG and IgM; one woman was positive for both anticardiolipin IgG and IgM. FVL heterozygotes did not have higher rates of antibodies when compared with controls (Table 1).

Overall, 47 of 362 (13.0%) women experienced the primary adverse pregnancy outcome, including 14 (3.9%) cases of preeclampsia and 36 (9.9%) cases of SGA $< 10\%$. Sixteen of the 36 SGA babies measured SGA $< 5\%$. Three women (0.8%) had both preeclampsia and SGA, and 3 women had early-onset preeclampsia necessitating delivery less than 34 weeks. Rates of preeclampsia and/or SGA based on anti- β_2 GPI and anticardiolipin antibody status among all women are displayed in Table 2. The rates of the primary outcome were also not different when women heterozygous for FVL were stratified by anti- β_2 GPI and anticardiolipin antibody status (Table 3).

For both sets of antibodies, data were further categorized by degrees of positivity. Additionally, "borderline" results (10–20 units for each of the antibodies) were considered. No significant differences in rates of the primary outcome were noted with these additional classifications, regardless of FVL mutation status (data not shown).

Among the five patients positive for lupus anticoagulant, there were no cases of preeclampsia/SGA. None of these 5 had positive anticardiolipin or anti B2GPI antibodies.

Discussion

Women diagnosed with preeclampsia and/or carrying SGA fetuses were not more likely to have either anticardiolipin or anti- β_2 GPI IgG and IgM antibodies. Overall, the rates of anticardiolipin and anti- β_2 GPI IgG and IgM antibodies were low. Women with the FVL mutation were not more likely to carry anticardiolipin and β_2 GPI antibodies. The 4.4% rate of anticardiolipin IgG antibodies was similar to other previously published reports on unselected obstetric patients, which have ranged from 2–7% (Yasuda et al. 1995, Lockwood

et al. 1989). Furthermore, the 4.1% rate of either anti- β_2 GPI IgG or IgM antibodies in our study was also similar to previous reports indicating that approximately 4% of women are positive for anti- β_2 GPI IgG or IgM antibodies in a general obstetric population (Faden et al. 1997).

Using a 20 GPL or MPL units cutoff, we found that nearly 15% of all patients studied were positive for either IgG or IgM anticardiolipin, an incidence somewhat higher than some other studies (Harris and Spinnato), but similar to that found in other reports (Lynch et al. 1995). The anticardiolipin assay, like many immunoassays, is subject to variability, particularly at the low and high ends of the assay performance curve. Indeed, the median positive anticardiolipin IgG and IgM results were 24 units, suggesting that a substantial proportion of positive anticardiolipin results were marginally positive, i.e., just into the positive range for the assay. This point serves to underscore the need for repeat testing for initially positive results in clinical practice (Miyakis et al). There was a very small proportion of patients with lupus anticoagulant (1.4%); patients with lupus anticoagulant neither adverse outcomes nor an increased incidence of anticardiolipin or anti- β_2 GPI antibodies.

This analysis has several strengths. We have studied a well-characterized group of prospectively collected patients from a multicenter study. Given the approximate 2% population frequency of asymptomatic FVL heterozygotes, our population of 117 pregnant, asymptomatic women with the FVL mutation is relatively large (Dizon-Townson et al. 2005). Furthermore, a modest percentage (47 women, 13.0%) had clearly defined adverse outcomes. Women with the FVL mutation have not been previously analyzed during pregnancy for the presence of anticardiolipin and anti- β_2 GPI antibodies. Thus, this is a unique study, designed to analyze whether the FVL mutation in the presence of aPL antibodies confers an increased risk of adverse pregnancy outcomes.

