Endocarditis

The Presence of Infection-Related Antiphospholipid Antibodies in Infective Endocarditis Determines a Major Risk Factor for Embolic Events

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OBJECTIVES	The impact of infection-associated antiphospholipid antibodies (APA) on endothelial cell activation, blood coagulation and fibrinolysis was evaluated in patients with infective endocarditis with and without major embolic events.
BACKGROUND	An embolic event is a common and severe complication of infective endocarditis. Despite the fact that APAs are known to be associated with infectious diseases, their pathogenic role in infective endocarditis has not been clearly defined.
METHODS	The relationship among the occurrence of major embolic events, echocardiographic vegetation size, endothelial cell activation, thrombin generation, fibrinolysis and APA was examined in 91 patients with definite infective endocarditis, including 26 patients with embolic events and 65 control subjects without embolic events.
RESULTS	Overall, 14.3% of patients exhibited elevated APA levels. Embolic events occurred more frequently in patients with elevated levels of APA than in patients without (61.5% vs. 23.1%; $p = 0.008$). Patients with elevated levels of APA showed higher levels of prothrombin-fragment F1+2 ($p = 0.005$), plasminogen-activator inhibitor 1 ($p = 0.0002$), von Willebrand factor ($p = 0.002$) and lower levels of activated protein C ($p = 0.001$) than patients with normal levels of APA. Thrombin generation and endothelial cell activation were both positively correlated with levels of APA. The occurrence of elevated APA levels was frequently associated with structural valve abnormalities ($p = 0.01$) and vegetations >1.3 cm ($p = 0.002$).
CONCLUSIONS	Infection-associated elevated APA levels in patients with infective endocarditis are related to endothelial cell activation, thrombin generation and impairment of fibrinolysis. This may contribute to the increased risk for major embolic events in these patients. (J Am Coll Cardiol 1999;33:1365–71) © 1999 by the American College of Cardiology

Embolic events in infective endocarditis remain a common and severe complication. They occur in 20% to 43% of cases, with the majority as cerebral emboli (1–3). Several studies report that the occurrence of embolic events is associated with a higher mortality in patients with infective endocarditis (4,5). During the last decade, the frequency of embolic events in infective endocarditis reported in the literature remains constant, despite improved antibiotic regimens and a tendency toward earlier surgical intervention (4,6,7). In infective endocarditis, it is believed that emboli are caused by fragmentation of valvular vegetations as a result of

turbulent blood flow within the left-sided cardiac chambers (8). Hence, several transthoracic and transesophageal echocardiographic studies have attempted to correlate the occurrence of an embolic event in infective endocarditis with the morphologic appearance of a valvular vegetation (9–11). However, results of such studies have been inconsistent. Several echocardiographic studies were unable to identify any specific vegetation-derived parameter that could be prospectively correlated with subsequent embolic events (12–14).

Several recent investigations have demonstrated that systemic bacterial infections, even in the absence of cardiac involvement, represent an independent risk factor for an embolic event (15–17). Inflammation-induced procoagulant changes and endothelial cell activation appear to play a major role in this setting. Evidence has been reported showing that the development of infection-related an-

tiphospholipid antibodies (APA) (e.g., independent from the primary antiphospholipid-antibody syndrome or a definable autoimmune disease) may have an impact on the occurrence of embolic events (17–19). The interrelationships between the presence of APA, alterations in platelet activation, coagulation pathways and the occurrence of embolic events have not been previously investigated in a large patient population with definite infective endocarditis. Of importance, infective endocarditis differs from other infectious diseases owing to the presence of cardiac vegetations that represent an additional independent risk factor for an embolic event.

Hence, the purpose of this study was to evaluate the hypothesis that the development of infection-related APA represents a major risk factor for embolic events in endocarditis, and such APAs are associated with in vitro endothelial cell activation and procoagulant abnormalities.

METHODS

Patients. The study group consisted of 91 patients with definite infective endocarditis according to the Duke criteria (20). They were selected out of 215 consecutive patients with suspected infective endocarditis who were referred to our hospital between November 1991 and January 1997. Morphologic findings were obtained in 45 patients during heart surgery and in 5 patients during autopsy; in these patients the diagnosis of infective endocarditis was confirmed by pathologic findings. In 41 patients the diagnosis was made on the basis of clinical criteria (two major criteria: n = 32; one major and three minor criteria: n = 9).

