Annexin V Expression and Anti-Annexin V Antibodies in Type 1 Diabetes

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Background: Annexin V (AnxV) has potent anticoagulant properties and regulatory functions for apoptosis and inflammation. Antibodies against annexin V (anti-AnxVs) may inhibit AnxV functions, leading to thrombosis during autoimmune diseases. Type 1 diabetes is an autoimmune disease and related with an ongoing autoimmune inflammation and thrombotic complications. There is no study evaluating anti-AnxVs/AnxV in a disease setting.

Objective: The aim of this study was to evaluate the status of AnxV and anti-AnxVs in patients with type 1 diabetes.

Methods: One hundred twenty-one patients with type 1 diabetes and 92 healthy controls were included in this study. Serum levels of AnxV and anti-AnxVs and expression of the *AnxV* gene and its common polymorphism in Kozak sequence (−1C>T) were studied. As a functional assay, the binding capacity of AnxV to platelets was evaluated.

Results: As compared with controls, type 1 diabetic patients had significantly low serum AnxV levels and *AnxV* gene expression. The number of anti-AnxV positivity and their serum levels were significantly higher in type 1 diabetic patients than controls. AnxV binding to platelets were significantly decreased in the type 1 diabetic patients. The frequencies of the −1C>T polymorphism of *AnxV* gene did not differ between groups.

Conclusions: This study demonstrated the significant changes in AnxV levels and its function in type 1 diabetic patients. These results support the hypothesis that the defective AnxV system may have a role in ongoing autoimmune activity and the development of thrombotic complications in type 1 diabetes. Further studies are necessary to elucidate the clinical impact of anti-AnxVs and dysregulated AnxV function in type 1 diabetes. **(*J Clin Endocrinol Metab* 99: 932–937, 2014)**

Annexin V (AnxV) belongs to a family of Ca^{2+} dependent phospholipid-binding proteins (1, 2) and exerts potent anticoagulant effects (3, 4). AnxV is also involved in the regulation of apoptosis and protection from both excessive coagulation and inflammatory activities (5–7). Antibodies against AnxV (anti-AnxVs) were determined in several autoimmune disorders (8–10). It was suggested that these antibodies might lead to thrombosis by interfering with the effects of AnxV (6, 8). Recent studies revealed that anti-AnxVs, associated with thrombotic and vasoocclusive events, also caused recurrent abortions in patients with different types of autoimmune disorders (8–11).

Hypercoagulability is a common feature of autoimmune disorders (12, 13). Inflammation and antiphospholipid autoantibodies are the prominent factors among the multiple risk factors that affect the coagulation status during autoimmune diseases (13, 14). Both venous thromboembolism and atherosclerosis through inflammation are linked in most of the autoimmune diseases (15, 16). As an autoimmune disease, type 1 diabetes is related with ongoing islet cell apoptosis and

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
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Received June 21, 2013. Accepted December 13, 2013.
First Published Online January 3, 2014

Abbreviations: anti-AnxV, antibodies against AnxV; AnxV, annexin V; AU, arbitrary unit; HbA1c, glycosylated hemoglobin.

Table 1. Subject Characteristics

Variable	Type 1 DM (n = 121)	Control (n = 92)	*P* Value[a]
Age, mean ± SD	29 ± 9	30 ± 9	NS
Male ,%	52.8	62	NS
BMI, kg/m^2	23.12 ± 4.49	22.78 ± 2.12	NS
Smoker, %	19	16	NS
Duration of diabetes, y	11 ± 9.5	ND	
Retinopathy, n	16	ND	
Nephropathy, n	13	ND	
Neuropathy, n	11	ND	

Abbreviations: BMI, body mass index; DM, diabetes mellitus; ND, not determined; NS, nonsignificant.

[a] Statistical comparisons were performed between patients with type 1 DM and control groups.

inflammation (17–19) and also associated with an increased thrombosis risk (12, 20–22). However, the risk factors other than diabetic ketoacidosis (23) and hyperglycemia (24) promoting a prothrombotic state are not well known.