There were several limitations of this study. This study was a secondary analysis, and was therefore not powered to detect our primary outcome. It is possible that high levels of anticardiolipin and/or anti- β_2 GPI antibodies among women with FVL mutation confers risk of only severe phenotypes, such as severe preeclampsia or severe SGA occurring mid-gestation. Unfortunately, this study cannot address this question because there were only 3 cases of early preeclampsia necessitating delivery less than 34 weeks gestation. Additionally, SGA is caused by numerous factors, and our lack of association here is limited to those cases caused by uteroplacental vasculopathy. Another potential drawback of our study is the use of archived samples which may have an impact on the stability of the antibodies tested. Although we have not addressed stability of these antibodies in this investigation, our previous studies (Tebo et al 2008, Branch et al 2001) and unpublished data shows that these antibodies are very stable if stored properly. In addition, the use of previously frozen sera has been used by others in studies of this kind (Lynch et al 1994, Lynch et al 1999, Yasuda et al 1995). Our study provides additional support to the notion that testing for anticardiolipin and anti- β_2 GPI is unlikely to predict adverse outcomes associated with placental insufficiency, such as preeclampsia and SGA, in women at low-to-moderate risk for such complications. Other studies have screened women for the presence of various antibodies in the setting of different risk factors. Branch, et al. assayed 5 different phospholipid antibodies (IgG and IgM) in women with a history of preeclampsia. Only modest positive predictive values were found to be associated with severe preeclampsia and SGA, and the authors concluded that testing for aPL antibodies to assess the risk of recurrent preeclampsia was of little prognostic value (Branch et al. 2001). Another study examined the levels of both anticardiolipin and anti- β_2 GPI antibodies in women with preeclampsia compared with normotensive controls and did not find a higher level of antibodies in those with preeclampsia (Lee et al. 2003). Our results were consistent with these findings, and

despite 1/3 of our cohort consisting of women with the FVL mutation, we did not find an increased risk of preeclampsia and/or SGA.

One criticism of studies analyzing aPL antibodies is that the cutoff values for “positive” and “negative” are arbitrary. We have analyzed our data using both the standard cutoffs for positivity as detailed by the manufacturer of the assay kit, as well as using lower, borderline “cutoffs” of 10–20 Units, technically at the upper limit of normal per manufacturer’s guidelines. Regardless of the definition of a “positive” assay, presence of these aPL antibodies is not associated with the adverse outcome. These findings are concordant with Silver, et al., who also concluded that women with low levels of IgG anticardiolipin are not at high risk for aPL-antibody like complications (Silver et al. 1996). Our study additionally provides evidence that these lower levels of antibodies in combination with the FVL mutation do not increase a woman’s risk of adverse pregnancy outcomes.

These data suggest that routine screening for anticardiolipin and anti- β_2 GPI antibodies among asymptomatic, low-risk women with the FVL mutation is of little clinical value when assessing risk for preeclampsia and/or SGA. Future research should address whether additional markers could be added in conjunction with these tests to improve the prediction of adverse pregnancy outcomes.

Acknowledgments

The author wishes to thank the following committee members who participated in protocol development and coordination between clinical research centers (Margaret Cotroneo, R.N.), protocol/data management and statistical analysis (Elizabeth Thom, Ph.D. and Valerija Momirova, M.S.), and protocol development and oversight (Donna Dizon-Townson, M.D., and Michael W. Varner, M.D.).

Funding: This work was supported by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (HD27869, HD21414, U01-HD36801, HD34208, HD27860, HD34116, HD34136, HD27861, HD34122, HD21410, HD27915, HD34210, HD27905, and HD27917) and the National Institute of Health’s Office of Research on Women’s Health (ORWH) and its contents are solely the responsibility of the authors and do not necessarily represent the official view of NICHD or ORWH.

This study was also supported in part by funding of the H.A. and Edna Benning Presidential Chair (DWB).

In addition to the authors, other members of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network are as follows:

University of Utah — M. Varner, D. Dizon-Townson, K. Anderson (University of Utah Health Sciences Center), F. Porter (Intermountain Healthcare), A. Guzman (McKay-Dee Hospital Center), K. Jolley (Utah Valley Regional Medical Center), J. Parsons (Utah Valley Regional Medical Center)

University of Alabama at Birmingham — D. Rouse, A. Northen, and K. Bailey

University of Chicago — A. Moawad, P. Jones, and G. Mallett

University of Cincinnati — T. Siddiqi, H. How, N. Elder, and W. Knox

University of Pittsburgh — M. Cotroneo, K. Lain, and T. Kamon

University of Miami — F. Doyle

The Ohio State University — J. D. Iams, F. Johnson, and C. Latimer

University of Tennessee — W. Mabie and R. Ramsey

University of Texas at San Antonio — O. Langer and D. Dudley

University of Texas Southwestern Medical Center — J. McCampbell, K. Leveno, and S. Williams

Thomas Jefferson University — A. Sciscione, M. Talucci, and M. DiVito

Wake Forest University Health Sciences University — P. Meis, M. Harper, M. Swain, and K. Lanier

