The Frequency of Anticardiolipin Antibodies and Genetic Mutations Associated with Hypercoagulability Among Patients with Wegener's Granulomatosis with and without History of a Thrombotic Event

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ABSTRACT. ***Objective.*** Venous thrombotic events (VTE), including both deep venous thrombosis and pulmonary emboli, are now recognized as an important complication of Wegener's granulomatosis (WG), but the mechanism(s) of this occurrence is unclear. The frequency of anticardiolipin antibodies (aCL), anti-$ß_2$-glycoprotein antibodies (anti-$ß_2$-GP), and several genetic hypercoagulable factors were examined in a large cohort of patients with WG.

Methods. One hundred eighty patients with active WG had serum and DNA samples collected upon entry into a clinical trial. Of the 180 patients, 29 patients had VTE — 13 before trial entry, 16 during trial. aCL (IgG, IgM, and IgA) and anti-$ß_2$-GP (IgG and IgM) were evaluated in 176 patients. Factor V Leiden (FVL), the prothrombin gene mutation (G20210A, PGM), and methylenetetrahydrofolate reductase (MTHFR) gene mutation were tested in the 29 patients with thrombotic events, and 36 patients without.

Results. aCL occurred with increased frequencies in patients with WG when compared to the general population (1%–5%): 12% had aCL and 3% had anti-$ß_2$-GP. There was no difference in the prevalences of aCL or anti-$ß_2$-GP based on clotting status. The prevalence of the genetic hypercoagulable factors examined in patients with WG was comparable to the reported rates in the general population.

Conclusion. Although the incidence of clinically significant VTE is increased in patients with WG, this increased risk is not explained by increased prevalences of aCL, anti-$ß_2$-GP, FVL, or mutations in PGM or MTHFR. These observations suggest a need to search for new genetic or acquired prothrombotic abnormalities to account for the increased thrombotic event rate in patients with active WG. (First Release Oct 1 2007; J Rheumatol 2007;34:2446–50)

Key Indexing Terms:
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THROMBOTIC EVENTS

Wegener's granulomatosis (WG) is a small-vessel systemic vasculitis with manifestations that often include upper and lower airway disease, glomerulonephritis, and other organ system manifestations. Recently, venous thrombosis has been associated with WG[1,2]. A 20-fold increase in venous thrombotic events (VTE) was observed among the 180

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patients enrolled in the Wegener's Granulomatosis Etanercept Trial (WGET) when compared to the general population[1,3]. Even more striking, perhaps, is the finding that the incidence of VTE in WG is more than 7 times higher than in systemic lupus erythematosus (SLE), an inflammatory rheumatic disease known to be associated with a hypercoagulable state. In contrast to SLE, however, in which a number of hypercoagulable risk factors have been identified, predisposing variables for clotting are unknown in WG. Although there appears to be an association between thrombosis and active vasculitis, the pathophysiology leading to thrombosis is not known.

Several genetic mutations and acquired risk factors have been associated with thrombosis in the general population and in groups of patients with other autoimmune diseases[4-6]. Evaluating the prevalence of such factors in WG is the first step in understanding the pathophysiology of VTE among patients with this disease and ultimately in devising effective therapies. Our study examined the prevalence of several hypercoagulable factors in patients with WG with and without VTE through the testing of baseline samples from the WGET. Understanding the relationships of hypercoagulable factors with VTE in WG could have important clinical implications and would provide further insight into the pathophysiology of VTE in this disease.

MATERIALS AND METHODS

Trial design. Details of the study design and primary results of the WGET have been reported[3,7,8]. The WGET was a randomized, double-blind, placebo-controlled trial of standard therapy with the addition of etanercept or placebo for patients with active WG.

Study subjects. The WGET had 180 study subjects. During the trial, 16 subjects had VTE. In addition, 13 subjects had VTE prior to enrollment in the study. WG status at the time of VTE in subjects that had events before enrollment was available for 12 of the 13 patients and was active in 10 of the 13 patients[1]. Thrombosis rates did not differ based on whether the patient received etanercept or placebo as a part of the trial[1].