Twenty-six patients were diagnosed as having either a cerebral (n = 22) or major peripheral (n = 4; 2 popliteal artery, 1 femoral artery, 1 subclavian artery) embolic event. In 9 patients, the embolic event occurred before admittance to the hospital (≤2 days) and in 17 patients after hospital admittance (≤2 days). Sixty-five patients with infective endocarditis but without embolic events served as the control group. The diagnosis of an embolic event was based on physical examination, a cerebral computed tomographic scan, a peripheral Doppler-ultrasonographic investigation

or an angiography. The diagnosis of a cerebral embolic event was in all cases made by an experienced neurologist who otherwise was not involved in this study. Patients with a cerebral hemorrhage or an uncertainty about the diagnosis of an embolic event were not included in the study.

All patients underwent transthoracic and transesophageal echocardiography during the acute phase of infective endocarditis (<3 days of admission). In all patients the presence of antiphospholipid-antibodies (e.g., LAC [lupus anticoagulant] and/or ACA [anticardiolipin antibodies]) and detailed coagulation system parameters were evaluated. Blood samples were taken within the first three days after admittance to the hospital. None of the patients had a history of the primary antiphospholipid-antibody syndrome or an autoimmune disease prior to acute infective endocarditis. There were 48 men and 43 women, with a mean age of 54 ± 15 years (range: 21 to 76 years). Patients being treated with anticoagulants or with prosthetic valve devices were excluded from the study group to avoid confounding by anticoagulation treatment. After discharge from hospital, all patients were followed regularly at the outpatient clinic at 12-month intervals for a maximum of 24 months. Mean follow-up was 12 ± 11 months.

Echocardiography. Transthoracic and transesophageal echocardiographic examinations were performed with commercially available ultrasound units (Hewlett-Packard Sonos 1500, Böeblingen, Germany; Vingmed CFM 800C, Wiesbaden, Germany). For transthoracic studies, 2.5-MHz transducers were used. A 5-MHz phased array transducer or mechanical sector scanner was used for multiplane transesophageal studies. The transesophageal examinations were performed in the left lateral decubitus position after obtaining patient's informed consent and after a 6-h fasting period. Before the probe was introduced into the esophagus a local pharyngeal anesthetic was administered. Sedation was routinely achieved with diazepam (2.5 to 10 mg IV). All investigations were carried out without any complications.

The presence of structural valve abnormalities and vegetations were evaluated according to transesophageal echocardiography by two independent investigators blinded as to the clinical diagnosis. The localization and maximal diameter of the vegetations were determined. A valvular vegetation was defined as an oscillating or fixed mass adherent to a leaflet, distinct in echogenic structure and with motion independent from the remainder of the involved leaflet. To be called a vegetation, the lesion had to be detectable throughout the complete cardiac cycle and to be visible in multiple views. Diffuse valvular irregularities or valvular thickening were not regarded as a vegetation (10,21). Interobserver and intraobserver variabilities for measurements of vegetation size were 7.8% and 5.4%, respectively. A structural valve abnormality was defined as the presence of valve sclerosis, stenosis, prolapse, aneurysm or perforation, with the specific diagnosis ascertained by previously described criteria (22,23).

Laboratory tests. Blood samples were obtained between 8 AM and 10 AM by antecubital venipuncture with a 21-gauge needle. Blood was used only when venous return was prompt throughout. The first 1 ml of blood was discarded. Venous blood samples were collected into tubes containing 3.8% trisodium citrate (ratio 9:1). The samples were centrifuged for 20 min at $2000g$ at $4-8°C$. Platelet-poor plasma was divided into small aliquots in plastic tubes and stored at $-80°C$ until further evaluation.

Diagnosis of LAC was established according to the recommended criteria (24): i) prolongation of a phospholipid-dependent clotting test (commercially available aPTT [activated prothrombin time] method; Actin FSL, Dade Behring, Frankfurt, Germany); ii) persistent prolongation of the aPTT after mixing the test plasma with normal pooled plasma; iii) modification of the clotting time according to various phospholipid concentrations (Lupus Anticoagulant Test, Baxter-Immuno, Vienna, Austria). LAC was additionally assayed by the use of hexagonal H (II) phase phospholipid molecules (Staclot LA, Stago, Mannheim, Germany) and an ELISA test to determine antiphospholipid IgG antibodies (Asserachrom APA IgG, Stago, Mannheim, Germany). Values were expressed as IgG-antiphospholipid (GPL)-units and considered negative at <5 U/ml. The ACAs were assayed with a commercially available ELISA (Varelisa Cardiolipin-Ab IgG, Mannheim, Germany). Values were expressed as GPL-units and considered negative at <12 U/ml.