Although there are several studies regarding AnxV/anti-AnxVs in autoimmune disorders, there is no study that investigates their probable associations with type 1 diabetes yet. In this study, we hypothesized that AnxV expression and function might be affected in patients with type 1 diabetes.

Materials and Methods

Subjects

One hundred twenty-five patients, who were previously diagnosed with type 1 diabetes, were recruited from the patient population attending the Endocrinology and Metabolism Clinic at Ankara University Medical School for this study. One hundred twenty-one of those patients, including 57 women and 64 men with a mean age of 29 ± 9 years, were included in the analyses. The exclusion criteria of the study were a personal history of any thromboembolic or hemorrhagic disease, the use of any medication or substance that affects hemostasis, and patients with any kind of diagnosed malignancy. Ninety-two healthy volunteers with normal fasting plasma glucose levels and without history of vascular, thromboembolic, or hemorrhagic diseases were included as the control group. Four patients who used acetylsalicylic acid were excluded from the study. The study was carried out with the approval from the local ethics committee board (Ankara University Medical School Ethics Committee, 18–596-12), and informed consents were obtained from each participant before enrollment. After an overnight fast of 10–12 hours, all venous blood samples were collected into EDTA, ACD [citric acid/citrate/dextrose, 5:1 (v/v)] containing, and serum separator tubes. EDTA-containing tubes were used for total RNA isolation and glycosylated hemoglobin (HbA1c) level measurements. ACD-containing tubes were used for platelet isolation, and serum separator tubes were used for all ELISA measurements. The samples were stored frozen at −80°C until assayed.

Biochemical measurements

HbA1c levels were analyzed in an ADAMS A1c HA-8160 analyzer (Arkray Inc) by HPLC.

Serum AnxV protein and anti-AnxV

Serum AnxV levels were detected by commercially available ELISA kits (eBioscience). The analytical sensitivity was 0.33 ng/mL. The calculated overall intraassay and interassay coefficients of variation were less than 10% and 11%, respectively. Levels of circulating anti-AnxVs IgG and IgM antibodies were determined by an ELISA technique using high-sensitivity commercial kits (American Diagnostic Inc). Results are expressed according to the A_{450} values obtained for samples and controls, using the calibration curve, and in units relative to the calibrator [arbitrary unit (AU) per milliliter] as follows: negative, less than 10 AU/mL; gray zone, 10–20 AU/mL; low positive, 20 or greater to less than 50 AU/mL; moderate positive, 50 or greater to less than100 AU/mL; and high positive, 100 AU/mL or greater. The calibrator defined as 50 AU/mL is equivalent to plasma containing IgG/IgM isotype autoantibodies to AnxV at a 1:100 dilution.

Isolation of blood platelets and flow cytometric measurement of AnxV binding to platelets

Blood samples from all participants were drawn into ACD solution [citric acid/citrate/dextrose; 5:1 (v/v)]-containing tubes, and platelets were isolated by differential centrifugation of blood (20 min at 200 × *g*). Platelet-rich plasma was separated and centrifuged for 20 minutes at 1000 × *g* to sediment platelets. Platelets were treated as described by Tomer (25). For the exposure of membrane anionic phospholipids, normal platelets were washed three times and resuspended with Ca^{++}/Mg^{++}-free PBS solution to a concentration of 2 to 2.5 × 10^9/L. Aliquots were stored at −80°C until assayed.

To determine the AnxV binding to platelets, the method described by Tomer et al (26) was used. Five microliters of thawed (37°C) platelet preparation were added to 50 μL of the patient plasma, and the samples were incubated with 2 μL phycoerythrin-labeled CD41a (eBioscience) and fluorescein isothiocyanate-labeled AnxV (BD Pharmingen) at room temperature after the

Table 2. Serum Levels of AnxV and Anti-AnxV and Annexin V mRNA Expression

	Type 1 DM (n = 121)	Control (n = 92)	*P* Value
AnxV, ng/mL	5.84 ± 2.39	6.86 ± 3.05	.017
Anti-AnxV IgG, AU/mL	20.05 ± 9.01	3.98 ± 1.89	.001
Anti-AnxV IgM, AU/mL	36.90 ± 13.13	11.01 ± 4.92	.001
AnxV expression, e^{-2a}	0.91 ± 0.38	2.50 ± 1.43	.001

[a] mRNA expression values (mean ± SD) were normalized against β-actin. $P < 0.05$ is considered as statistically significant.

