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Diagnostic significance of anti-annexin-A5 antibody determination

Review Article

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Abstract: Anti-annexin A5 antibodies are directed against annexin A5 - a phospholipid-binding protein that belongs to the ubiquitous annexin family. These antibodies were first discovered in 1994 by Matsuda et al. in women with recurrent fetal loss or preeclampsia and in patients with systemic lupus erythematosus and positive lupus anticoagulant and/or anticardiolipin antibodies. Since then anti-annexin A5 antibodies have been the focus of research. In addition to their well known prothrombotic and procoagulant activities the authors discuss the involvement of these antibodies in the pathogenesis of antiphospholipid syndrome, recurrent pregnancy loss, systemic lupus erythematosus and other immune and non-immune disorders. Controversial reports are presented and a possible interpretation of the results is given. The authors suggest the significance of anti-annexin A5 antibodies as an additional diagnostic marker and discuss the necessity of more extensive research on their clinical significance.

Keywords: *Anti-annexin A5 antibodies • Antiphospholipid syndrome • Pregnancy loss • Systemic lupus erythematosus*

1. Introduction

Annexin A5 (A5) is a 36 kDa glycoprotein present in a wide variety of tissues and cells, such as the placenta, endothelium and peripheral blood cells. A5 has a high calcium-dependent affinity to negatively-charged phospholipids and is known to participate in the regulation of at least two crucial physiological mechanisms: the coagulation cascade (via marked anticoagulant activity) and apoptosis.

Antibodies against annexin-A5 (a-A5) have a procoagulant effect and induce endothelial cell apoptosis. These autoantibodies were first described in 1994 by Matsuda et al. [1,2].

2. Significance of anti-annexin-A5 antibodies in patients with the antiphospholipid syndrome and poor pregnancy outcome/pregnancy loss

Antiphospholipid syndrome (APS) is characterized by thrombosis, low platelet count and pregnancy loss in the first, second or third trimester. Anticardiolipin antibodies (ACL) and lupus anticoagulant (LA) are the major laboratory characteristics of APS patients. These antibodies were first considered to be directed against negatively charged or neutral phospholipids [3,4]. Research published in 1990 however revealed that in fact ACL are directed against a complex consisting of cardiolipin and a plasma co-factor – beta-2-glycoprotein-I (b2GPI) [5-7]. It is well known that the antiplospholipid antibodies (APL) may be directed against a large number of antigens, including cardiolipin, beta-2-glycoprotein-I, prothrombin, phosphatidylserine,

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annexin A5, etc. Due to the wide variety of antibodies described in APS patients and the fact that some of them are directed against several proteins, in 1992 Vermylen and Arnaut [8] proposed the term "*antiphospholipid-protein antibodies*". Anti-annexin A5 antibodies (a-A5) belong to this particular group of antiphospholipid-protein antibodies.

After the initial report by Matsuda et al. [1] of a women with a-A5 and a history of recurrent pregnancy loss and preeclampsia but without APS, Nakamura et al. [9] studied the effect of these antibodies on the APTT test and found an overlap between a-A5 and LA activity suggesting the role of a-A5 in the autoimmune mechanisms of LA production.

In 1995 Kuragaki et al. [10] discussed the significance of serum and plasma a-A5 levels and suggested that these antibodies are directed against antigenic domains different from the LA binding sites. In 1997 Rand et al. [11] found that APL reduce A5 levels and accelerate plasma coagulation in trophoblast-endothelial cells culture. They postulated that this could be one of the major mechanisms of thrombogenesis and pregnancy loss in APS patients. This article was discussed by two other groups of investigators [12,13] furthering the hypothesis of Rand et al. [11] by discussing the mechanisms of APL action upon A5. Another researcher, Cheng [13], also suggested the participation of a-A5. In response to these studies Rand and Lockwood [14] once again emphasized upon the relationship between APL and A5.