Anticardiolipin antibodies (aCL) and anti-ß$_2$-glycoprotein antibodies (anti-ß$_2$-GP) were evaluated in 176 of the 180 WGET subjects. Four patients did not have baseline serum samples available for analysis. The 29 subjects with VTE were evaluated for genetic hypercoagulable factors. An additional 36 subjects without thrombosis were randomly selected for the same genetic testing.

Ascertainment of VTE. Details of the methods used to diagnose and confirm cases of VTE (deep vein thrombosis and/or pulmonary embolism) has been published[1]. For all cases, including those that occurred prior to the study, the VTE in WGET were diagnosed by clinical symptoms and then confirmed by diagnostic tests including vascular ultrasound, impedance plethysmography, ventilation-perfusion scan, computed tomographic angiography, spiral computed tomography, venography, or angiography. Study physicians completed a standardized thrombosis form for each event, recording information from the patient, non-study physicians, and medical records. These forms included the date of event, clinical details, diagnostic test results, and WG disease activity status[1].

Study specimens. Serum and whole blood (DNA) specimens were collected from all subjects in WGET at their baseline visit. They were stored at –80°C and shipped on dry ice. All laboratory personnel were blinded to the VTE status of all subjects.

aCL and anti-ß$_2$-glycoprotein I (anti-ß$_2$-GPI) studies. The titers of aCL (IgG, IgM, and IgA) and anti-ß$_2$-GPI (IgG and IgM) were measured at the Morehouse School of Medicine Antiphospholipid Standardization Laboratory using an ELISA test with a positivity cutoff of 10 units[9-13]. The standardized aCL ELISA was established initially with 200 samples and recently reevaluated in a joint study with The Binding Site in the UK using 1000 samples from healthy donors, with no change in the aCL cutoff values[14]. Anti-ß$_2$-GPI IgG and IgM titers were obtained using the QUANTA-Lite™ kit (Inova Diagnostics, San Diego, CA, USA) following the manufacturer's instructions. Twenty standard M units (SMU) was used as a cutoff point, as established by the manufacturer in 1996 at the 7th International Symposium on Antiphospholipid Antibodies[15]. Samples from the baseline visit were selected for investigation in this study because all participants had active disease at the time of enrollment and the majority of thrombotic events occurred prior to baseline or within several weeks of the baseline evaluation.

Genetic studies. Testing for the identification of the factor V Leiden (FVL) mutation, the prothrombin gene mutation (G20210), and the methylenetetrahydrofolate reductase (MTHFR) gene mutation was performed in the Boston University Whitaker Cardiovascular Institute Laboratory. Genomic DNA was obtained from leukocytes by standard phenolchloroform extraction. FVL, the G20210A mutation in the prothrombin gene, and the 677 C–T substitution in the MTHFR gene were determined by polymerase chain reaction (PCR) and restriction digestion, according to published methods[16-18]. Digested PCR products were separated by electrophoresis on 2.5% ethidium-bromide stained agarose gel[19].

RESULTS

aCL. The results of testing for aCL and anti-ß$_2$-GP antibodies are summarized in Table 1. Twenty-one of the 176 samples tested (12%) had elevated titers of aCL. However, none of the patients evaluated had high positive titers of IgG, IgM, or IgA aCL. Three of the 29 patients (10%) with WG and VTE were positive for aCL IgM (titers 11.2 MPL, 20.3 MPL, and 38.9 MPL, respectively, corresponding to medium- or low-titer results). One patient who did not experience a VTE had a positive aCL IgG, with a titer of 16.6 GPL. Seventeen patients without VTE had elevated aCL IgM. Among these patients, 60% were in the low-positive range (10.1 to 20.0 MPL) and 40% were in the moderately-positive range (between 20.1 and 80 MPL). No patient had detectable IgA aCL at their baseline visit.

anti-ß$_2$-GP antibodies. No participants with thrombosis had increase titers of anti-ß$_2$-GP; 5 of the 147 patients without thrombosis (3%) were positive for anti-ß$_2$-GP.

