Risk Factors Associated with Thrombosis in Patients with Antiphospholipid Antibodies

KAREN E. HANSEN, DAVID F. KONG, KAREN D. MOORE, and THOMAS L. ORTEL

ABSTRACT. ***Objective.*** To define risk factors associated with thrombosis in patients with antiphospholipid antibodies (aPL).

Methods. Ninety-nine patients with aPL, most of whom had prior thrombosis, were evaluated for the presence of acquired and inherited thrombophilic states. Genomic testing was performed for factor V_{R506Q}, 3' prothrombin (PTG) and methylene tetrahydrofolate reductase (MTHFR) polymorphisms. Clinical records were reviewed for the presence of acquired risk factors (RF) for thrombosis and events associated with aPL. Univariate statistical analysis was performed using Fisher's exact testing. A neural network statistical model was also used to identify which thrombophilic risk factors were most important in development of arterial and venous thrombosis.

Results. For arterial thrombosis, hypertension, tobacco use, hyperlipidemia, and diabetes mellitus were the most important predictors of thrombosis. By contrast, tobacco use, the 3' PTG and factor V_{R506Q} polymorphisms, and previous cardiac surgery were the most important predictors of venous thrombosis.

Conclusion. In this hypothesis-generating retrospective study, acquired risk factors were most important in arterial thrombosis, while the presence of factor V_{R506Q} and 3' PTG polymorphisms were more important in the development of venous thrombosis. These findings are being validated in an ongoing, prospective study. (J Rheumatol 2001;28:2018-24)

Key Indexing Terms:

ANTIPHOSPHOLIPID ANTIBODIES | DIABETES MELLITUS | FACTOR V LEIDEN
HYPERLIPIDEMIA | HYPERTENSION | 3' PROTHROMBIN

Antiphospholipid antibodies (aPL) are of clinical importance in the development of thrombosis. Between 40 and 60% of patients with systemic lupus erythematosus (SLE) and aPL develop thrombosis[1]. It is not fully understood, however, why some individuals with aPL develop thrombosis while others suffer no such event. For example, we recently described 7 families with an inherited form of the antiphospholipid antibody syndrome, in whom 7 of 30 family members (23%) with aPL were asymptomatic[2]. An emerging concept is that other factors also contribute to the prothrombotic state (the so-called two hit hypothesis); the coexistence of other inherited or acquired risk factors is also thought to contribute to subsequent clotting events.

Recent authors have demonstrated that individuals with aPL have a variable incidence of additional prothrombotic risk factors, inherited as well as acquired. Inherited risk factors, such as factor V Leiden (factor V_{R506Q}) and the methylene tetrahydrofolate reductase (MTHFR) polymorphism, have been associated with an increased risk of thrombosis in patients with aPL in certain studies[3,4] but not in others[5-7]. Another study found that hypertension, hyperlipidemia, high homocysteine levels, elevated anti-DNA antibody levels, low complement levels, and aPL were all independent predictors of thrombosis in patients with SLE[8]. However, this study did not determine the effect of multiple variables in a single patient.

In our hypothesis-generating retrospective study, we sought to define the clinical impact of both acquired and inherited causes of thrombosis in 99 patients with aPL. Acquired causes of thrombosis included estrogen use, hypertension, diabetes mellitus, hyperlipidemia, tobacco use, and obesity. Inherited prothrombotic risk factors included factor V_{R506Q}, MTHFR, and the 3' prothrombin gene (PTG) polymorphisms.

We postulated that the coexistence of a second prothrombotic state would modulate the clinical presentation of patients with aPL. We also theorized that foreknowledge of a constellation of thrombophilic risk factors might help

From the Department of Medicine, Divisions of Rheumatology, Cardiology and Hematology, and the Department of Pathology, Duke University Medical Center, Durham, NC, 27710.

Supported in part by a grant (M01-RR-30) from the National Center for Research Resources and the General Clinical Research Centers Program, National Institute of Health. Dr. Ortel's work was supported by a Research Grant from the Arthritis Foundation and a Midcareer Investigator Award in Patient-Oriented Research (K24 AI01603-01).

K.E. Hansen, MD, Fellow, Division of Rheumatology; D.F. Kong, MD, Fellow, Division of Cardiology; K.D. Moore, BS, Research Technician; T.L. Ortel, MD, PhD, Associate Professor of Medicine.