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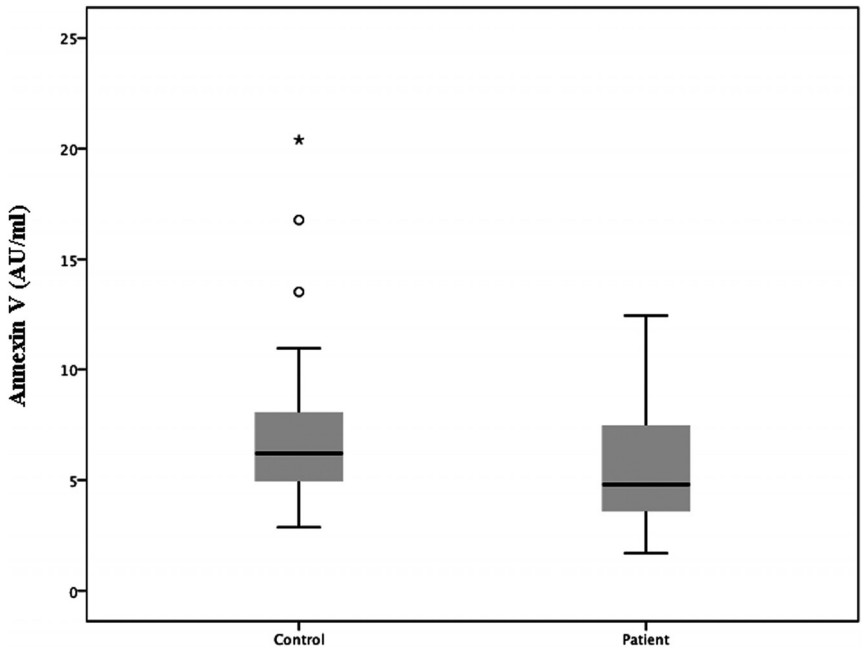

Figure 1. Box plots showing AnxV protein levels of patients with type 1 diabetes (n = 121) and healthy controls (n = 91). The box includes observations from the 25th to the 75th percentile. The horizontal line within the box represents the median value. The upper and lower lines outside the box represent the highest and lowest value, respectively. *, represents significant difference: $P < .05$.

incubation samples were analyzed by flow cytometry (BD FACSCanto II). The results of the AnxV binding to the platelets were expressed in arbitrary fluorescence units.

Genetic analyses

AnxV gene expression

Total RNA was isolated from whole blood samples collected into EDTA-containing tubes using the high pure RNA isolation kit (Roche). cDNA was generated with the PCR system (Thermo Hybaid) using a transcriptor high fidelity cDNA synthesis kit (Roche). Quantitative real-time PCR was performed using the Roche Light Cycler 480 system. *β-Actin* was used as a housekeeping gene for normalization. Samples were analyzed in duplicates. The forward and reverse primers with the identification numbers given below were loaded to RT-PCR according to the manufacturer's recommendations: *AnxV* Roche single assay identification number 107409; β-actin Roche single assay identification number 101125.

Table 3. AnxV IgG and IgM Antibody Results According to the Study Groups

	Antibody Negative	Gray Zone	Antibody Positive
Type 1 DM (n = 121)			
Anti-AnxV IgG, n, %	11 (9.09)	13 (10.74)	97 (80.17)
Anti-AnxV IgM, n, %	9 (7.44)	8 (6.61)	104 (85.95)
Control (n = 92)			
Anti-AnxV IgG, n, %	90 (97.8)	1 (1.1)	1 (1.1)
Anti-AnxV IgM, n, %	67 (72.8)	12 (13.1)	13 (14.1)

Results are expressed as follows: negative, less than 10 AU/mL; gray zone, 10–20 AU/mL; and positive, 20 AU/mL or greater.

