Published in final edited form as:

Am J Obstet Gynecol. 2007 February ; 196(2): 167.e1–167.e5.

Excessive complement activation is associated with placental injury in patients with antiphospholipid antibodies

Jaime M. Shamonki, MD, Jane E. Salmon, MD, Elizabeth Hyjek, MD, and Rebecca N. Baergen, MD

From the Department of Pathology, New York Presbyterian Hospital-Weill Medical College (Drs Shamonki, Hyjek, and Baergen), and the Department of Medicine, Hospital for Special Surgery-Weill Medical College (Dr Salmon), Cornell University, New York, NY

Abstract

OBJECTIVE—Studies that use a murine model of antiphospholipid syndrome have demonstrated a critical role for complement activation that leads to fetal and placental injury in the presence of antiphospholipid antibodies (APAs). We examined the placentas of patients with APAs to demonstrate a similar association with tissue injury in humans.

STUDY DESIGN—Immunohistochemical analyses with the use of antibodies to the complement products C4d, C3b, and C5b-9 were performed on paraffin-embedded tissue sections of placentas from 47 patients with APAs and 23 normal control patients.

RESULTS—We found evidence of increased complement deposition in the trophoblast cytoplasm (C4d and C3b), trophoblastic cell and basement membrane (C4d), and extravillous trophoblasts (C4d) of patients with APAs, compared with control patients. We report a correlation between placental pathologic features and complement deposition (C4d) in the trophoblastic cytoplasm, cell membrane, and basement membrane.

CONCLUSION—These findings are consistent with murine studies that implicate complement as a critical factor in the fetal tissue injury observed in antiphospholipid syndrome.

Keywords

antiphospholipid antibodies; antiphospholipid syndrome; complement; placenta; pregnancy

The antiphospholipid syndrome (APS) is characterized by arterial or venous thrombosis and poor obstetric outcomes that include intrauterine fetal death and growth restriction in the presence of antiphospholipid antibodies (APAs).^{1–3} In pregnancy, circulating APAs are associated with histopathologic changes in the placenta that reflect decreased uteroplacental perfusion (which include villous infarction, decidual vasculopathy, decidual vascular thrombosis) and “accelerated” villous maturity.^{3–6} It is presumed that the specific antigenic reactivity of APAs is critical to their effects; however, the pathogenic mediators that cause fetal and placental tissue injury are understood poorly.^{7,8} Although traditional experimental models have emphasized the role of thrombosis in placental tissue, recent studies demonstrate that the trophoblastic basement membrane is a particular target for APAs, which suggests that these antibodies may be directed specifically at the placenta. These and other proinflammatory

Reprints: Rebecca N. Baergen, MD, Department of Pathology, New York, Presbyterian Hospital-Weill Medical College of Cornell University, 525 East 68th St, New York, NY 10021; rbaergen@med.cornell.edu.

Presented at the United States and Canadian Academy of Pathology meetings in San Antonio, TX (Feb. 2005) and Atlanta, GA (Feb. 2006).

factors may also contribute to the characteristic pathologic changes that are observed in the placentas from these patients.^{4,9,10}

A murine model has demonstrated a critical role for complement in mediating fetal tissue damage in APS. Passive transfer of human immunoglobulin G from patients with high titer APAs induces fetal death and/or fetal growth restriction in murine pups.^{11–13} In the placentas, a marked deciduitis and immunohistochemical evidence of APAs and complement deposition are present. Mice deficient in either C3, C4, or C5 are protected from fetal death that normally would ensue when exposed to APAs, despite evidence of APAs in their placentas. Similar results are found in APA-exposed mice that had been administered specific inhibitors of C3,⁹ C5a,¹⁴ or factor Bb.^{14,15} These studies demonstrate that, although the nature of the antigen recognized by APAs directs their deposition, complement activation is critical for the induction of tissue injury and fetal loss that is induced by APAs.¹⁶ It is suggested that complement activation products recruit and activate inflammatory cells into the placenta and either directly or indirectly induce injury.