Genetic studies. The results of the genetic studies are summarized in Table 2. Among the 65 subjects with WG evaluated for genetic abnormalities, 1 patient (1.5%) had FVL mutation, 2 patients (3%) had the prothrombin gene mutation, and 2 patients (3%) were homozygous for the MTHFR mutation. One patient had both prothrombin and MTHFR gene mutations. Among the patients without VTE, 4 (12%) were homozygous for the MTHFR gene mutation. None of the patients with genetic abnormalities were positive for aCL or anti-ß$_2$-GP. Of note, all 3 of the patients found to have FVL and prothrombin gene abnormalities experienced clinically significant VTE. Overall, 57/65 (88%) with WG had none of the abnormalities investigated. Four (14%) of the 29 patients with a history of VTE and 4 (11%) of the 36

Table 1. Anticardiolipin and anti-ß$_2$-GP antibodies in study subjects with Wegener's granulomatosis with and without thrombosis.

	aCL IgG	aCL IgM	aCl IgA	Any aCl Antibody	Anti-ß$_2$-GP IgG	Anti-ß$_2$-GP IgM	Any Anti-ß$_2$-GP	Any aCL or Any Anti-ß$_2$-GP	Any Anti-ß$_2$-GP and Any aCL
VTE, n = 29	0	3	0	3 (10%)	0	0	0	3	0
No VTE, n = 147	1	17	0	18 (13%)	1	5	5* (3.4%)	23	3
Total, n = 176	1	20	0	21 (12%)	1	5	5* (2.8%)	26	3

aCL: anticardiolipin antibodies; anti-ß$_2$-GP: ß$_2$-glycoprotein antibodies; VTE: venous thrombotic event. *1 patient both anti-ß$_2$-GP IgM and IgG-positive.

Table 2. Genetic hypercoagulable factors in study subjects with Wegener's granulomatosis with and without thrombosis.

	Factor V Leiden Mutation Heterozygous	Prothrombin Gene Mutation (G20210A) Heterozygous	MTHFR Mutation Homozygous	Any of 3 Abnormalities	None of 3 Abnormalities
VTE, n = 29 (%)	1 (3)	2* (7)	2* (7)	4 (14)	25 (86)
No VTE, n = 36 (%)	0	0	4 (12)	4 (12)	32 (88)

MTHFR: methylenetetrahydrofolate reductase mutation; VTE: venous thrombotic event. *1 patient both heterozygous for the prothrombin gene mutation and homozygous for MTHFR mutation.

patients without a history of VTE had at least one of the 3 genetic mutations evaluated. Additionally, heterozygosity for the MTHFR mutation was found in 34 of 65 (52%) of the patients with WG: 14/29 (48%) with and 20/36 (56%) without VTE. However, MTHFR heterozygosity is not considered a significant risk factor for thrombotic events.

DISCUSSION

Our results refute the concepts that aCL, anti-ß$_2$-GP, or the common genetic risk factors evaluated in our study explain the high incidence of VTE in patients with WG. This knowledge is important because it allows investigators to pursue different explanations in understanding this phenomenon. Although aCL antibodies occurred with an increased frequency (12%) in our cohort with WG compared to the general population, they were found generally in low titers and were not increased specifically among the group of patients with WG who experienced VTE. aCL are found in 1%–5% of healthy controls, with slight increase in prevalence among elderly and chronically ill patients[6,20]. In patients who have idiopathic VTE, aCL and anti-ß$_2$-GP antibodies have been reported in increased prevalence of 5% to 30%[6,20-22].

In this cohort of patients with WG, the rates of FVL (1%), prothrombin (3%), and MTHFR (9%) gene mutations were not different when compared to the rate in the general population. In the general population, the rates of FVL, prothrombin gene mutation, and MTHFR range up to 8%, 6.5%, and 15%, respectively, with some estimates in each case being substantially lower[5,6,19,23,24]. Among the 29 patients with WG and with a history of VTE, only 4 (14%) were positive for at least 1 of the 3 genetic mutations evaluated. In contrast, in a study of 162 patients with idiopathic VTE, 75 (40%) were found to have mutations in FVL, prothrombin gene mutation, or the MTHFR mutation[5].

Of note, all 3 of the patients found to have FVL and prothrombin gene abnormalities had clinically significant thrombosis. One patient with a documented VTE had both prothrombin and the MTHFR gene mutations. The combination of a mutation in MTHFR with either FVL or the prothrombin gene mutation greatly increases the risk of venous thrombosis in the general population[24]. Although these mutations may have been a factor in the development of a VTE in these 3 patients, these factors clearly cannot explain most of the increased risk of VTE in WG. Thus, while the hypercoagulable factors examined in our study, when present, add to the risk for thrombosis in patients with WG, the number of study subjects with these abnormalities was quite small, and definitive recommendations in regard to screening for these genetic mutations cannot be made.