Dr. Hansen's current address, Denver Arthritis Clinic, Suite 510, 4545 East 9th Avenue, Denver, CO.

Address reprint requests to Dr. T.L. Ortel, Division of Hematology, Duke University Medical Center, Box 3422, Durham, NC 27710. E-mail:ortel001@mc.duke.edu

Submitted September 8, 2000; revision accepted February 16, 2001.

predict patients at highest risk for thrombosis. This knowledge could be used to focus therapeutic interventions for an individual, and to design therapeutic interventions in future studies.

MATERIALS AND METHODS

Patients. Ninety-nine patients with aPL seen at Duke University Medical Center agreed to participate in this study. Any patient diagnosed with an aPL (lupus anticoagulant and/or anticardiolipin antibody) through the Clinical Coagulation and/or Clinical Immunology Laboratories between 1995 and 1998 was eligible for this study (about 500 individuals). Patients were randomly recruited through the Rheumatology and High Risk Thrombosis clinics, or by review of testing results from the clinical laboratories. During the span of this study, we estimate approximately half of the patients with aPL were followed in our clinics (about 250 patients), with the remainder being seen in other clinics or during hospitalization only.

A lupus anticoagulant (LAC) was defined as 1) a prolonged phospholipid-dependent screening assay, 2) lack of correction after a 1:1 mix with pooled normal plasma, and 3) correction of a prolonged screening assay by addition of excess phospholipid. Anticardiolipin antibody (aCL) IgG and IgM levels were determined as described by Loizou and colleagues[9], using 10% fetal bovine serum for blocking. The antiphospholipid antibody results for the 99 patients in this study are summarized in Table 1. Seventy-seven patients had aPL documented on 2 (or more) occasions greater than 4 weeks apart. The institutional review board approved this study, and all patients gave informed consent.

Information was collected by systematic chart review on clinical events associated with aPL, comorbid diseases, and acquired risk factors for thrombosis. Thrombotic events included venous thrombosis (deep vein thrombosis, pulmonary embolus) and arterial thrombosis (myocardial infarction at age less than 65 years, stroke at age less than 60 years with no other apparent cause[10], or transient ischemic attack). Additional clinical events included miscarriage, vascular access occlusion, and avascular necrosis. SLE was classified by the presence of 4 American College of Rheumatology criteria as updated in 1997[11]. Primary antiphospholipid antibody syndrome (APS) was defined as the presence of aPL in association with a clinical event (an arterial or venous thromboembolic event, as described above, and/or recurrent miscarriages), and in the absence of connective tissue disease or other secondary cause of aPL[12].

Comorbid diseases or acquired risk factors for thrombosis included hypertension, hyperlipidemia, malignancy, diabetes mellitus, obesity (body mass index > 27), use of oral contraceptives or hormone replacement therapy and tobacco use. Age, gender, race, and prior cardiac surgery were also recorded in the database. Twenty-five patients were evaluated for risk factors at the time of the event. The remainder were seen for thrombosis elsewhere and were then referred to Duke University for evaluation. Most referrals occurred within several months of the thrombotic event, and since the majority of acquired risk factors for thrombosis are chronic rather than acute (i.e. obesity, diabetes), we assumed for this retrospective study that the given risk factor was present at the time of thrombosis.

Table 1. Summary of patients with aPL.

Antiphospholipid Antibody	Patient (n = 99)
aCL	74
Isotype	
IgG only	29
IgM only	18
IgG and IgM	27
Titer (units)	
IgG (16–40 GPL)	21
IgG (41–80 GPL)	14
IgG (> 80 GPL)	21
IgM (11–20 MPL)	14
IgM (21–40 MPL)	18
IgM (> 40 MPL)	13
Lupus anticoagulant (LAC)	83
LAC only	25
LAC and aCL	58

Genomic DNA was prepared from peripheral blood leukocytes or from buccal cell brushings, as described[2]. Laboratory testing for thrombophilic genetic states included analyses for factor V_{R506Q}[2], thermolabile MTHFR[13] and 3' PTG[14] polymorphisms. Serum homocysteine levels were available for fewer than half the patients, and this variable was therefore not analyzed in this study.

Statistical analyses. The associations between clinical events, acquired risk factors, and inherited causes of thrombosis were examined using Fisher's exact tests. Two tailed p values are reported as a measure of the unadjusted univariate association between thrombophilic states and outcomes.