In 2001 Di Simone et al. [15], using a monoclonal antibody against A5, found that the binding of a-A5 with A5 with subsequent syncytiotrophoblastic apoptosis and the inhibition of trophoblast gonadotropin could be one of the underlying mechanisms of the unfavourable effects of a-A5 on embryo implantation and pregnancy outcome.

Using monoclonal human APL and atomic force microscopy (AFM) in 2004, Rand et al. [16] found that plasma samples of patients with positive APL and thromboembolism had a reduction in both the binding of A5 to phospholipids and the anticoagulant activity of A5. Several years earlier, in series of experiments, Hanly and Smith [17] showed that the blocking of A5 binding to procoagulant phospholipids surfaces is anti-b2GPI antibody dependent. This discovery suggests a role for A5 in the pathogenesis of APS. Bas de Laat et al. [18] found that plasma samples from patients with anti-b2GPI antibodies directed against domain I of b2GPI, responsible for the LA activity, lead to a marked increase in A5 resistance.

Studying different antibodies to conventional and non-conventional phospholipids and free phospholipid proteins in female patients with unexplained early fetal loss in 2000, Gris et al. [19] found that only LA, positive IgM antiphosphatidyl and ethanolamine antibodies, positive IgG anti-b2GPI antibodies and positive IgG a-A5 are independent risk factors for the diagnosis of unexplained early fetal loss. Although no clear association with thrombotic complications could be proven, the presence of these antibodies would suggest that initiation of aspirin treatment would be of benefit as aspirin is well tolerated in pregnancy. The authors suggest that A5, also known as placental anticoagulant protein I, is located on the apical surface of the placental syncytiotrophoblast and, with the development of the placenta during pregnancy, a massive exposition of A5 to the circulating immune cells could occur. As T-cell tolerance towards self antigens is limited by the presence of these proteins [20], the abnormally high concentration of endogenous A5 could induce the development of antibodies directed against this substance. Research done in mice by Rand et al. [11] and Wang et al. [21], showed that the infusion of a-A5 decreases the binding of A5 to the apical surfaces of syncytiotrophoblasts in the placenta and leads to placental thrombosis, necrosis and fetal loss. In the current review, we discuss whether a-A5 in humans could induce early local placental thrombosis during the development of the trophoblast and disrupt placental blood flow.

In a study by Ogawa et al. [22], the authors concluded that despite the fact that positive a-A5 were found in some of the patients with primary and secondary APS, the pathogenic role of these autoantibodies is uncertain. This conclusion was based on data showing that the results differed when different plates were used (pretreated vs. not pretreated with gamma-rays). A possible explanation of this phenomenon is that A5 could undergo conformational changes when coming in contact with the irradiated plates. Siaka et al. [23] and Arai et al. [24] also suggest that, in patients with APS and spontaneous abortions, a-A5 are rarely found and they are not a sensitive method for APL detection. Arnold et al. [25] found positive a-A5 in 35% of the ACL-positive and in 29% of the ACL-negative female patients with fetal loss but suggested that a-A5 are, however, not a risk factor for a fetal loss.

In a cohort of female patients with recurrent pregnancy loss, Ulcova-Gallova et al. [26], found positive a-A5 in 13.5% of the cases. Using FITC-labeled a-A5 and an apoptosis investigation kit on trophoblast-placenta material from these women, the authors found apoptotic and necrotic cells in the trophoblast and microthromboses in some intervillous spaces and placental vessels.

Bizzaro et al. [27], found positive a-A5 in 17% of women with recurrent miscarriages. An important finding in this study is that 19% of the ACL-negative patients were a-A5 positive. Moreover, 50% of the patients with one positive APL had positive a-A5. The latter strongly correlated with the presence of recurrent miscarriages. In 2006, Zammiti et al. [28] found that positive a-A5 and anti-b2GPI antibodies are independent risk factors for recurrent pregnancy loss.