No study to date has demonstrated complement activation in the presence of APAs in the human placenta. In the human placenta, maternal blood has direct contact with the trophoblast, which exposes fetal tissue to circulating maternal antibodies. The extravillous trophoblast, although not involved in maternofetal exchange, is also associated intimately with maternal tissue that is present singly and in small clusters throughout the implantation site and other extravillous sites.¹⁷

The classic pathway of complement activation is mediated by antibody-antigen complexes and is where we directed our preliminary investigation. Early in the common pathway, C4 splits to generate C4d, among several products. Eventual activation of C3 marks the common pathway, the convergence of the classic and alternative pathways. The split products of C3 are C3a, a powerful anaphylatoxin, and C3b. C3b further splits C5 into C5a, another anaphylatoxin, and C5b, the primary contributor to C5b-9 or “membrane attack complex.” In this study, we use immunohistochemical methods to analyze placentas from patients with APAs for deposition of complement activation products from the classic (C4d), early common (C3b), and terminal common (C5b-9) pathways in the villous interface between mother and fetus.

Materials and Methods

Subjects

Patients were identified by retrospective review of surgical pathologic reports from 2001 to 2004.¹⁸ Control cases were selected on the basis of a clinical history of normal pregnancy and absence of significant placental pathologic findings. The study population included 47 patients with a clinician-provided history of positive APAs. We predicted cases with clinical APS, fetal death, or immaturity not only may trigger greater complement activation but also may produce more nonspecific immunohistochemical staining as the result of profound histopathologic changes that occur frequently in these states. To better isolate the effect of APAs alone on complement deposition, only placentas of viable term infants from patients with APAs, and not with APS, were included in the study. All clinical information was provided by the submitting obstetrician. Histopathologic diagnoses were rendered on all placentas by 1 pathologist at the time of specimen submission to pathology, independently of this study. The study was approved by the Institutional Review Board of the Committee for Human Rights in Research of New York Presbyterian Hospital-Weill Medical College of Cornell University.

Immunohistochemistry

Samples were obtained from surgical pathology files of New York Presbyterian Hospital-Cornell. Tissue samples were processed in formalin and were embedded in paraffin. A 5- μ m-thick full-thickness section of each placenta was prepared for each immunohistochemical stain. Tissue that was uninvolved by pathologic lesions was selected because it was more representative of the placental function as a whole and more accurately reflected the dynamic processes. Immunohistochemical staining was performed with monoclonal mouse antibodies anti-SC5b-9, anti-iC3b (Quidel Corp, San Diego, CA) and polyclonal rabbit anti-human C4d antibody (ALPCO Diagnostics, Windham, NH). Immunohistochemical staining for all antibodies was performed with an automated immunostainer (TechMate 500; Ventana Medical Systems, Tucson, AZ), according to a modified monoimmunoperoxidase protocol (Ventana Medical Systems). C5b-9 and C3b staining was performed with the ChemMate ABC Peroxidase Secondary Detection System (Ventana Medical Systems). Sections were retrieved with Dako target retrieval solution (DakoCytomation, Carpinteria, CA). Sections were incubated with anti-C5b-9(dilution 1:250) and anti-C3b (dilution 1:5000) for 50 minutes and with secondary and tertiary antibodies for 25 minutes each. Peroxidase reaction was developed with diaminobenzidine liquid chromogen. C4d staining was performed with the Mouse EnVision Plus Horseradish peroxidase detection system (DakoCytomation). Sections were retrieved with 10 mmol/L citrate buffer, pH 6.0. Sections were incubated with anti-C4d antibody (dilution of 1:50) for 1 hour then with secondary and tertiary antibodies for 30 minutes each. Peroxidase reaction was developed with the use of diaminobenzidine + liquid chromogen (DakoCytomation). All sections were counterstained with hematoxylin. Appropriate positive and negative isotypic controls, which included normal placenta, were used for each antibody.

Evaluation of immunoreactivity

The intensity of immunoreactivity and percentage of cells that was stained in each case were evaluated for each antibody to calculate an H-score. The H-score has been previously described and is defined as $\Sigma Pi(I)$, where I is the intensity of staining with a value of 0, 1, or 2 (none-to-minimal, moderate, or strong, respectively), and Pi is the percentage of cells that were stained for each intensity, varying from 0-100% for a maximum score of 200.¹⁹ Scores were recorded for each cell type (cytotrophoblast, syncytiotrophoblast, extravillous trophoblast), location (cytoplasm, cell membrane, basement membrane) and antibody (C3b, C4d, and C5b-9). When the slides were scored, the pathologist was blinded to cases vs control patients.