The entire population of 99 patients was used to construct multivariate computer models for predicting arterial or venous thrombosis in the setting of aPL. Eighteen candidate variables were identified in the data set. These included age at first thrombotic event, race, gender, use of oral contraceptives or hormone replacement therapy, hypertension, hyperlipidemia, tobacco use, diabetes mellitus, body mass index, rheumatologic diagnosis, prior cardiac surgery, neoplasia, IgM and IgG aCL titers, and the presence of factor V_{R506Q}, MTHFR, and 3' PTG polymorphisms. Arterial and venous thrombotic outcomes were measured as binary endpoints.

Of the 99 patients in the study population, lipid status, body mass index, estrogen use, and tobacco use were known for 56, 72, 94, and 96 patients, respectively. Median values from the remainder of the population were used to impute values for the missing data. There was no relationship between missing data for lipid status or body mass index and the occurrence of venous or arterial thromboembolism.

The limited size of the patient population and number of candidate variables precluded fitting of a traditional logistic regression model. Artificial neural network (ANN) techniques[15-18] were applied to identify the most important candidate variables and create an initial set of models for predicting the likelihood of arterial and venous thrombosis. The neural networks were fitted with a single hidden layer to screen for nonlinear relationships among the independent variables. This ANN architecture can emulate complex nonlinear transformations and interactions among the predictors[19].

To avoid overfitting and improve applicability of the models to patients with aPL in general, a bootstrap resampling technique was used to internally validate the neural network models[20]. To bootstrap a sample of 50 patients, one randomly performs 50 draws from the population (with replacement). The new bootstrapped data set contains 50 cases, although some cases are replicates, and not all of the original cases are represented. This process was repeated 100 times, to create 100 bootstrapped data sets, each of size 50. The bootstrapped data sets are samples of the original data set, in the same fractal sense that the original data set is a sample of the larger universe of data to which we wish to generalize. This allows us to derive statistics that apply approximately to the behavior of the original data set, relative to the larger universe of all patients with aPL.

The most important property of a predictive model is discrimination, the ability of a model to distinguish between patients who are likely to have a thrombotic event and those who are not. In this study, the discrimination of the neural network models was measured using the c-index (concordance probability)[21] , which is equal to the area under a receiver operating characteristic (ROC) curve[22]. In other words, the c-index is the probability that, of 2 patients drawn randomly from the population, one who had a thrombotic event and the other who did not, the patient with the thrombotic event would have the higher prediction of thrombosis. A perfect predictor has a c-index of 1.0, while a chance predictor (flipping a coin) has a c-index of 0.5.

For each outcome, a c-index was calculated for a full model using the original data set, and an average c-index was calculated for a series of

models fitted on bootstrapped data sets. Because the bootstrapped data sets represent a subset of information contained in the full data set, c-indices for models fitted to bootstrapped data sets will usually be lower than when the full data set is used to fit the model.

RESULTS

Patient population. Fifty-eight of the 99 patients had primary APS, 25 had SLE, and 6 had other connective tissue diseases (1 with rheumatoid arthritis and 5 with undifferentiated connective tissue disease). This distribution was similar for the subset of 77 patients with aPL documented on 2 or more occasions at Duke. Fifty-eight patients were female. Seventy-eight patients were Caucasian, 19 were African American, and 2 were of other ethnicity. Five had a neoplasm (one each with throat cancer, chronic lymphocytic leukemia, and colon cancer, one with cervical and metastatic colon cancer, and one with lymphoma and breast cancer).

Seventy-eight patients had sustained one or more thromboembolic event; 41 patients had venous, 25 had arterial, and 12 had both arterial and venous events. The average age at time of first clinical event was 40 yrs (range 15-77 yrs). Other clinical manifestations included avascular necrosis (2 patients), miscarriage (16 patients, 4 in isolation), and isolated vascular access graft occlusion (3 patients). Since the numbers of patients with these events was small, further statistical analyses were not performed on these subsets. Twelve patients had no aPL-associated clinical event.

Seventeen patients had an ischemic stroke. The median age at the time of the event was 42 yrs (range 19-57 yrs). Four also had venous thrombosis and another 3 had one or more miscarriages. Hypertension was present in 11, hyperlipidemia in 7, tobacco use in 8, estrogen use in 5, and diabetes in 2, respectively.