3. Significance of the determination of a-A5 in patients with systemic lupus erythematosus and APS

In 1994, Matsuda et al. [2] first described positive a-A5 in 26% of patients with systemic lupus erythematosus (SLE) and suggested that this antibody could be an independent risk factor in these patients. Three years later, Kaburaki et al. [29] found positive a-A5 in 19% of SLE patients and emphasized that these antibodies are found more frequently in patients with arterial and/or venous thromboses, intrauterine fetal loss and prolonged APTT, as well as in SLE patients with negative APL. In 1999 Satoh et al. [30] published similar results and underlined the importance of the determination of a-A5 in SLE patients.

According to Lakos et al. [31] the determination of a-A5 and anti-prothrombin antibodies could support the diagnosis of APS in patients with autoimmune diseases, including SLE. The presence of these autoantibodies is associated with positive anti-b2GPI. Bearing in mind that b2GPI, prothrombin and A5 bind the negatively-charged phosphatidylserine on the surface of activated platelets, the authors suggest that despite their different physiological role, the antibodies against these substances could have the same pathogenic effect. Anti-prothrombin and a-A5 antibodies have low sensitivity (51.4% and 34.2%, respectively) but they are highly specific (85.7% and 94.2%, respectively) for the diagnosis of APS. The high specificity of a-A5 test (94.2%) is comparable to the high specificity of anti-b2GPI antibodies (80%) and LA (97.1%).

Nojima et al. [32] evaluated the significance of a large number of antibodies – those against b2GPI, prothrombin, proteins C and S, A5 – and emphasized that the highest concentration and the prevalence of a-A5 is found in patients with SLE and pregnancy loss. Moreover, in this study the only significant risk factor for fetal loss in these patients were a-A5. On the other hand, de Laat et al. [33] share the contrary opinion that a-A5 are not a risk factor for thrombosis or a pregnancy loss in patients with APS and SLE but the mutations in the A5 gene are an independent risk factor for thromboses and fetal loss, apart from the APS.

Bizzaro et al. [27] found positive a-A5 in 13% of all investigated SLE patients and in 9% of ACL-negative SLE patients. The authors showed that unlike the patients with spontaneous abortions where a-A5 are an important risk factor, the a-A5 positive SLE patients had the lowest risk for thrombosis as discussed above.

4. Anti-annexin A5 antibodies in other conditions

There have been some reports of positive a-A5 in patients with the following diseases and conditions:

- Myocardial infarction [34] – positive a-A5 have been found in 2/62 patients with myocardial infarction under the age of 45. The authors suggest that in these patients the hypercoagulation stated is unrelated to the presence of APS.
- Cerebrovascular disease [35] – positive a-A5 have been found in 2/37 patients with cerebrovascular disease without underlying autoimmune disease. Despite the absence of underlying autoimmune disease, the authors suggest that these autoantibodies may represent an additional risk factor for thrombosis in these patients.
- Scleroderma [36] - positive a-A5 were found in 18.2% of the investigated patients and their presence was associated with digital ischemia.
- Rheumatoid polyarthritis [37] – the authors found high a-A5 concentration and suggest that these antibodies have pathogenic role because they inhibit the effect of annexins on the pro-inflammatory phospholipase A2.
- Takayasu's arteriitis [38] – positive a-A5 were found in 36% of these patients. In patients with active disease this prevalence increases to 53%. The a-A5 are associated with anti-endothelial antibodies, correlate with disease activity and likely have pathogenic role in the development of vasculitis.
- Skin diseases [39] – positive autoantibodies against annexin A1, A2, A3, A4, A5 and A6 have been found in different dermatological conditions, such as autoimmune diseases of the skin, psoriasis, leg ulcerations, malignant melanoma, etc. The distribution of these autoantibodies, including a-A5 is quite homogenous and therefore their determination is not recommended as diagnostic criterion in any of the stated conditions.