Statistical methods

Statistical analysis was performed with SPSS software (version 11.0.1 for Windows; SPSS Inc and LEAD Technologies, Inc, Chicago, IL). Patient characteristics and immunohistochemical results were analyzed using *t*-test for independent samples or χ^2 test where applicable. Correlations were performed with the Pearson correlation coefficient. Probability values of <.05 were considered statistically significant.

Results

Clinical history and outcome

Clinical information is summarized in Table 1. Patients with APAs had significantly more previous spontaneous abortions and, accordingly, greater gravidity (a difference which approached significance). There were no other clinical differences that were observed. Patients were of similar age and parity. The pregnancies of both groups produced placentas and fetuses of similar weights. Because only placentas from full-term viable infants were included in the study, fetuses were of a comparable gestational age.

Histopathologic analyses of placentas

The APA group showed the following pathologic lesions on routine microscopy: deciduitis, decidual necrosis, increased syncytial knots, “accelerated” villous maturity, avascular villi and villous infarcts, retroplacental hematomas, intervillous thrombosis, and decidual vasculopathy. Decidual vasculopathy includes fibrinoid necrosis of the vascular wall, thrombosis, atherosclerosis, and persistence of vascular smooth muscle. The histopathologic findings are summarized in Table 2. Pathologic lesions were present in 62% of APA cases. The patients with APAs and normal control patients differ significantly in this aspect (χ^2 , 24.29; $P < .001$).

Evidence for complement activation in placentas from patients with APAs

C4d reactivity was observed in 3 areas within the placenta: villous trophoblast (syncytiotrophoblast and cytotrophoblast) cytoplasm (Figure 1), villous trophoblast cell and basement membrane (Figure 2), and the extravillous trophoblast of the basal plate (Figure 3). Reactivity to C3b and C5b-9 were observed only in the extravillous trophoblast and villous trophoblast cytoplasm. For all complement components, intensity of immunoreactivity was uniformly strong in the extravillous trophoblast, although intensity of staining was more variable in the villous trophoblast.

Immunoreactivity to C4d protein was significantly stronger in the villous trophoblast cytoplasm ($P < .001$), basement membrane ($P < .001$), and extravillous trophoblast ($P < .001$) in APA cases compared with control patients. Significantly greater immunoreactivity to C3b ($P = .005$) was also observed in the villous trophoblast cytoplasm in placentas of patients with APAs compared with normal control patients. In contrast, we found significantly less deposition of C5b-9 in the villous trophoblast cytoplasm ($P = .005$) of APA cases vs control patients. Strong immunoreactivity for C3b and C5b-9 was extensively present in the extravillous trophoblast of both APS cases and control patients; thus, a significant difference between the 2 groups was not detected. These findings are summarized in Table 3.

Additionally, we found a significant correlation between the presence of pathologic lesions (as previously described) and the deposition of C4d in the trophoblast cytoplasm ($P < .001$) and cellular and basement membranes ($P = .001$). A trend was observed that correlated pathologic condition to C4d deposition in the extravillous trophoblast of decidua ($P = .089$); however, the results are not statistically significant. These findings are summarized in Table 4.

Comment

We have documented a novel finding in the placentas of patients with APAs by demonstrating an increased deposition of complement factors C4d and C3b, compared with normal control patients. Even when clinically silent, by measures such as fetal birth weight, APAs produce histopathologic changes in the placenta and, accordingly, are associated with increased complement deposition.

As in the murine models of APS, these results support the role of complement in the induction of placental tissue injury in the presence of APAs. In vitro studies of human placentas have shown that the trophoblastic cell membranes are targets for APAs¹⁴; however, pathologic findings in placentas from women with APAs consist of lesions that are associated commonly with malperfusion. Taken together, these studies suggest that proinflammatory factors that stimulate complement activation may precede the changes that ultimately lead to ischemia, tissue injury, and fetal loss.