Wayne State University — M. Dombrowski, G. Norman, P. Lockhart, and C. Sudz

The George Washington University Biostatistics Center — E. Thom, V. Momirova, and A. Arrieta

Eunice Kennedy Shriver National Institute of Child Health and Human Development — M. Klebanoff, S. Pagliaro, and D. McNellis

MFMU Steering Committee Chair (Vanderbilt University Medical Center) — S. Gabbe

References

- Battaglia FC, Lubchenco LO. A practical classification of newborn infants by weight and gestational age. *J Pediatr* 1967;71:159–163. [PubMed: 6029463]
- Branch DW, Andres R, Digre KB, Rote NS, Scott JS. The association of antiphospholipid antibodies with severe preeclampsia. *Obstet Gynecol* 1989;73:541–545. [PubMed: 2494618]
- Branch DW, Porter TF, Rittenhouse L, et al. Antiphospholipid antibodies in women at risk for preeclampsia. *Am J Obstet Gynecol* 2001;184:825–833. [PubMed: 11303189]
- Branch DW. Antiphospholipid antibodies and fetal compromise. *Thrombosis Research* 2004;114:415–418. [PubMed: 15507272]
- Crowther MA, Kelton JG. Congenital thrombophilic states associated with venous thrombosis: a qualitative overview and proposed classification system. *Ann Intern Med* 2003;138:128–134. [PubMed: 12529095]
- Dizon-Townson D, Miller C, Sibai B, et al. The relationship of the factor V leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol* 2005;106:517–524. [PubMed: 16135581]
- Faden D, Tincani A, Tanzi P, et al. Anti-beta 2 glycoprotein I antibodies in a general obstetric population: preliminary results on the prevalence and correlation with pregnancy outcome. *Eur J Obstet Gynecol Reprod Biol* 1997;73:37–42. [PubMed: 9175687]
- Harris EN, Spinnato JA. Should anticardiolipin tests be performed in otherwise healthy pregnant women? *Am J Obstet Gynecol* 1991;165:1272–1277. [PubMed: 1957844]
- Jahn A, Razum O, Berle P. Routine screening for intrauterine growth retardation in Germany: low sensitivity and questionable benefit for diagnosed cases. *Acta Obstet Gynecol Scand* 1998;77:643–648. [PubMed: 9688242]
- Lee RM, Brown MA, Branch DW, Ward K, Silver RM. Anticardiolipin and anti-β2-glycoprotein-I antibodies in preeclampsia. *Am J Obstet Gynecol* 2003;102:294–300.
- Lim W, Crowther MA, Eikelboom JW. Management of antiphospholipid antibody syndrome. *JAMA* 2006;295:1050–1057. [PubMed: 16507806]
- Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbins JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. *Am J Obstet Gynecol* 1989;161:369–373. [PubMed: 2504043]
- Lynch A, Marlar R, Murphy J, Davila G, Santos M, Rutledge J, Emlen W. Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. *Ann Int Med* 1994;120:470–475. [PubMed: 8093135]
- Lynch A, Byers T, Emlen W, Rynes D, Shetterly SM, Hamman RF. Association of antibodies to beta2-glycoprotein 1 with pregnancy loss and pregnancy induced hypertension: a prospective study in low-risk pregnancy. *Obstet Gynecol* 1999;93:193–198. [PubMed: 9932554]
- Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R, Derksen RH, DE Groot PG, Koike T, Meroni PL, Reber G, Shoenfeld Y, Tincani A, Vlachoyiannopoulos PG, Krilis SA. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). *J Thromb Haemost* 2006;4:295–306. [PubMed: 16420554]
- Silver RM, Porter TF, Leeuwen IV, Jeng G, Scott JR, Branch DW. Anticardiolipin antibodies: clinical consequences of “low titers.”. *Obstet Gynecol* 1996;87:494–500. [PubMed: 8602297]