Seven patients had a myocardial infarction. The median age at the time of the event was 49 yrs (range 34-66 yrs). Two patients had venous thrombosis as well. Cardiac catheterization was performed in 5 patients and showed normal vessels in one, non-occlusive disease in 2, and coexisting atherosclerosis in 2. Hypertension was present in 2, hyperlipidemia in 3, tobacco use in 3, and high body mass index in 3 patients, respectively.

To determine important coexistent risk factors for thrombosis, we compared those patients with venous and arterial thrombosis to asymptomatic patients with aPL in a statistical model. There were not adequate numbers of patients with the other clinical manifestations associated with these antibodies to allow reliable statistical analysis of important coexistent thrombophilic factors in those settings.

Inherited thrombophilic disorders. Eighteen patients had one or more thrombophilic polymorphisms; 8 were heterozygous for factor V_{R506Q}, 3 were heterozygous for the 3' PTG polymorphism, and 9 were homozygous for the MTHFR polymorphism (including 2 who also carried the factor V_{R506Q} polymorphism). The clinical events noted in the patients with thrombophilic polymorphisms are summarized in Table 2.

Sixteen of the 18 patients with thrombophilic polymorphisms had venous and/or arterial thrombosis ($p = 0.347$). One individual homozygous for the MTHFR polymorphism had no aPL-associated clinical events; another had avascular necrosis. The presence of factor V_{R506Q} or the 3' PTG polymorphism was associated with a significantly increased risk of venous thrombosis (10 of 11 compared to 43 of 88 patients, $p = 0.0095$) but not arterial thrombosis. Only 5 patients with the MTHFR polymorphism had venous thrombosis, including 2 who were heterozygous for factor V_{R506Q}. The MTHFR polymorphism was not statistically associated with any form of thrombosis.

The number of clinical events per person was 2.67 in those with the 3' PTG polymorphism, 2.13 in those with factor $V_{R506Q,}$ 1.67 in patients with homozygous MTHFR polymorphism, and 1.8 in patients with aPL alone. One of the 3 patients with 3' PTG polymorphism had an aggressive prothrombotic state with multiple recurrent thrombotic events despite high-intensity anticoagulation, influencing the high number of events per person in this subgroup.

An inherited thrombophilic polymorphism was found in

Table 2. Summary of thrombophilic genotypes and phenotypes in 99 patients with aPL.

	Factor V_{R506Q} (n = 8)	MTHFR Homozygotes (n = 9)*	3′ Prothrombin Gene Polymorphism (n = 3)	aPL Alone (n = 81)
Venous thrombosis (%)	4 (50	4* (44.4)	2 (66.6)	32 (39.5)
Arterial thrombosis (%)	1 (12.5)	2 (22.2)	0	22 (27.1)
Arterial and venous thrombosis (%)	3 (37.5)	1 (11.1)	1 (33.3)	8 (9.9)
Miscarriage (%)	2 (25)	1 (11.1)	0	13 (16.0)
Avascular necrosis	0	1 (11.1)	0	2 (2.5)
No event	0	1 (11.1)	0	11 (13.6)
Total clinical events per person	2.13	1.67	2.67	1.8
Average age at first clinical event (yrs)	41.3	45.6	45.7	29.8
Acquired risk factors per person	1.38	1.56	1.67	1.70

* Two also carried factor V_{R506Q} polymorphism, one with both arterial and venous, another with venous thrombosis.

1 asymptomatic patient with aPL, compared to 17 of 87 patients who experienced an aPL-associated event. The odds ratio (OR) of thrombosis in a patient with both aPL and a thrombophilic polymorphism was 2.67 [95% confidence interval (CI) 0.32-22.15]. An average of 1.38 acquired risk factors per person was present in the group with aPL and no clinical events, compared to 1.5 risk factors per person in the primary APS group, and 1.62 risk factors per person in the group with SLE and thrombosis.

Univariate statistical analysis. Table 3 summarizes data and statistical analysis on the impact of acquired risk factors for thrombosis, using Fisher's exact test. Hypertension, elevated lipids, diabetes and tobacco use were each statistically associated with arterial thrombosis. The combination of factor V_{R506Q} or 3' PTG mutations with aPL was statistically associated with venous thrombosis. Estrogen use, high body mass index, prior cardiac surgery, and cancer were not statistically associated with an increased incidence of venous or arterial thrombosis.