5. Discussion

The diagnostic significance of a-A5 antibodies has been studied by many authors. Yet, the results of these studies remain controversial. Some authors focus their efforts on animal models and investigate fundamental issues concerning the role of annexin A5 and a-A5 antibodies in the process of thrombogenesis. Of particular interest is the paper by Lieby et al. [40]. The authors studied 5 randomly selected monoclonal antiphospholipid antibodies from a single patient with APS and their thrombogenic properties, particularly their ability to induce fetal loss in pregnant mice. The results of the study revealed that the only antibody capable ofinducing fetal loss appeared to be the antibody directed against annexin A5. Moreover, its antiphospholipid activity was dependent on annexin A5 and the authors clarified the mutations responsible for its activity. To elucidate the importance of the stated mutations for the antibody pathogenicity, they performed in vitro reversion to the germ-line configuration. The resulting germ-line antibody reacted with several self-antigens, showed only partial loss of its antiphospholipid reactivity, lost its annexin A5 dependence and, which is more importantly, lost its pathogenicity. The results of this study shed new light upon the mechanisms of a-A5 production and pahogenicity.

In terms of the clinical importance of these antibodies, many authors suggest that a-A5 are an additional diagnostic marker in patients with different thrombotic conditions, reproductive problems and autoimmune diseases [2,9,15,19,28,31,32,38].

The study by Nojima et al. [32] suggests that a-A5 are an important risk factor for the development of thromboses in women with fetal loss. Moreover, the authors studied several known thrombogenic risk factors and showed that the only significant risk factor for fetal loss in these patients were a-A5.

Other investigators have not found enough data to support the diagnostic role of these antibodies [22-25,33,39]. The discrepancies among these studies are mainly due to the different diagnostic criteria used, the different disease stages and the different methods of determination of a-A5 [41].

More extensive results on larger populations of patients are needed for the determination of the diagnostic significance of these autoantibodies.

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References

[1] Matsuda J., Gotoh M., Saitoh N., Gohchi K., Tsukamoto M., Yamamoto T., Anti-annexin V antibody in the sera of patients with habitual fetal loss or preeclampsia, Thromb. Res., 1994, 75, 1, 105-6

[2] Matsuda J., Saitoh N., Gohchi K., Tsukamoto M., Anti-annexin V antibody in systemic lupus erythematosus patients with lupus anticoagulant and/or anticardiolipin antibody, Am. J. Hematol., 1994, 47, 1, 56-58

[3] Pengo V., Thiagarajan P., Shapiro S.S., Heine M.J., Immunologic specificity and mechanism of action of IgG lupus anticoagulant, Blood, 1987, 70, 69-76

[4] Harris E.N., Antiphospholipid antibodies, Br. J. Haematol, 1990, 74, 1-9

[5] McNeil H.P., Simpson R.J., Chesterman C.N., Krilis S.A., Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: beta-2-glycoprotein I (apolipoprotein H), Proc. Natl. Acad. Sci. U.S.A., 1990, 87, 4120-4

[6] Galli M., Comfurius P., Maassen C., Hemker H.C., de Baets M.H., van Breda-Vriesman P.J. et al., Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma cofactor, Lancet, 1990, 335, 1554-1557

[7] Matsuura E., Igarashi Y., Fujimoto M., Ichikawa K., Koike T., Anticardiolipin cofactor (s) and differential diagnosis of autoimmune disease, Lancet, 1990, 336, 177-178

[8] Vermylen J., Arnout J., Is the antiphospholipid syndrome caused by antibodies directed against physiologically relevant phospholipid-protein complexes, J. Lab. Clin. Med., 1992, 120, 10-12

[9] Nakamura N., Kuragaki C., Shidara Y., Yamaji K., Wada Y., Antibody to annexin V has anti-phospholipid and lupus anticoagulant properties, Am. J. Hematol., 1995, 49, 347-348

[10] Kuragaki C., Kidoguchi K., Nakamura N., Wada Y., Anti-annexin V antibodies in plasma and serum from patients with lupus anticoagulant, Am. J. Hematol., 1995, 50, 68

[11] Rand J.H., Wu X.X., Andree H.A., Lockwood C.J.,

Guller S., Scher J., Harpel P.C., Pregnancy loss in the antiphospholipid antibody syndrome – a possible thrombogenic mechanism, N. Engl. J. Med., 1997, 337, 154-160