- Simioni P, Tormene D, Spiezia L, Tognin G, Rossetto V, Prandoni P. Inherited thrombophilia and venous thromboembolism. *Semin Thromb Hemost* 2006;32:700–708. [PubMed: 17024597]
- Tebo AE, Jaskowski TD, Hill HR, Branch DW. Clinical relevance of multiple antibody specificity testing in anti-phospholipid syndrome and recurrent pregnancy loss. *Clin Exp Immunol* 2008;154:332–338. [PubMed: 18826497]
- Tsapanos V, Kanellopoulos N, Cardamakis E, et al. Anticardiolipin antibodies levels in healthy pregnant and non-pregnant women. *Arch Gynecol Obstet* 2000;263:111–115. [PubMed: 10763838]
- Vila P, Hernandez MC, Lopez-Fernandez MF, Batlle J. Prevalence, follow-up, and clinical significance of the anticardiolipin antibodies in normal subjects. *Thromb Haemost* 1994;72:209–213. [PubMed: 7831653]
- Yasuda M, Takakuwa K, Tokunaga A, Tanaka K. Prospective studies of the association between anticardiolipin antibody and outcome of pregnancy. *Obstet Gynecol* 1995;86:555–559. [PubMed: 7675379]

Table 1

Incidence of positivity for anti- β_2 GPI and anticardiolipin antibodies among the entire cohort and stratified by FVL mutation, given as the number of observed events over the total number of patients in the corresponding column (%).

		All Patients	FVL negative	FVL heterozygote	p-value
anti- β_2 GPI IgG	(-)	360/362 (99.4)	244/245 (99.6)	116/117 (99.1)	0.54
	(+)	2/362 (0.6)	1/245 (0.4)	1/117 (0.9)	
anti- β_2 GPI IgM	(-)	349/362 (96.4)	239/245 (97.6)	110/117 (94.0)	0.13
	(+)	13/362 (3.6)	6/245 (2.4)	7/117 (6.0)	
aCL IgG	(-)	346/362 (95.6)	234/245 (95.5)	112/117 (95.7)	0.93
	(+)	16/362 (4.4)	11/245 (4.5)	5/117 (4.3)	
aCL IgM	(-)	322/362 (89.0)	216/245 (88.2)	106/117 (90.6)	0.49
	(+)	40/362 (11.0)	29/245 (11.8)	11/117 (9.4)	

Abbreviations: anti- β_2 GPI (anti-beta2 glycoprotein I), aCL (anticardiolipin).

Table 2

Rates of preeclampsia and/or SGA based on anti- β_2 GPI and anticardiolipin antibody status among all women, given as the number of observed events over the total number of patients in the corresponding row (%).

		No PreE and/or SGA	PreE and/or SGA	p-value
anti-β_2 GPI IgG	(-)	314/360 (87.2)	46/360 (12.8)	0.24
	(+)	1/2 (50.0)	1/2 (50.0)	
anti-β_2 GPI IgM	(-)	303/349 (86.8)	46/349 (13.2)	>0.99
	(+)	12/13 (92.3)	1/13 (7.7)	
aCL IgG	(-)	301/346 (87.0)	45/346 (13.0)	>0.99
	(+)	14/16 (87.5)	2/16 (12.5)	
aCL IgM	(-)	279/322 (86.6)	43/322 (13.4)	0.55
	(+)	36/40 (90.0)	4/40 (10.0)	

Abbreviations: PreE (preeclampsia), SGA (small for gestational age), anti- β_2 GPI (anti-beta2 glycoprotein I), aCL (anticardiolipin).

Table 3

Rates of preeclampsia and/or SGA based on anti- β_2 GPI and anticardiolipin antibody status among women heterozygous for FVL mutation, given as the number of observed events over the total number of patients in the corresponding row (%).

		FVL Heterozygotes		
		No PreE and/or SGA	PreE and/or SGA	p-value
anti-β_2 GPI IgG	(-)	102/116 (87.9)	14/116 (12.1)	>0.99
	(+)	1/1 (100)	0/1 (0)	
anti-β_2 GPI IgM	(-)	97/110 (88.2)	13/110 (11.8)	>0.99
	(+)	6/7 (85.7)	1/7 (14.3)	
aCL IgG	(-)	98/112 (87.5)	14/112 (12.5)	>0.99
	(+)	5/5 (100)	0/5 (0)	
aCL IgM	(-)	93/106 (87.7)	13/106 (12.3)	>0.99
	(+)	10/11 (90.9)	1/11 (9.1)	

Abbreviations: PreE (preeclampsia), SGA (small for gestational age), anti- β_2 GPI (anti-beta2 glycoprotein I), aCL (anticardiolipin).