The placenta provides a highly stimulating substrate for complement activation. Maternal blood is in direct contact with fetal tissue; thus, the mother is exposed to paternal antigens on the trophoblastic surface.⁹ Here fetal tissue becomes susceptible to complement activation and

damage. The established relative hypoxic environment of the normal placenta, although believed to drive trophoblast differentiation,²⁰ is also a trigger for the initiation of the complement cascade. Accordingly, we detected deposition of complement activation products in the villi and deciduas in normal placentas. Despite the potential for continuous complement activation, extensive tissue damage is not seen in the normal placenta because it is protected from spontaneous complement activation by the complement regulatory proteins DAF, MCP, and CD59, which are expressed highly on cytotrophoblasts.²¹ In contrast, placentas from patients with APAs showed extensive deposition of complement activation products, which suggests that protection by complement regulatory proteins is overwhelmed by exposure to APAs. Because the complement classic pathway is activated directly through antibody-antigen complexes, which includes those that are bound to APAs,^{9,16,17} it is most likely that complement deposition that is observed is due to the increased activation of the system, rather than because of a depletion or inactivation of complement regulatory proteins.

Complement activation products have the capacity to activate leukocytes and endothelial cells and thereby induce a prothrombotic phenotype. As such, complement activation may be a critical event that precedes the thrombosis that defines APS. Heparin therapy may actually prevent APA-associated miscarriage by inhibiting the proinflammatory factors that lead to fetal tissue injury.^{16,22} A body of evidence has mounted that demonstrates the complement-inhibitory effects of heparin,^{23–25} which may account for its ability to avert miscarriage or fetal morbidity in the presence of APAs. This argument is furthered by Girardi et al,^{16,22,26} who demonstrated that, unlike heparin, anticoagulants that do not inhibit complement activation (fondaparinux and hirudin) do not prevent pregnancy loss in mice that are exposed to APAs.

An unexpected finding was that we observed a decrease in C5b-9 complement protein in APA cases compared with control patients. C5b-9, the membrane attack complex, leads to tissue injury through permeabilizing cell membranes and acting as an ion channel to trigger cell activation. It is unclear why there was a discrepancy between deposition of complement proteins from the proximal aspects of the cascade (C4d and C3b) compared with the terminal component (membrane attack complex). In addition to producing the membrane attack complex, activation of the complement common pathway releases the anaphylatoxins C3a and C5a, which are independent inflammatory mediators that are capable of leading to tissue damage. The pathologic condition that was observed in these APA cases correlates significantly with deposition of C4d and may be the effect of anaphylatoxin release (C3a, C5a) rather than of the direct effects of the membrane attack complex.

The proposed etiologic mechanisms that result in placental and fetal injury in the presence of maternal APAs are many. In support of the extensive work in the murine model, a demonstration of complement deposition in human placentas raises new questions about fetomaternal interaction and complement regulation. More specific therapy, namely an agent that specifically inhibits complement activation, may be useful in the future treatment of pregnant patients with APAs.²⁷ Future studies, to include patients with more severe clinical symptoms and adverse obstetric outcomes, will address specifically the differences in complement deposition between patients with clinical APSs vs the presence of APAs alone. We hope such studies will improve our understanding of the mechanism by which complement activation leads to placental tissue injury in humans and will better define a therapeutic role for anticomplement agents.

References

1. De Groot PG, Derksen RHW. Pathophysiology of the antiphospholipid syndrome. *J Thromb Haemost* 2005;3:1854–60. [PubMed: 16102052]