[12] Rote N.S., Antiphospholipid antibodies, annexin V and pregnancy loss, (Letter) N. Engl. J. Med., 1997, 337, 1630-1631

[13] Cheng H.M. Antiphospholipid antibodies, annexin V and pregnancy loss, (Letter) N. Engl. J. Med., 1997, 337, 1630-1631

[14] Rand J.H., Lockwood C.J. Antiphospholipid antibodies, annexin V and pregnancy loss, (Letter) N. Engl. J. Med., 1997, 337, 1630-1631

[15] Di Simone N., Castellani R., Caliandro D., Caruso A., Monoclonal anti-annexin V antibody inhibits trophoblast gonadotropin secretion and induces syncytiotrophoblast apoptosis, Biology of reproduction, 65, 2001, 1766-1770

[16] Rand J.H., Wu X.X., Lapinski R., van Heerde W.L., Reutelingsperger C.P., Chen P.P. et al., Detection of antibody mediated reduction of annexin V anticoagulant activity in plasmas of patients with antiphospholipid syndrome, Blood, 2004, 104, 9, 2783-2790

[17] Hanly J.G., Smith S.A., Anti-beta2-glycoprotein I (GPI) antibodies, annexin V binding and the anti-phospholipid syndrome, Clin. Exp. Immunol., 2000,120, 3, 537-543

[18] de Laat B., Wu X.X., van Lummel M., Derksen R.H., de Groot P.G., Rand J.H., Correlation between antiphospholipid antibodies that recognize domain I of beta2-glycoprotein I and a reduction in the anticoagulant activity of annexin, Blood, 2007,109,4,1490-4

[19] Gris J.C., Quéré I., Sanmarco M., Boutiere B., Mercier E., Amiral J., et al., Antiphospholipid and antiprotein syndromes in non-thrombotic, non-autoimmune women with unexplained recurrent early foetal loss, The Nimes Obstetricians and Haematologists Study – NOHA., Thromb. Haemost, 2000, 84, 228-36

[20] Schild H., Rötzschke O., Kalbacher H., Rammensee H.G., Limit of T cell tolerance to self proteins by peptide presentation, Science, 1990, 247, 1587-1589

[21] Wang X., Campos B., Kaetzel M.A., Dedman J.R. Annexin V is critical in the maintenance of murine placental integrity, Am. J. Obstet. Gynecol., 1999, 180, 1008-1016

[22] Ogawa H., Zhao D., Dlott J.S., Cameron G.S., Yamazaki M., Hata T., et al., Elevated anti-annexin V antibody levels in antiphospholipid syndrome and their involvement in antiphospholipid antibody specificities, Am. J. Clin. Pathol., 2000, 114, 619-628

[23] Siaka C., Lambert M., Caron C., Amiral J., Hachulla E., Hatron P.Y., et al., Low prevalence of anti-annexin V antibodies in antiphospholipid syndrome with fetal loss, Rev. Med. Intern., 1999, 20, 9, 762-765

[24] Arai T., Matsubayashi H., Sugi T., Kondo A., Shida M., Suzuki T., et al., Anti-annexin A5 antibodies in reproductive failures in relation to antiphospholipid antibodies and phosphatidylserine, Am. J. Reprod. Immunol., 2003, 50, 3, 202-208

[25] Arnold J., Holmes Z., Pickering W., Farmer C., Regan L., Cohen H., Anti-beta 2 glycoprotein 1 and anti-annexin V antibodies in women with recurrent miscarriage, Br. J. Haematol., 2001, 113, 911-914

[26] Ulcová-Gallová Z., Mukengnábl P., Hadravská S., Bibková K., Slechtová J., Kyselová V. et al., Placenta and annexin V receptors, antibodies against annexin V and against other phospholipids in patients with recurrent pregnancy loss, Ceska Gynekol., 2006, 71, 469-73