Prothrombin fragment 1+2 (F1+2), a marker of thrombin generation, was analyzed by ELISA (Enzygnost, F1+2, Behring). The von Willebrand factor (vWF), a specific marker for endothelial cell injury, was measured by ELISA (Asserachrom vWF, Stago, Mannheim, Germany) with values expressed as percent of those obtained with normal pooled plasma. Plasminogen activator inhibitor-1 (PAI-1) is regarded as a major determinant of fibrinolytic activity (25). It also represents a function of endothelial release and activation, although platelet granules also contain PAI-1, which may complicate plasma measures (26). Activated protein C (aPC) represents a marker of fibrinolysis. It is a potent endogenous anticoagulant and an inhibitor of PAI-1 (27). Both aPC (Berichrom, Behring, Frankfurt, Germany) and PAI-1 (Berichrom, Behring) were assayed by chromogenic substrate assays. All assays had a day-to-day variation and an intra-assay coefficient of variation of <12% and <7%, respectively.

Statistics. Differences in the frequency of embolic events, structural valve abnormalities and endocarditis-related valve involvement in relation to the presence or absence of elevated APA levels were analyzed by the Fisher exact test. Mean values and the standard deviation (SD) were calculated for continuous variables. Differences between data of multiple groups were analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) test with the Dunn's multiple comparison post hoc test. The
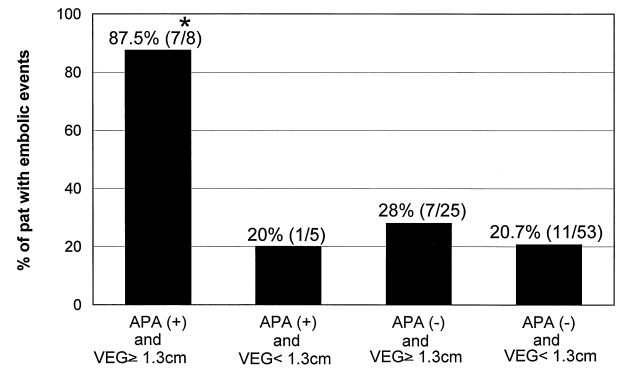
Figure 1. Frequency of embolic events in four different groups of patients according to vegetation size and presence of APA (*p < 0.05).

nonparametric Mann-Whitney U test was used to test for differences between data from the two groups. Correlations between coagulation or fibrinolytic parameters were examined by linear regression and Spearman's rank correlation analysis. A p-value <0.05 was considered to be significant.

RESULTS

Presence of antiphospholipid antibodies, correlation to embolic events and follow-up. In 13/91 (14.3%) patients, elevated APA levels were found. Elevated ACA levels only were present in 10/91 (11%) patients. Elevated LAC and ACA levels were present in 3/91 (3.3%) patients. In 78/91 (85.7%) patients, no elevated APA levels were found. Embolic events occurred in APA(+) (elevated levels of APA) and APA(−) (normal levels of APA) patients in 8/13 (61.5%) and 18/78 (23.1%), respectively (p = 0.008). All patients in whom LAC was present suffered from an embolic event.

Twelve months after acute infective endocarditis, APAs were still present in 3/10 (30%) and after 24 months in 1/7 (14%) of the initially APA(+) patients. Lupus anticoagulant was not present in any of the three patients after 12 months. In parallel, no pathologic values for coagulation or fibrinolytic parameters were found during follow-up when normal APA levels were present in initially APA(+) patients. In terms of relapses of infective endocarditis within 24 months after hospital discharge, no significant differences occurred between APA(−) patients (no relapse) and APA(+) patients (1/32; 3%; p = NS).