Neural network multivariate models. A summary of the distribution of raw predictions from neural networks fitted using the bootstrapped data sets is illustrated in Figure 1 which depicts the relative ability of the neural network models to separate those patients who are likely to have an event from those who are not. These relationships can be quantified using the c-index. For arterial thrombosis, the model had a c-index of 0.83 when fitted on the full data set, and an average c-index of 0.80 when fitted on bootstrapped data sets. Hypertension, tobacco use, hyperlipidemia, and diabetes mellitus were the major predictors of arterial thrombotic events. For venous thrombosis, the model had a c-index of 0.85 when fitted on the full data set, and an average c-index of 0.78 when fitted on bootstrapped data sets. Tobacco use, 3'PTG and factor V_{R506Q} polymorphisms, and previous cardiac surgery were the principal contributors to venous thrombotic events.

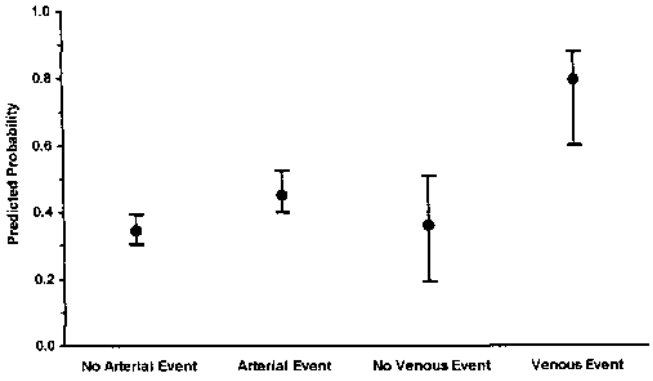

Figure 1. Distribution of neural network predicted probabilities, by outcome, for 99 study patients. For each distribution, the center dot indicates the 50th percentile; dash, 25th and 75th percentiles.

Ethnic influence. Ethnic differences in inherited and acquired thrombophilic risks are summarized in Table 4. Factor $V_{R506Q,}$ MTHFR, and 3' PTG polymorphisms were seen in 0, 1, and 1 of African American patients, compared to 8, 8, and 2 Caucasian patients respectively. African Americans sustained more arterial events ($p = 0.066$) and statistically fewer venous thrombotic events ($p = 0.038$) than Caucasian patients. African American patients also sustained a higher number of aPL-associated clinical events per person than Caucasians (2.16 versus 1.81 events per person). There was no statistical difference between ethnic groups regarding miscarriage, avascular necrosis, or the absence of clinical events. African American patients had a higher number of acquired risk factors (2.3 versus 1.5 risk factors per person) and were statistically more likely to have hypertension, elevated lipids and diabetes per person than Caucasian patients.

Rheumatologic diagnosis. There was no statistical difference between SLE and primary APS subgroups in the type of clinical events or incidence of acquired risk factors, with the exception of estrogen use, which was more common in patients with SLE ($p = 0.0031$). In patients with SLE, estrogen use (oral contraceptives or hormone replacement

Table 3. Acquired risk factors for arterial and venous thrombosis. For estrogen use, hyperlipidemia, tobacco use, and body mass index, clinical data were not available for all subjects. Individuals for whom data was not available were not included in the particular statistical analysis for that risk factor.

Risk Factor	Arterial Thrombosis	p	Venous Thrombosis	p
Estrogen use, n = 18 of 94	6 of 18, vs 30 of 76	NS	11 of 18, vs 40 of 76	NS
Hypertension n = 39 of 99	22 of 39, vs 15 of 60	0.0027	15 of 39, vs 38 of 60	0.0229
Elevated lipids, n = 21 of 56	16 of 21, vs 7 of 35	< 0.0001	5 of 21, vs 26 of 35	0.0003
Tobacco use, n = 33 of 96	18 of 33, vs 19 of 63	0.0273	12 of 33, vs 39 of 63	0.0196
Diabetes mellitus, n = 10 of 99	7 of 10, vs 30 of 89	0.0371	3 of 10, vs 50 of 89	NS
Body mass index > 27, n = 45 of 72	16 of 45, vs 10 of 27	NS	25 of 45, vs 14 of 27	NS
Cardiac surgery, n = 9 of 99	6 of 9, vs 31 of 90	NS	2 of 9, vs 51 of 90	NS
Factor V_{R506Q} & 3′ PTG mutations, n = 11 of 99	5 of 11, vs 32 of 88	NS	10 of 11, vs 43 of 88	0.0095
Neoplasia, n = 5 of 99	2 of 5, vs 35 of 94	NS	3 of 5, vs 50 of 94	NS

Statistical analysis was performed using Fisher's exact test. NS: not statistically significant.