[27] Bizzaro N., Tonutti E., Villalta D., Tampoia M., Tozzoli R., Prevalence and clinical correlation of anti-phospholipid-binding protein antibodies in anticardiolipin-negative patients with systemic lupus erythematosus and women with unexplained recurrent miscarriages, Arch. Pathol. Lab. Med., 2005, 129, 61-68

[28] Zammiti W., Mtiraoui N., Kallel C., Mercier E., Almawi W.Y., Mahjoub T., A case-control study on the association of idiopathic recurrent pregnancy loss with autoantibodies against beta2-glycoprotein I and annexin V, Reproduction 2006, 131, 817-822

[29] Kaburaki J., Kuwana M., Yamamoto M., Kawai S., Ikeda Y., Clinical significance of anti-annexin V antibodies in patients with systemic lupus erythematosus, Am. J. Hematol., 1997, 54, 209-213

[30] Satoh A., Suzuki K., Takayama E., Kojima K., Hidaka T., Kawakami M., et al., Detection of anti-annexin IV and anti-annexin V antibodies in patients with antiphospholipid syndrome and systemic lupus erythematosus, J. Rheumatol., 1999, 26, 1715-1720

[31] Lakos G., Kiss E., Regeczy N., Tarjan P., Soltesz P., Zeher M. et al., Antiprothrombin and anti annexin V antibodies imply risk of thrombosis in patients with systemic autoimmune diseases, J. Rheumatol., 2000, 27, 924-929

[32] Nojima J, Kuratsune H, Suehisa E, Futsukaichi Y, Yamanishi H, Machii T., et al., Association between the prevalence of antibodies to β2-glycoprotein I,

prothrombin, protein C, protein S, and annexin V in patients with systemic lupus erythematosus and thrombotic and thrombocytopenic complications, Clin. Chem. 2001, 47, 6, 1008-1015

[33] de Laat B., Derksen R.H., Mackie I.J., Roest M., Schoormans S., Woodhams B.J., et al., Annexin A5 polymorphism (-1C-->T) and the presence of anti-annexin A5 antibodies in the antiphospholipid syndrome, Ann. Rheum. Dis., 2006, 65, 11, 1468-72

[34] Roldán V., Marín F., Pineda J., Marco P., Corral J., Climent V., et al., Annexin V levels in survivors of early myocardial infarction, Rev. Esp. Cardiol., 2002, 55, 12, 1230-1234

[35] Gaspersic N., Rot U., Cucnik S., Kveder T., Bozic B., Rozman B., Anti-annexin V antibodies in patients with cerebrovascular disease, Ann. Rheum. Dis., 2003, 62, 188-189

[36] Sigiura K., Muro Y., Anti-annexin V antibodies and digital ischemia in patients with scleroderma, J. Rheumatol., 1999, 26, 2168-2172

[37] Dubois T, Bisagni-Faure A, Coste J, Mavoungou E, Menkes CJ, Russo-Marie F., et al., High levels of antibodies to annexin V and VI in patients with rheumatoid arthritis, J. Rheumatol., 1995, 22, 1230-1234

[38] Tripathy N.K., Sinha N., Nityanand S., Anti-annexin V antibodies in Takayasu's arteritis: prevalence and relationship with disease activity, Clin. Exp. Immunol., 2003, 134, 360-364

[39] Bastian B.C., Nuss B., Römisch J., Kraus M., Bröcker E.B., Autoantibodies to annexins: a diagnostic marker for cutaneous disorders?, J. Dermatol. Sci., 1994, 8, 194-202

[40] Lieby P., Poindron V., Roussi S., Klein C., Knapp A.-M., Garaud J.-C., et al., Pathogenic antiphospholipid antibody: an antigen-selected needle in a haystack, Blood, 104, 2004, 6, 1711-1715

[41] Galli M., Barbui T., Prevalence of different anti-phospholipid antibodies in systemic lupus erythematosus and their relationship with antiphospholipid syndrome, Clin. Chem., 47, 2001, 6, 985-987