2. Derksen RH, Khamashta MA, Branch DW. Management of the obstetric antiphospholipid syndrome. *Arthritis Rheum* 2004;50:1028–39. [PubMed: 15077285]
3. Salafia CM, Parke AL. Pregnancy and rheumatic disease: placental pathology in systemic lupus erythematosus and phospholipid antibody syndrome. *Rheum Dis Clin North Am* 1997;23:85–97. [PubMed: 9031376]
4. Magid MS, Kaplan C, Sammaritano LR, Peterson M, Druzin ML, Lockshin MD. Placental pathology in systemic lupus erythematosus: a prospective study. *Am J Obstet Gynecol* 1998;179:226–34. [PubMed: 9704792]
5. Out HJ, Kooijman CD, Bruinse HW, Derksen RH. Histopathological findings in placentae from patients with intra-uterine fetal death and antiphospholipid antibodies. *Eur J Obstet Gynecol Reprod Biol* 1991;41:179–86. [PubMed: 1936501]
6. Stone S, Pijnenborg R, Vercruysse L, et al. The placental bed in pregnancies complicated by primary antiphospholipid syndrome. *Placenta* 2006;27:457–67. [PubMed: 16005063]
7. Rand JH, Wu XX, Andree HA, et al. Pregnancy loss in the antiphospholipid-antibody syndrome: a possible thrombogenic mechanism. *N Engl J Med* 1997;337:154–60. [PubMed: 9219701]
8. Simantov R, LaSala JM, Lo SK, et al. Activation of cultured vascular endothelium by antiphospholipid antibodies. *J Clin Invest* 1995;96:2211–9. [PubMed: 7593607]
9. Holers VM, Girardi G, Mo L, et al. Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J Exp Med* 2002;195:212–20.
10. Levine LS, Branch DW, Rauch J. The antiphospholipid syndrome. *N Engl J Med* 2002;346:752–63. [PubMed: 11882732]
11. Blank M, Cohen J, Toder V, Shoenfeld Y. Induction of antiphospholipid syndrome in naïve mice with mouse lupus monoclonal and human polyclonal antibodies. *Proc Natl Acad Sci U S A* 1991;88:3069–73. [PubMed: 2014226]
12. Branch DW, Dudley DJ, Mitchell KA, et al. Immunoglobulin G fractions from patients with antiphospholipid antibodies cause fetal death in BALB/c mice: a model for autoimmune fetal loss. *Am J Obstet Gynecol* 1990;163:210–6. [PubMed: 2197866]
13. Piona A, La Rosa L, Tinacani A, Faden D, Magro G, Grasso S. Placental thrombosis and fetal loss after passive transfer of mouse lupus monoclonal or human polyclonal anti-cardiolipin antibodies in pregnant naïve BALB/c mice. *Scand J Immunol* 1995;41:427–32. [PubMed: 7725061]
14. Girardi G, Berman J, Redecha P, et al. Complement C5a receptors and neutrophils mediate fetal injury in the antiphospholipid syndrome. *J Clin Invest* 2003;112:1644–54. [PubMed: 14660741]
15. Thurman JM, Kraus DM, Girardi G, et al. A novel inhibitor of the alternative complement pathway prevents antiphospholipid antibody induced pregnancy loss in mice. *Mol Immunol* 2005;42:87–97. [PubMed: 15488947]
16. Girardi G, Bulla R, Salmon JE, Tedesco F. The complement system in the pathophysiology of pregnancy. *Mol Immunol* 2006;43:68–77. [PubMed: 16023727]
17. Baergen RN. Manual of Benirschke and Kaufmann's pathology of the human placenta. New York: Springer; 2005. p. 81-90.
18. Edens RE, Linhardt RJ, Bell CS, Weiler JM. Heparin and derivatized heparin inhibits zymosan and cobra venom factor activation of complement in serum. *Immunopharmacology* 1994;27:145–53. [PubMed: 8014028]
19. Myatt L. Placental adaptive responses and fetal programming. *J Physiol* 2006;572:25–30. [PubMed: 16469781]
20. Salmon JE, Girardi G, Volers VM. Complement activation as a mediatory of antiphospholipid antibody induced pregnancy loss and thrombosis. *Ann Rheum Dis* 2002;61:46–50.
21. Harris CL, Morgan BP. The many faces of the membrane regulators of complement. In: Szebeni, J., editor. *The complement system: novel roles in health and disease*. Boston: Kluwer Academic Publishers; 2004. p. 129-66.
22. Girardi G. Heparin treatment in pregnancy loss: potential therapeutic benefits beyond anticoagulation. *J Reprod Immunol* 2005;66:45–51. [PubMed: 15949561]
23. Ecker E, Gross P. Anticomplementary power of heparin. *J Infect Dis* 1929;44:250–7.

24. Kazatchkine MD, Fearon DT, Metcalfe DD, Rosenberg RD, Austen KF. Structural determinants of the capacity of heparin to inhibit the formation of the human amplification C3 convertase. *J Clin Invest* 1981;67:223–8. [PubMed: 6778897]
25. Ninomiya H, Kawashima Y, Nagasawa T. Inhibition of complement-mediated hemolysis in paroxysmal nocturnal hemoglobinuria by heparin or low-molecular weight heparin. *Br J Haematol* 2000;109:875–81. [PubMed: 10929045]
26. Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. *Nat Med* 2004;10:1222–6. [PubMed: 15489858]
27. Hillmen P, Hall C, March JCW, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med* 2004;350:552–9. [PubMed: 14762182]

FIGURE 1. Villous trophoblast cytoplasm

Strong immunohistochemical reactivity to C4d in the villous trophoblast (original magnification, $\times 40$).