Table 4. Ethnic differences in inherited and acquired risk factors and clinical events.

	Caucasian, n = 78	African American, n = 19	p Value
Factor V_{R506Q} (%)	8 (10.3)	0	NS
3′ PTG (%)	2 (2.56)	1 (5.26)	NS
MTHFR (%)	8 (10.3)	1 (5.26)	NS
Venous thrombosis (%)	47 (60.2)	6 (31.6)	0.038
Arterial thrombosis (%)	26 (33.3)	11 (57.9)	0.066
Miscarriage (%)	15 (19.2)	1 (5.26)	NS
Avascular necrosis (%)	2 (2.56)	1 (5.26)	NS
No clinical event (%)	9 (11.5)	2 (10.5)	NS
Clinical events per person	1.8	2.2	
Use of oral contraceptives	8	0	NS
Hormone replacement therapy	9	2	NS
Hypertension	26	13	0.0082
Elevated lipids	14 of 45	7 of 10	0.033
Tobacco use	25	7	NS
Diabetes mellitus	5	5	0.023
Body mass index ≥ 27	34 of 55	9 of 14	NS

NS: not statistically significant.

therapy) was noted in 8 of 15 women with thrombosis, compared with 1 of 4 without thrombosis (OR 3.4, 95% CI 0.29-40.97). In patients with SLE and venous thrombosis, estrogen use was noted in 6 of 10 women, compared to 2 of 8 with SLE and no venous thrombosis (OR 4.5, 95% CI 0.58-34.63).

DISCUSSION

We sought to explain why some individuals with aPL develop thrombosis, while others do not. In this preliminary, hypothesis-generating retrospective study, we sought candidate variables that influenced not only the development of thrombosis, but also the site at which the event would occur. In both univariate and multivariate analyses, acquired risk factors appear to be important in predicting arterial thrombotic events including hypertension, tobacco use, hyperlipidemia and diabetes mellitus. In contrast, the 3'PTG and factor V_{R506Q} polymorphisms, along with tobacco use and prior cardiac surgery, were important in predicting venous thrombosis.

In support of these findings, Petri, *et al* have identified several factors that increase the risk of thrombosis in patients with aPL, including hypertension, hyperlipidemia, and elevated homocysteine levels[8,23,24]. While our study did not demonstrate a relationship between the MTHFR polymorphism and thrombosis, homocysteine levels were not measured in the majority of our patients and so we cannot comment on the importance of this variable. Several studies have demonstrated that the coexistence of 2 prothrombotic states increases the risk of thrombosis in a given individual[25-28]. For example, the combination of the 3' PTG and factor V_{R506Q} polymorphisms has been associated with recurrent venous thrombosis[28], and other investigators have shown that the combination of aPL with one of these polymorphisms can increase the risk of a thrombotic event in a given individual[3,4]. Although these polymorphisms have been associated with arterial thrombosis in certain patient subsets[29-32], we did not find them to be additional risk factors for arterial events in our patients with aPL.

In vitro and *in vivo* work has demonstrated putative mechanisms by which some of the acquired risk factors cause thrombosis. Matsuda, *et al* reviewed the literature on hypercoagulability in diabetes mellitus[33]. Studies have demonstrated increased platelet aggregation and thromboxane production, and increased fibrinogen, thrombin-antithrombin complexes, and von Willebrand factor levels[33]. Schafer summarized effects of hyperlipidemia on the hypercoagulable state[34]. These include increased platelet aggregation and platelet thromboxane production[34]. Pravastatin was shown to decrease platelet thrombosis on injured endothelium in patients with hyperlipidemia[35]. There are little data in the literature on the influence of tobacco on hemostasis. Moschos, *et al* studied the effects of passive smoke in beagles, and found increased turnover of fibrinogen, particularly in dogs on a high lipid diet[36]. Also noted were shortened clotting times and partial thromboplastin times along with decreased platelet counts and increased platelet aggregation, suggesting but not proving a state of accelerated coagulation.