Valvular morphology and vegetation size. Among the 26 patients with embolic events, significantly larger vegetations were observed in the presence of elevated APA levels (1.6 ± 0.4 cm) as compared to patients with normal levels of APA (1.1 ± 0.4 cm) (p = 0.002). Using a vegetation size of 1.3 cm as an arbitrary cutoff value, patients with elevated APA levels revealed substantial differences in the frequency of embolic events (p = 0.002). In contrast, lesser differences in the frequency of embolic events were found in APA(−)

**Table 1.** Frequency of Structural Valve Abnormalities in Patients With Normal and Elevated Levels of APA

	APA(+) (n = 13)	APA(−) (n = 78)
Sclerosis	2 (15%)	5 (6%)
Stenosis	0	2 (3%)
Leaflet-prolapse	3 (23%)	5 (6%)
Leaflet-aneurysm	1 (7%)	1 (1%)
Leaflet-perforation	3 (23%)	3 (4%)

Numbers = frequency of structural valve abnormalities; a patient can be mentioned more than once; numbers in brackets = frequency in percent of patients.

patients at this cutoff value (Fig. 1). In APA(+) patients, the mitral valve was involved in 9/13 (69%), whereas in APA(−) patients, this valve was involved in 32/78 (41%; p = NS). No significant differences occurred between APA(+) and APA(−) patients in the mean number of vegetations per valve (1.2 ± 0.4 vs. 1.3 ± 0.5; p = NS) and in the number of multiple valve endocarditis (8% vs. 6%; p = NS). Infective endocarditis was accompanied by a significantly higher amount of structural valve abnormalities in APA(+) patients (7/13; 54%) than in APA(−) patients (15/78; 19%) (p = 0.01) (Table 1).

Relationship between antiphospholipid antibodies, endothelial activation and thrombin generation. In 7/8 (88%) APA(+) patients with embolic events, elevated F1+2 levels were found. Among APA(−) patients with embolic events 10/18 (56%) revealed elevated F1+2 levels. Among the controls, 23 patients (35%) revealed elevated F1+2 levels. The highest F1+2 levels were present in APA(+) patients with embolic events (p = 0.005) (Fig. 2).

Patients with embolic events revealed significantly higher PAI-1 levels (15.6 ± 4.1 IU/ml) when elevated APAs were present, as compared to patients with normal levels of APA (7.2 ± 4.6 IU/ml) (p = 0.0002) (Fig. 3). In contrast, the mean level of PAI-1 in patients without embolic events was 3.8 ± 1.4 IU/ml with only minor differences between APA(−) and APA(+) patients. In the patients with embolic events, PAI-1 levels were positively correlated to APA levels (r = 0.7; p < 0.0001).
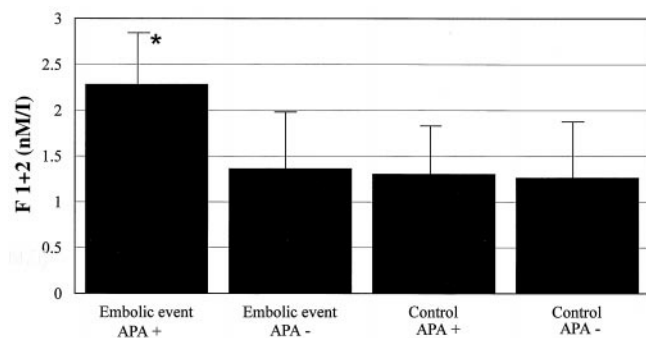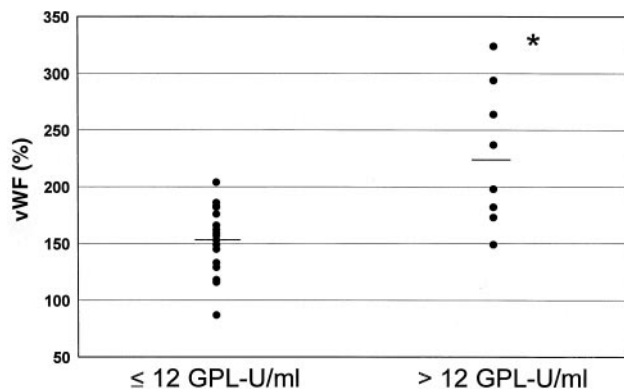