−1C>T polymorphism of *AnxV* gene

The −1C>T polymorphism of the *AnxV* gene (rs11575945) was evaluated by polymerase gene reaction amplification with the use of two oligonucleotide primers: *AnxV* forward primer (Hu Annex V F), TAAGCCCGAGGTTTCTT CTC, and *AnxV* reverse primer (Hu Annex V R) AAGAAAAGTGGAAGC GATGTC. The sequences of the fluorescent-labeled probe were ACCTGTGCCATAGCGACTAC and CAGGT CAGGGGAAGGTGAAGCA for the anchored probe. All primer and probes are designed by TIB MOLBIOL GmbH Eresburgstraße. The melting curve analysis is performed by monitoring the temperature-dependent hybridization of the hybridization probe to single-stranded DNA. Because the hybridization probe was T nucleotide specific, the samples that melted off at a higher temperature were considered as T nucleotide.

Statistical analysis

The statistical analyses were performed by SPSS (version 15; SPSS Inc). The data for AnxV protein and anti-AnxVs were expressed as mean ± SD and compared using a Student's *t* test. Categorical parameters were analyzed using the χ^2 test. Correlation analyses were performed within the Pearson correlation test. Values of $P < .05$ were considered as statistically significant.

Results

Demographic and clinical characteristics of the study groups are shown in Table 1. Age, gender, body mass index, and smoking habits were similar between patients with type 1 diabetes and control subjects.

Results of the mean levels of serum AnxV and anti-AnxVs are shown in Table 2. Serum AnxV levels were significantly low in patients with type 1 diabetes when compared with the controls ($P = .017$, Figure 1). In the type 1 diabetic group, 97 patients (80.17%) were anti-AnxV IgG positive and 104 patients (85.95%) were anti-AnxV IgM positive (Table 3). The serum anti-AnxV IgG levels of the patients with type 1 diabetes (20.05 ± 9.01 AU/mL) were significantly higher than the control group (3.98 ± 1.89 AU/mL, $P = .001$). The anti-AnxV IgM levels (36.90 ± 13.13 AU/mL) of the same patient group was also significantly higher than the controls (11.01 ± 4.92 AU/mL, $P = .001$). Correlation analyses between AnxV binding and AnxV protein/anti-AnxV antibody levels showed a weak negative correlation between AnxV binding and serum AnxV level ($r = -0.332$, $P = .051$) and no