Nine of 99 patients had undergone cardiac surgery. Bovine thrombin is used for local hemostasis during many forms of surgery, including cardiovascular surgery. We have demonstrated the production of antibodies to bovine thrombin in patients undergoing surgery with known use of bovine thrombin, with sera prior to surgery being negative[37]. In patients undergoing cardiac surgery, bovine thrombin exposure may also result in the production of aPL, which may potentially be associated with thrombotic complications[38]. We are studying this issue in a separate project.

The use of estrogens in women with both SLE and aPL is felt to be prothrombotic by some clinicians[39]. In our study, women with SLE and estrogen use had a high OR for venous thrombosis, but with CI crossing one. In the statistical models, estrogen use was lower in importance than several other variables. Further study is needed to answer the question of whether estrogen use is prothrombotic in women with SLE and aPL.

It is possible that selection bias may have influenced the results of our study. Individuals referred to an academic institution may not perfectly represent all patients with aPL in terms of the number of comorbid illnesses, acquired risk factors for thrombosis, severity of thrombotic state, or other factors. Misclassification of events could contribute to the high impact of acquired risk factors in arterial events. Nevertheless, of the 99 patients studied, 21 had never sustained a thromboembolic event. Another weakness of the

study is its retrospective nature. The presence of acquired risk factors for thrombosis was based on chart review of each patient, and only about 25% of the patients had clear documentation of the acquired risk factors at the time of thrombosis. The remainder of patients had a thrombotic event elsewhere and were later referred to our institution, so that assumptions regarding the presence of these risk factors were made. Since most acquired risk factors for thrombosis are chronic, this assumption is a reasonable one. Nevertheless, a prospective study would be ideal in its ability to determine cause and effect.

Finally, the statistical methods used in this study are based on the large number of variables examined in a relatively small number of patients. Indeed, we were unable to use traditional logistic regression analysis in this study because of our data set. However it is reassuring that both statistical models found the same results, and that other groups have found similar results regarding the importance of hypertension and hyperlipidemia.

We are currently recruiting patients with aPL with and without aPL-associated thrombotic events. In a prospective study, we plan to test our initial findings, using individuals from both a community-based private practice and an academic institution. A larger study is planned with power calculations to allow confirmation of our initial findings. Our ultimate goal is to provide an individual with aPL information on his/her relative risk of thrombosis. Intervention studies using warfarin or anti-platelet agents could be designed for patients at highest risk of thrombosis.

ACKNOWLEDGMENT

The authors would like to thank Michael Schiff for his thoughtful review of the manuscript, and Keith F. Klemp for assistance with patient genotyping.