Figure 2. Mean F1+2 levels and standard deviations in patients with embolic events and control subjects. (Upper range of normal values = 1.1 nM/l; nM/l = nmol per liter.)
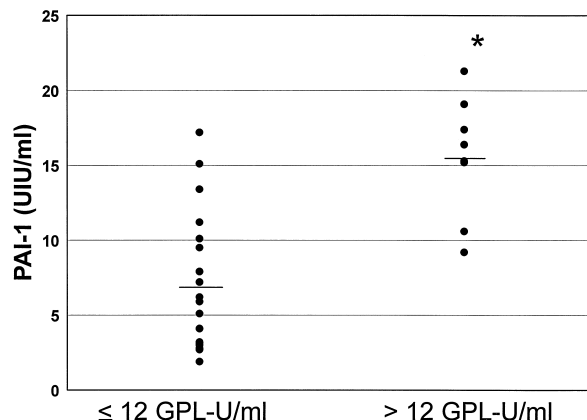
Figure 3. PAI-1 levels in 26 patients with embolic events according to normal and elevated anticardiolipin-antibody titers. A majority of values in both patient groups exceed the normal range of PAI-1. However, 2/18 (11%) and 6/8 (75%) of patients with normal and elevated ACA titers have PAI-1 levels >15 UIU/ml, respectively. (Upper range of normal values = 3.5 UIU/ml; the **horizontal bars** indicate the mean values in each patient group; UIU = urokinase inhibiting units.)
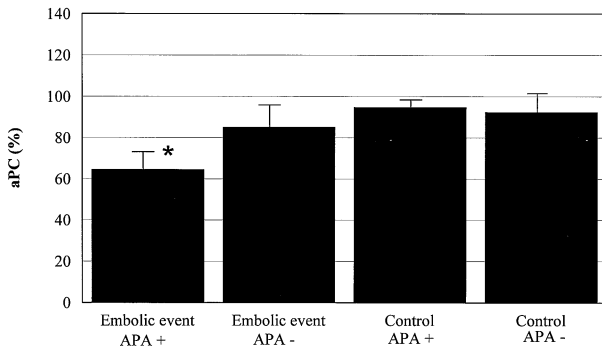
In patients with embolic events, vWF levels were significantly higher in APA(+) patients than in APA(−) patients (228 ± 62% vs. 153 ± 29%; p = 0.002) (Fig. 4). Controls revealed mean vWF levels of 145 ± 30%, with only slight differences between APA(−) and APA(+) patients. Levels of vWF were positively correlated to levels of APA (r = 0.66; p = 0.0002).

Relationship between antiphospholipid antibodies and fibrinolysis. Mean circulating aPC levels were lowest in APA(+) patients with embolic events (p = 0.001) (Fig. 5). No substantial differences occurred between patients in whom aPC was evaluated before or after the embolic event.

Figure 4. The vWF levels in 26 patients with embolic events according to normal and elevated anticardiolipin-antibody titers. A majority of values in both patient groups exceed the normal range of vWF. This is not surprising as vWF is an acute-phase reactant. Nevertheless, 1/18 (6%) and 4/8 (50%) of patients with normal and elevated ACA titers have vWF levels >200%, respectively. (Upper range of normal values = 150%.)

Figure 5. Mean aPC levels and standard deviations in patients with embolic events and control subjects. (Upper range of normal values = 70%.)

In patients with embolic events, aPC was reduced in 75% (6/8) and 13% (2/16) according to elevated and normal APA levels, respectively. None of the APA(+) patients without embolic events revealed reduced aPC levels. The aPC levels were inversely related to APA levels (r = −0.48; p = 0.01) in patients with embolic events.

DISCUSSION

Until now it has not been clear whether the presence of infection-related APA adds to the underlying pro-thrombotic state and impacts on the frequency of embolic events in infective endocarditis. Therefore, it is important to investigate the impact of APA on the clinical course of patients with infective endocarditis, in which valvular vegetations already represent an independent risk factor for an embolic event. The study provides three major findings: 1) The presence of infection-related APA is significantly associated with alterations of endothelial cells, coagulation-pathway abnormalities and inhibition of fibrinolysis; 2) infection-related APA can be divided in two groups, the biologically active and the biologically inactive, with the latter having no impact on endothelial cell activation or the procoagulant state; and 3) the presence of infection-related APA adds substantially to the risk of an embolic event in infective endocarditis.