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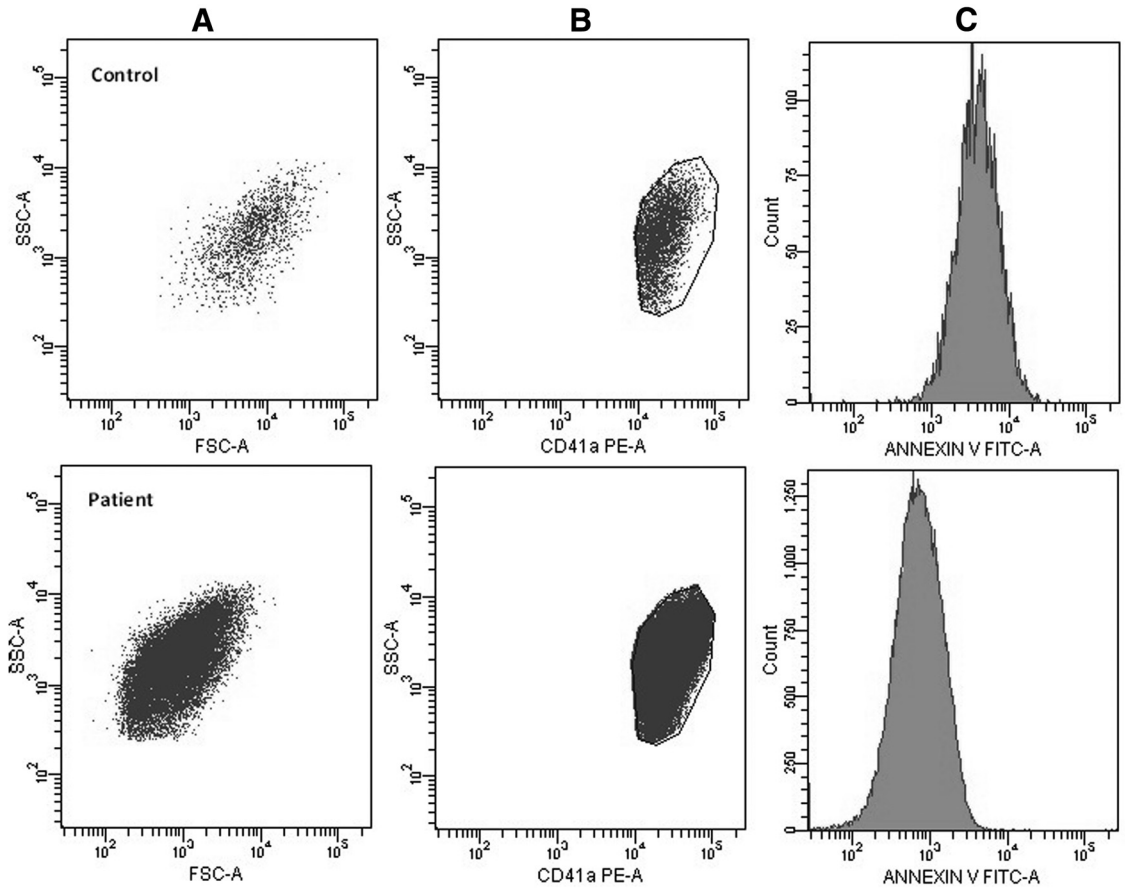

Figure 2. Flow cytometric detection of AnxV binding to platelets. The platelet population was detected by the characteristic light scatter (A) and then resolved from electronic noise and cell debris by the specific immunofluorescence of the phycoerythrin (PE)-labeled anti-CD41a antibody (B). The platelet population was selected and analyzed for the binding of fluorescein isothiocyanate-labeled AnxV. The fluorescence histogram (C) shows an approximately 2-fold decrease in the binding of AnxV in the presence of type 1 diabetic patient plasma.

significant correlation between AnxV binding and anti-AnxV IgG and IgM levels (r = 0.135, $P = .42$, and r = 0.194, $P = .36$, respectively).

The mean HbA1c level of diabetic subjects was 9.6% (normal range; 4.5–6.0). Correlation analysis of HbA1c levels of patients with type 1 diabetes revealed weak, negative correlation with *AnxV* mRNA expression and weak positive correlations with anti-AnxVs IgG and IgM (r=-0.158, 0.052, 0.025, respectively).

Expression of *AnxV* mRNA was significantly reduced in patients with type 1 diabetes when compared with controls ($P < .001$, Table 2). The analysis of −1 C>T polymorphism of the *AnxV* gene showed that there was no significant difference at genotype frequencies (CC, 82.70% and 81.52%, CT, 15.50% and 17.39%, TT, 1.80% and 1.09%, respectively) and allele frequencies between patients with type 1 diabetes and the control group (allele C 0.905 vs 0.902, allele T 0.095 vs 0.098, respectively).

The fluorescence histogram showed an approximately 2-fold decrease in the binding of AnxV in the presence of type 1 diabetic patient plasma ($P = .03$, Figure 2).