FIGURE 2. Villous trophoblast cell and basement membranes
Strong immunohistochemical reactivity to C4d in the cell membrane and basement membrane of the villous trophoblast (original magnification, $\times 40$).

FIGURE 3. Extravillous trophoblasts of the basal plate

Individual extravillous trophoblasts are present throughout the decidua and show strong immunohistochemical reactivity to C4d (original magnification, $\times 40$). V, chorionic villi of the basal plate; D, decidua.

TABLE 1

Clinical characteristics of APA cases and control patients

Characteristic	APA cases (n = 47)	Control patients (n = 23)	P value
Maternal age (y)	34.7 ± 5.6	34.9 ± 6.3	.93 (NS)
Gravidity (n)	3.7 ± 2.7	2.3 ± 1.8	.17 (NS)
Parity (n)	0.85 ± 1.4	1.0 ± 1.8	.735 (NS)
History of spontaneous abortions (n)	1.80 ± 2.1	0.18 ± 0.5	.001*
Fetal weight (g)	3006 ± 59.4	3031 ± 66.4	.88 (NS)
Placental weight (g)	425 ± 122	424 ± 101	.97 (NS)
Gestational age (wk)	38.0 ± 1.74	38.7 ± 1.7	.15 (NS)

Data are expressed as mean ± SD. NS, not significant.

* Significance at $P \leq .05$.

TABLE 2

Histopathologic findings found in APA cases and control patients

Histopathologic findings	APA cases (n = 47)	Control patients (n = 23)
Acute and/or chronic deciduitis (n)	9 (19%)	0
Decidual necrosis (n)	3 (6%)	0
Increased syncytial knots (n)	11 (23%)	0
Accelerated villous maturity (n)	3 (6%)	0
Avascular villi and villous infarcts (n)	13 (28%)	0
Retroplacental hematomas (n)	9 (19%)	0
Intervillous thrombi (n)	9 (19%)	0
Decidual vasculopathy (n)	9 (19%)	0

TABLE 3

Comparison of immunohistochemical staining

Variable	APA cases (n = 47)	Control patients (n = 23)	P value
C4d cytoplasm of villous trophoblast	105.3 ± 34	20 ± 4	<.001 [*]
C4d cellular and basement membrane of villous trophoblast	87.2 ± 59	28.4 ± 25	<.001 [*]
C4d extravillous trophoblast of decidua	98.8 ± 57	42.2 ± 37	<.001 [*]
C3b cytoplasm of villous trophoblast	59.3 ± 36	36.3 ± 28	.005 [*]
C3b extravillous trophoblast of decidua	95.5 ± 55	70.8 ± 55	.090 (NS)
C5b-9 cytoplasm of villous trophoblast	64.5 ± 57	113.8 ± 59	.005 [*]
C5b-9 extravillous trophoblast of decidua	162 ± 22	146 ± 34	.065 (NS)

Data are expressed as mean ± SD. H-scores for C4d, C3b, and C5b-9 in patients with APAs and in normal control patients. (H score is the $\Sigma \text{Pi(I)}$, where I is the intensity of immunostaining.) NS, not significant.

* Significance at $P \leq .05$.

TABLE 4

Correlations of placental pathologic condition with complement results

Variable	Correlation coefficient	P value
C4d cytoplasm of villous trophoblast	0.567	<.001*
C4d cellular and basement membrane of villous trophoblast	0.384	.001*
C4d extravillous trophoblast of decidua	0.218	.089 (NS)
C3b cytoplasm of villous trophoblast	0.206	.105 (NS)
C3b extravillous trophoblast of decidua	0.109	.397 (NS)
C5b-9 cytoplasm of villous trophoblast	−0.73	.548 (NS)
C5b-9 extravillous trophoblast of decidua	0.007	.954 (NS)

Correlation coefficient was calculated with complement immunostaining in specific locations in the placenta in patients with APAs vs control patients; Pearson method was used. *NS*, not significant.

* Significant at $P < .05$ (2-tailed).