REFERENCES

1. Love PE, Santoro SA. Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Ann Intern Med 1990;112:682-98.
2. Goel N, Ortel TL, Bali D, et al. Familial antiphospholipid antibody syndrome: criteria for disease and evidence for autosomal dominant inheritance. Arthritis Rheum 1999;42:318-27.
3. Ames PRJ, Tommasino C, D'Andrea G, Iannaccone L, Brancaccio V, Margaglione M. Thrombophilic genotypes in subjects with idiopathic antiphospholipid antibodies - prevalence and clinical significance. Thromb Haemost 1998;79:46-9.
4. Simantov R, Lo SK, Salmon JE, Sammaritano LR, Silverstein RL. Factor V Leiden increases the risk of thrombosis in patients with antiphospholipid antibodies. Thromb Res 1996;84:361-5.
5. Bentolila S, Ripoll L, Drouet L, Crassard I, Tournier-Lasserve E, Piette J-C. Lack of association between thrombosis in primary antiphospholipid syndrome and the recently described thrombophilic 3'- untranslated prothrombin gene polymorphism. Thromb Haemost 1997;78:1415-21.
6. Sasso EH, Suzuki LA, Thompson AR, Petri MA. Hereditary resistance to activated protein C: an uncommon risk factor for thromboembolic disease in lupus patients with antiphospholipid antibodies. Arthritis Rheum 1997;40:1720-1.
7. Dizon-Townson D, Hutchison C, Silver R, Branch DW, Ward K. The factor V Leiden mutation which predisposes to thrombosis is not common in patients with antiphospholipid syndrome. Thromb Haemostas 1995;74:1029-31.
8. Petri M. Thrombosis and systemic lupus erythematosus: The Hopkins lupus cohort prospective study. Scand J Rheum 1996;25:191-3.
9. Loizou S, McCrea JD, Rudge AC, Reynolds R , Boyle CC, Harris EN. Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. Clin Immunol Immunopathol 1985; 62:738-45.
10. Levine SR, Brey RL, Sawaya KL, Salowich-Palm L, Kokkinos J, Kostrzema B. Recurrent stroke and thrombo-occlusive events in the antiphospholipid syndrome. Ann Neurol 1995;38:119-24.
11. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:17-25.
12. Piette J-C. 1996 diagnostic and classification criteria for the antiphospholipid/ cofactors syndrome: a 'mission impossible'? Lupus 1996;5:354-63.
13. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nature Genet 1995;10:111-3.
14. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-703.
15. Goodman PH. NevProp software version 4, Reno, NV: University of Nevada. URL:http://www.scs.unr.edu/nevprop/
16. Kappen HJ, Neijt JP. Neural network analysis to predict treatment outcome. Ann Oncol 1993;4 Suppl 4:S31-S34.
17. Itchhaporia D, Snow PB, Almassy RJ, Oetgen WJ. Artificial neural networks: current status in cardiovascular medicine. J Am Coll Cardiol 1996;28:515-21.
18. Baxt WG. Application of artificial neural networks to clinical medicine. Lancet 1995;346:1135-8.
19. Goodman PH, Harrell FE. Neural networks: advantages and limitations for biostatistical remodeling. 1998: URL:http://www.scs.unr.edu/nevprop/
20. Efron B, Tibshirani R. An introduction to the bootstrap. New York: Chapman & Hall; 1993.
21. Harrell FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. JAMA 1982;247:2543-6.
22. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29-36.
23. Petri M, Hellmann D, Hochberg M, Meyerhoff J, Bell W, Goldman D. Arterial thrombotic events (TE) in SLE: The Baltimore lupus cohort study [abstract]. Arthritis Rheum 1994;37 Suppl 9:S297.
24. Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J, Rosenberg IH. Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet 1996;348:1120-4.
25. Cattaneo M, Tsai MY, Bucciarelli P, et al. A common mutation in the methylenetetrahydrofolate reductase gene (C677T) increases the risk for deep-vein thrombosis in patients with mutant factor V (Factor V:Q^{506}). Arterioscler Thromb Vasc Biol 1997;17:1662-6.
26. Koeleman BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C-deficient families. Blood 1994;84:1031-5.
27. Vandenbroucke JP, Koster T, Briet E, Reitsma PH, Bertina RM, Rosendaal FR. Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. Lancet 1994;344:1453-7.
28. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep vein thrombosis among heterozygous carriers of both

factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999;341:801-6.
29. De Stefano V, Chiusolo P, Paciaroni K, et al. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular disease in young adults. Blood 1998;91:3562-5.
30. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood 1997;90:1747-50.
31. Rosendaal FR, Siscovick DS, Schwartz SM, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood 1997;89:2817-21.
32. Simioni P, De Ronde H, Prandoni P, Saladini M, Bertina RM, Girolami A. Ischemic stroke in young patients with activated protein C resistance. A report of three cases belonging to three different kindreds. Stroke 1995;26:885-90.
33. Matsuda T, Morishita E, Jokaji H, Asakura H, Saito M, Yoshida T. Mechanism on disorders of coagulation and fibrinolysis in diabetes. Diabetes 1996;45:S109-S110.
34. Schafer AI. The hypercoagulable states. Ann Intern Med 1985;102:814-28.
35. Lacoste L, Lam JYT, Hung J, Letchacovski G, Solymoss CB, Waters D. Hyperlipidemia and coronary disease: correction of the increased thrombogenic potential with cholesterol reduction. Circulation 1995;92:3172-7.
36. Moschos CB, Ahmed SS, Lahiri K, Regan TJ. Chronic smoking in an animal model: effects on clotting and fibrinolysis. Atherosclerosis 1976;23:437-42.
37. Ortel TL, Neal MC, Thames EH, Moore KD, Lawson JH. Immunologic impact and clinical outcomes after surgical exposure to bovine thrombin. Ann Surg 2001;233:88-96.
38. Ortel TL, Moore KD, Fleming J, Slade K, Neal MC, Thames EH: Antiphospholipid antibodies developing in the setting of vascular surgery frequently result from exposure to bovine thrombin. Blood 1997;90 Suppl 1:300a.
39. Petri M. Pathogenesis and treatment of the antiphospholipid antibody syndrome. Med Clin North Am 1997;81:151-77.