Discussion

This cross-sectional study is the first to demonstrate that type 1 diabetic patients had significantly low levels of serum AnxV protein and *AnxV* gene expression when compared with healthy controls. In addition, serum levels of anti-AnxVs and the number of their positivity were found to be significantly higher in type 1 diabetic patients than in controls. The AnxV binding capacity to platelets and a common mutation in the *AnxV* Kozak sequence (C-1T), which may carry a functional effect (27), were also investigated. Although a significant decrease in the binding capacity of AnxV were shown in type 1 diabetic subjects, the frequencies of the −1C>T polymorphism did not differ between the two study groups.

The anticoagulant activities of AnxV are derived from its inhibitory effect on both prothrombin activation (3) and endothelial cell-mediated thrombin formation (4). AnxV particularly binds to phosphatidylserine when it is exposed on the activated cell surface and in that way inhibits the activation of factor X and prothrombin in the blood coagulation cascade (5). These mechanisms regu-

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late apoptosis and protect from both excessive phospholipid-dependent coagulation and proinflammatory activities (5–7). Antibodies against AnxV were detected at first in patients with systemic lupus erythematosus (28). Subsequently, clinical studies have shown the association of anti-AnxVs with the incidence of thrombotic events and recurrent abortions in systemic lupus erythematosus patients with or without antiphospholipid syndrome as well as vasoocclusion in patients with scleroderma (6, 7). These results suggested that anti-AnxVs might interfere with AnxV and lead to thrombosis (6, 8). Furthermore, it was shown that exposure of the antiphospholipid antibodies could reduce AnxV in both placental villi and cultured placental trophoblasts in patients with antiphospholipid-antibody syndrome (29, 30). This antibody-mediated reduction of AnxV was associated with acceleration of plasma coagulation (2, 30).

A more recent study also reported that anti-AnxVs were detected in a significant percentage of patients with acute cerebral ischemia (31). Like most autoimmune disorders, type 1 diabetes is also associated with both arterial and venous thrombotic complications (12, 16, 20–22). According to the suggestion that antiplatelet-anionic-phospholipid autoantibodies interfere with the activity of natural anticoagulant protein AnxV (26), the binding of AnxV to platelets was investigated using the flow cytometry technique. In the presence of type 1 diabetic patients' plasma, a significant decrease in the binding of AnxV to platelets was shown. In addition to the high levels of anti-AnxV, this result also supports the possible functional role of anti-AnxVs in type 1 diabetes.

Recent studies have reported an ongoing islet cell apoptosis and regeneration (17, 19), even in patients with longstanding type 1 diabetes (18). It was previously shown that the treatment of diannexin, a recombinant homodimer of annexin V, was associated with reduced inflammation and β-cell apoptosis and improved the early function of islet grafts after islet transplantation in a murine model (32). In the present study, *AnxV* gene expression and AnxV protein levels were found to be significantly decreased in type 1 diabetic patient. Therefore, the impact of the defective AnxV system and anti-AnxVs in type 1 diabetes, especially regarding ongoing inflammation and apoptosis of β-cells, needs to be further investigated.

A common polymorphism, C to T change at position −1 of the Kozak sequence of the *AnxV* gene, was previously described (33). Gonzalez-Conejero et al (34) reported that the presence of the −1T allele was associated with an increased level of AnxV and reduced the risk of myocardial infarction. They also studied the prognostic impact of the −1C/T polymorphism in the development of a new cardiac event in patients with premature myocardial infarction and suggested that the protective role of the −1T allele was maintained in the long-term follow-up (35). These results were, however, not confirmed by the other studies (36, 37), and the functional impact of this polymorphism is still part of an ongoing debate. In the present study, the CT/TT genotype prevalence was lower than CC, and there was no significant difference between the C and T allele frequencies. No significant association was observed between the −1C>T transition and the levels of AnxV and/or anti-AnxVs as well.

In conclusion, this is the first study to evaluate AnxV protein and anti-AnxV antibodies in type 1 diabetic patients. As compared with healthy controls, AnxV protein and *AnxV* gene expression as well as the AnxV binding capacity are decreased, and anti-AnxV levels are significantly increased in patients with type 1 diabetes. Further studies are necessary to elucidate the pathogenetic and clinical impact of changes in the AnxV system in type 1 diabetes.