Previous literature. The APA react with negatively charged phospholipids. They require beta-$_2$-glycoprotein 1 as a cofactor to bind to phospholipids; beta-$_2$-glycoprotein 1 inhibits factor Xa synthesis on activated platelets. Thus, APA may interfere with this inhibition leading to factor Xa generation and thrombin formation (28,29). Previous reports demonstrated that immunoglobulin fractions from patients with APA can bind to endothelial cells (30,31). This results in endothelial cell activation and phenotypic changes, with induction of a pro-adhesive, pro-thrombotic surface causing a subsequent perturbation of the endothelium-platelet axis and thrombin generation (32,33). In an inflammatory state like infective endocarditis, the activation of endothelial cells is induced by cytokines such as

TNF-alpha, interleukin-1 or bacterial endotoxins (34,35). Recently it has been reported that LAC may bind to heparan sulfate on endothelial cells. This interferes with the antithrombin III–heparan downregulation of serine proteases (36). The presence of LAC as described in autoimmune diseases or the primary antiphospholipid syndrome causes interference with the coagulation pathway and leads to an alteration of various enzymatic or cellular functions linked to hemostasis. It appears to be associated with a higher risk of thrombosis and embolic events (36,37).

Specific data about the development of APA in the setting of definite infective endocarditis are scarce. It is well known that APA occur frequently in infectious diseases (19,38). However, most studies suggest that these infection-induced APA have no pathogenic role and are not associated with LAC activity (39,40). Several recent reports demonstrated that transitory APA in infectious diseases seem to have a pathogenic role for venous thrombosis or arterial embolization (41,42). In patients with infection-associated stroke Macko et al. described a significantly lower level of aPC, and a higher level of APA compared to controls, suggesting an interference with the aPC activation reaction, which has been described earlier in vitro (32,43,44). In patients with stroke, Ameriso et al. (18) reported elevated levels of d-dimer and ACA when an infection occurred prior to the stroke.

Role of antiphospholipid antibodies in infective endocarditis. In our study, there were apparently two different subsets of APA. In one subset (five patients) the APA did not affect hemostasis and was not associated with an increased frequency of embolic events. In the other subset (eight patients) the presence of APA was associated with endothelial cell activation, thrombin generation and reduced fibrinolysis leading to a higher frequency of embolic events.

The lack of any symptoms compatible with the primary antiphospholipid syndrome prior to the onset of infective endocarditis, and the decline in APA titers after treatment of infective endocarditis, strongly suggest that the APA syndrome in infective endocarditis was acquired and was infective endocarditis-related. However, data from this study do not exclude the transitory development of APA consequent to previous infectious diseases other than infective endocarditis. In this regard, it is conceivable that the higher number of structural valve abnormalities and mitral valve involvement in patients with elevated levels of APA might be a result of preexisting APA. Previous studies reported a correlation between the presence of APA and the occurrence of valvular heart disease in autoimmune diseases or the primary antiphospholipid syndrome (45,46).

Endothelial cell activation and thrombin generation as assessed by vWF, PAI-1 and F1+2 levels were significantly higher in APA(+) patients with embolic events. A previous study demonstrated a close association between endothelial cell activation and thrombin generation in a group of patients with systemic lupus erythematosus and elevated

APA levels (33). However, PAI-1 is also released by activated platelets, which have a direct impact on thrombin generation. Therefore, platelet activation might play an additional role in this setting. Intriguingly, endothelial cell activation and consecutive thrombin formation is a common finding in systemic infectious diseases as well as in certain noninfectious diseases (47–50). Data of this study demonstrate that endothelial cell activation and thrombin formation is a common finding also in acute infective endocarditis even without elevated APA levels, suggesting the presence of other APA-independent mechanisms for the activation of the clotting system. It should be emphasized, however, that the presence of APA in infective endocarditis adds to this potential for thrombin generation by increased endothelial cells activation, resulting in the highest thrombin generation in this subset of patients.

Our data would also suggest that in these APA(+) patients with infective endocarditis, elevated thrombin generation is associated with increased growth of vegetations. In this context, vegetation size is a meaningful parameter to identify individuals at high risk for an embolic event within the group of APA(+) patients. Vegetation size in APA(−) patients did not allow the accurate prediction of embolic events in an individual patient, although embolic events occurred more frequently in patients with vegetations larger than 1.3 cm.