In our study, 21 of 176 (12%) patients with WG had aCL, including 3 of 29 (10%) patients with thrombosis. The prevalence of aCL among patients with WG has been investigated, and those studies are summarized in Table 3. Similar to our study, all of the listed studies demonstrate an increased frequency of aCL in patients with WG; however, there does not appear to be a relationship between aCL and the increase in thromboses.

There are several notable strengths of our study. This project involved prospectively collected data from a large cohort of patients with well characterized WG. Further, a standardized protocol was used to ascertain and confirm thrombotic events. Laboratory procedures for the assay of hypercoagulable factors were performed by experts in the

Table 3. Frequencies of anticardiolipin antibodies (aCL) and anti-ß$_2$GP antibodies among patients with Wegener's granulomatosis.

Reference*	Sample Size of Patients with WG	Prevalence of aCL (either IgG or IgM) (%)	No. with Thrombosis	Prevalence of aCL in Patients with Thrombosis (%)	Prevalence of Anti-ß$_2$-GP (%)
Hergesell[25]	29	1 (3.4)	NA	NA	NA
Merkel[26]	52	2 (3.8)	NA	NA	NA
Hansen[27]	36	7 (19)	6	2 (29)	0 (0)
Lamprecht[28]	67	32 (48)	11	4 (36)	NA**
Meyer[29]	26	3 (12)	0	0 (0)	NA
Von Scheven[30]	5	3 (60)	5	3 (60)	1 (20)
Present study	176	21 (12)	29	3 (10)	5 (2.8)

* An additional study examined aCL among patients with WG but did not specify the frequency of positive tests[31]. ** Did not separate the presence of aCL from anti-ß$_2$-GP. NA: not available.

field. This is the largest study to measure aCL and anti-ß$_2$-GP in patients with WG and the first study to screen a population of patients with WG for the currently known genetic risk factors for hypercoagulability.

Our study also has some limitations. Levels of aCL and anti-ß$_2$-GP may be affected by immunosuppressive medications; however, the baseline visit in the WGET came within one month of initiation of new immunosuppressive medication doses and glucocorticoids in all patients. Although levels of aCL and anti-ß$_2$-GP would ideally have been measured at the time of thrombosis, such timing was not possible and it is unlikely that it would have changed the conclusion that these antibodies are not responsible for the increased rate of thrombosis in the patients with WG. Second, the types of samples available for testing did not permit us to screen for the frequencies of protein C and S deficiencies and lupus anticoagulant activity, all of which are established risk factors for hypercoagulable states. However, these potential risk factors (along with the antithrombin III gene mutation) have been investigated by others, without the identification of positive associations[2].

Our study provides evidence that aCL, anti-ß$_2$-GP antibodies, FVL, the prothrombin gene mutation, or the MTHFR gene mutation do not explain, either alone or in combination, the increased prevalence of thrombosis in patients with WG. The contribution of many acquired risks for VTE, such as proteinuria, neuropathy, bed rest, obesity, smoking, and hormone replacement, were partially evaluated in this cohort of patients previously published by Merkel, *et al*[1], as well as in a case series of patients in Weidner, *et al*[2]. In addition to these acquired risk factors, inflammation, inflammatory cytokines, endothelial cell damage, endothelial cell antibodies, or a combination of the above with acquired risk factors could all play a role in the pathophysiology of VTE in patients with WG, and have not yet been thoroughly investigated, but should be the focus of future WG research. Additionally, the venous involvement observed in WG differs substantially from patterns of vasculitic involvement known to occur in other major forms of vasculitis, e.g., polyarteritis nodosa, giant cell arteritis, and Takayasu's arteritis, and may be an important contributing factor to the occurrence of VTE in this population.

These observations suggest a need to search for additional prothrombotic abnormalities including genetic mutations, inflammatory mediators, or endothelial cell abnormalities, to account for the increased thrombotic event rate in patients with active WG.

APPENDIX

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