Acknowledgments

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This work was supported by Scientific and Technological Research Council of Turkey Grant SBAG-111S249.

Disclosure Summary: The authors have nothing to disclose.

References

1. **Bandorowicz-Pikula J.** *Annexins: Biological Importance and Annexin-Related Pathologies*. Springer; 2003.
2. **Rand JH, Wu XX, Andree HA, et al.** Antiphospholipid antibodies accelerate plasma coagulation by inhibiting annexin-V binding to phospholipids: a "lupus procoagulant" phenomenon. *Blood*. 1998; 92:1652–1660.
3. **Andree HA, Stuart MC, Hermens WT, et al.** Clustering of lipid-bound annexin V may explain its anticoagulant effect. *J Biol Chem*. 1992;267:17907–17912.
4. **van Heerde WL, Poort S, van 't Veer C, Reutelingsperger CP, de Groot PG.** Binding of recombinant annexin V to endothelial cells: effect of annexin V binding on endothelial-cell-mediated thrombin formation. *Biochem J*. 1994;302 (Pt 1):305–312.
5. **Reutelingsperger CP, van Heerde WL, Annexin V.** The regulator of phosphatidylserine-catalyzed inflammation and coagulation during apoptosis. *Cell Mol Life Sci*. 1997;53:527–532.
6. **Esposito G, Tamby MC, Chanseaud Y, Servettaz A, Guillevin L, Mouthon L.** Anti-annexin V antibodies: are they prothrombotic? *Autoimmun Rev*. 2005;4:55–60.
7. **Bozic B, Irman S, Gaspersic N, Kveder T, Rozman B.** Antibodies against annexin A5: detection pitfalls and clinical associations. *Autoimmunity*. 2005;38:425–430.
8. **Nakamura N, Shidara Y, Kawaguchi N, et al.** Lupus anticoagulant autoantibody induces apoptosis in umbilical vein endothelial cells:

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involvement of annexin V. *Biochem Biophys Res Commun*. 1994; 205:1488–1493.
9. **Sugiura K, Muro Y.** Anti-annexin V antibodies and digital ischemia in patients with scleroderma. *J Rheumatol*. 1999;26:2168–2172.
10. **Lakos G, Kiss E, Regeczy N, et al.** Antiprothrombin and antiannexin V antibodies imply risk of thrombosis in patients with systemic autoimmune diseases. *J Rheumatol*. 2000;27:924–929.
11. **Ogawa H, Zhao D, Dlott JS, 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.
12. **Zoller B, Li X, Sundquist J, Sundquist K.** Risk of pulmonary embolism in patients with autoimmune disorders: a nationwide follow-up study from Sweden. *Lancet*. 2012;379:244–249.
13. **Zoller B, Li X, Sundquist J, Sundquist K.** Autoimmune diseases and venous thromboembolism: a review of the literature. *Am J Cardiovasc Dis*. 2012;2:171–183.
14. **Xu J, Lupu F, Esmon CT.** Inflammation, innate immunity and blood coagulation. *Hamostaseologie*. 2010;30:5–6, 8–9.
15. **Poredos P, Jezovnik MK.** The role of inflammation in venous thromboembolism and the link between arterial and venous thrombosis. *Int Angiol*. 2007;26:306–311.
16. **Zoller B, Li X, Sundquist J, Sundquist K.** Risk of subsequent coronary heart disease in patients hospitalized for immune-mediated diseases: a nationwide follow-up study from Sweden. *PloS One*. 2012;7:e33442.
17. **Dor Y, Brown J, Martinez OI, Melton DA.** Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. 2004;429:41–46.
18. **Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC.** Sustained β cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia*. 2005;48:2221–2228.
19. **Bending D, Zaccone P, Cooke A.** Inflammation and type one diabetes. *Int Immunol*. 2012;24:339–346.
20. **Ramagopalan SV, Wotton CJ, Handel AE, Yeates D, Goldacre MJ.** Risk of venous thromboembolism in people admitted to hospital with selected immune-mediated diseases: record-linkage study. *BMC Med*. 2011;9:1.
21. **Bergqvist D, Arnadottir M, Bergentz SE, et al.** Juvenile diabetes mellitus—a risk factor for postoperative venous thromboembolism? *Acta Med Scand*. 1985;217:307–308.
22. **Stein PD, Goldman J, Matta F, Yaekoub AY.** Diabetes mellitus and risk of venous thromboembolism. *Am J Med Sci*. 2009;337:259–264.
23. **Carl GF, Hoffman WH, Passmore GG, et al.** Diabetic ketoacidosis promotes a prothrombotic state. *Endocr Res*. 2003;29:73–82.
24. **Lemkes BA, Hermanides J, Devries JH, Holleman F, Meijers JC, Hoekstra JB.** Hyperglycemia: a prothrombotic factor? *J Thrombos Haemost*. 2010;8:1663–1669.
25. **Tomer A.** Antiphospholipid antibody syndrome: rapid, sensitive, and specific flow cytometric assay for determination of anti-platelet phospholipid autoantibodies. *J Lab Clin Med*. 2002;139:147–154.
26. **Tomer A, Bar-Lev S, Fleisher S, Shenkman B, Friger M, Abu-Shakra M.** Antiphospholipid antibody syndrome: the flow cytometric annexin A5 competition assay as a diagnostic tool. *Br J Haematol*. 2007;139:113–120.
27. **Kenis H, Doggen CJ, Vos HL, Reutelingsperger CP, van Heerde WL.** The C-1T mutation in the annexin A5 Kozak sequence slightly increases the risk of myocardial infarction in men. *J Thromb Haemostas*. 2003;1:2688–2689.
28. **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.
29. **Rand JH, Wu XX, Guller S, Scher J, Andree HA, Lockwood CJ.** Antiphospholipid immunoglobulin G antibodies reduce annexin-V levels on syncytiotrophoblast apical membranes and in culture media of placental villi. *Am J Obstet Gynecol*. 1997;177:918–923.
30. **Rand JH, Wu XX, Andree HA, et al.** Pregnancy loss in the antiphospholipid-antibody syndrome—a possible thrombogenic mechanism. *N Engl J Med*. 1997;337:154–160.
31. **Lee KO, Kim WJ, Na SJ, Heo JH, Lee KY.** Clinical significance of anti-annexin V antibody in acute cerebral ischemia. *J Neurol Sci*. 2011;305:53–56.
32. **Cheng EY, Sharma VK, Chang C, et al.** Diannexin decreases inflammatory cell infiltration into the islet graft, reduces β-cell apoptosis, and improves early graft function. *Transplantation*. 2010; 90:709–716.
33. **van Heerde WL, Jumilly AL, Lux P, et al.** Mutations in the annexin V gene, a new risk factor for venous thrombosis? *Thromb Haemost*. 1999;1:537–537.
34. **Gonzalez-Conejero R, Corral J, Roldan V, et al.** A common polymorphism in the annexin V Kozak sequence (−1C>T) increases translation efficiency and plasma levels of annexin V, and decreases the risk of myocardial infarction in young patients. *Blood*. 2002; 100:2081–2086.
35. **Roldan V, Marin F, Gonzalez-Conejero R, Corral J, Vicente V.** Prognostic value of annexin A5–1 C/T polymorphism in a long term follow-up after premature myocardial infarction. *J Thromb Haemost*. 2007;5:862–863.
36. **van Heerde WL, Kenis H, Schoormans S, Lap P, Reutelingsperger CP.** The −1C>T mutation in the annexin A5 gene does not affect plasma levels of annexin A5. *Blood*. 2003;101:4223–4224; author reply 4224–4225.
37. **Franchi F, Viscardi Y, Cetin I, Bucciarelli P, Martinelli I, Biguzzi E.** Annexin V C/T-1 polymorphism and pregnancy complications. *Haematologica*. 2006;91:864.

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