Antiphospholipid antibodies had an inhibitory effect on fibrinolysis as seen by the inverse relationship between APA and aPC levels. Data are strikingly similar to the study of Macko et al. (43) in patients with infection-associated stroke. Previous reports have shown that patients with continuously decreased aPC levels are less likely to develop clinical symptoms than patients with a transitory aPC decline (51,52). Follow-up data of this study demonstrate that pathologic aPC levels in APA(+) patients with acute infective endocarditis appear to be transitory, therefore increasing the likelihood for the clinical relevance of this finding. In support of this notion, elevated APA levels in the presence of acute infective endocarditis might indicate a possible trigger mechanism for embolic events. It has already been demonstrated that APA interfere with the generation of aPC on the endothelial cell surface (44). Activated protein C adds to fibrinolysis by inhibiting PAI-1 (43). Impaired fibrinolysis promotes increased fibrin generation in the subgroup of APA(+) patients with embolic events, adding to a larger vegetation size.

Conclusions. Data of this study demonstrate that within the group of APA(+) patients with definite infective endocarditis a subset exists with a high risk for major embolic events. The subset can be readily identified by echocardiographic quantitation of vegetation size and by evaluation of coagulation and fibrinolysis parameters. The pathways involved in promoting the risk of embolization include endothelial cell activation, elevated thrombin formation and impaired fibrinolysis. The APA may trigger embolic events by pertubing these pathways and enhancing the growth of a vegetation, with subsequent fragmentation from shear forces in the turbulent bloodstream. The identification of APA(+) patients with infective endocarditis as being at high risk for embolization might influence the decision algorithm for an early and preventive surgical intervention to avoid the deleterious consequences of an embolic event.

REFERENCES

1. Nissen H, Nielsen PF, Frederiksen M, et al. Native valve infective endocarditis in the general population: a 10-year survey of the clinical picture during the 1980s. Eur Heart J 1992;13:872–7.
2. Hart GH, Foster JW, Luther MF, Kanter MC. Stroke in infective endocarditis. Stroke 1990;21:695–700.
3. Stewart JA, Silimperi D, Harris P, et al. Echocardiographic documentation of vegetative lesions in infective endocarditis: clinical implications. Circulation 1980;61:374–80.
4. Jones HR Jr, Siekert RG. Neurological manifestations of infective endocarditis: review of clinical and therapeutical challenges. Brain 1989;112:1295–1315.
5. Jaffe WM, Morgan DE, Pearlman AS, Otto CM. Infective endocarditis, 1983–1988: echocardiographic findings and factors influencing morbidity and mortality. J Am Coll Cardiol 1990;15:1227–33.
6. Larbalestier RI, Kinchla NM, Aranki SF, et al. Acute bacterial endocarditis: optimizing surgical results. Circulation 1992;86 Suppl II:68–74.
7. Verheul HA, van den Brink RBA, van Vreeland T, et al. Effects of changes in management of active infective endocarditis on outcome in a 25-year period. Am J Cardiol 1993;72:682–7.
8. Weinstein L, Schlesinger JJ. Pathoanatomic, pathophysiologic and clinical correlations in endocarditis. N Engl J Med 1974;291:832–7, 1122–6.
9. Sanfilippo AJ, Picard MH, Newell JB, et al. Echocardiographic assessment of patients with infectious endocarditis: prediction of risk for complications. J Am Coll Cardiol 1991;18:1191–9.
10. Erbel R, Rohmann S, Drexler M, et al. Improved diagnostic value of echocardiography in patients with infective endocarditis by transesophageal approach: a prospective study. Eur Heart J 1988;9:43–53.
11. Mügge AM, Daniel WG, Frank G, Lichtlen PR. Echocardiography in infective endocarditis: reassessment of prognostic implications of vegetation size determined by the transthoracic and the transesophageal approach. J Am Coll Cardiol 1989;14:631–8.
12. Werner GS, Schulz R, Fuchs JB. Infective endocarditis in the elderly in the era of transesophageal echocardiography: clinical features and prognosis compared with younger patients. Am J Med 1996;100:90–97.

13. Steckelberg JM, Murphy JG, Ballard D, et al. Emboli in infective endocarditis: the prognostic value of echocardiography. Ann Int Med 1991;114:635–40.
14. Hwang JJ, Shyu KG, Chen JJ, et al. Infective endocarditis in the transesophageal echocardiographic era. Cardiology 1993;83:250–7.
15. Valtonen V, Kuikka A, Syrjänen J. Thrombo-embolic complications in bacteremic infections. Eur Heart J 1993;14 Suppl K:20–23.
16. Macko RF, Ameriso SF, Barndt R, et al. Precipitants of brain infarction: roles of preceding infection/inflammation and recent psychological stress. Stroke 1996;27:1999–2004.
17. Syrjänen J, Valtonen VV, Iivanainen M, et al. Preceding infection as an important risk factor for ischemic brain infarction in young and middle aged patients. Br Med J 1988;296:1156–60.
18. Ameriso SF, Wong VLY, Quismorio FP, Fisher M. Immunhematologic characteristics of infection-associated cerebral infarction. Stroke 1991;22:1004–9.
19. Vaarala O, Palosuo T, Kleemola M, Aho K. Anticardiolipin response in acute infections. Clin Immunol Immunpathol 1986;41:8–15.
20. Durack DT, Lukes AS, Bright DK. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. Am J Med 1994;96:200–209.
21. Shapiro SM, Young E, De Guzman S, et al. Transesophageal echocardiography in diagnosis of infective endocarditis. Chest 1994;105:377–82.
22. Weyman AE. Left ventricular inflow tract: I. The mitral valve. In Weyman AE, editor. Principles and Practice of Echocardiography. Philadelphia: Lea & Febiger, 1994:391–471.
23. Weyman AE, Griffin BP: Left ventricular outflow tract: The aortic valve, aorta and subvalvular outflow tract. In Weyman AE, editor. Principles and Practice of Echocardiography. Philadelphia: Lea & Febiger, 1994:498–575.
24. Exner T, Triplett DA, Taberner D, Machin SJ. Guidelines for testing and revised criteria for lupus anticoagulants. SSC Subcommittee for the standardization of lupus anticoagulants. Thromb Haemost 1991;65:320–2.
25. Humphries SE, Panahloo A, Montgomery HE, et al. Gene–environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis. Thromb Haemost 1997;78:457–61.
26. Suffredini AF, Harpel PC, Parillo JE. Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N Engl J Med 1989;320:1165–72.
27. Esmon CT. The protein C anticoagulant pathway. Arterioscler Thromb Vasc Biol 1992;12:135–45.
28. Shi W, Chong BH, Hogg PJ, Chesterman CN. Anticardiolipin antibodies block the inhibition by beta 2-glycoprotein I of the factor Xa-generating activity of platelets. Thromb Haemost 1993;70:342–8.
29. Machin SJ. Platelets and antiphospholipid antibodies. Lupus 1996;5:386–7.
30. Del Papa N, Meroni PL, Tincani A, et al. Relationship between anti-phospholipid and anti-endothelial cell antibodies: further characterization of the reactivity on resting and cytokine-activated endothelial cells. Clin Exp Rheumatol 1992;10:37–42.
31. McCrae KR, DeMichele A, Samuels P, et al. Detection of endothelial cell-reactive immunoglobulin in patients with anti-phospholipid antibodies. Br J Haematol 1991;79:595–605.
32. Simantov R, Lasala JM, Lo SK, et al. Activation of cultured vascular endothelial cells by antiphospholipid antibodies. J Clin Invest 1995;96:2211–9.
33. Ferro D, Pittoni V, Quintarelli C, et al. Coexistence of anti-phospholipid antibodies and endothelial pertubation in systemic Lupus erythematosus patients with ongoing prothrombotic state. Circulation 1997;95:1425–32.
34. Schleef RR, Bevilacqua MP, Sawdey M, et al. Cytokine activation of vascular endothelium. J Biol Chem 1988;263:5797–5803.
35. Scarpati EM, Sadler JE. Regulation of endothelial cell coagulant properties. J Biol Chem 1989;264:20705–13.
36. Triplett DA. Antiphospholipid-antibodies, lupus anticoagulants and thromboembolic disease. Haematologica 1995;80:122–6.
37. Shapiro SS. The lupus anticoagulant/antiphospholipid syndrome. Annu Rev Med 1995;47:533–53.
38. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders: prevalence and clinical significance. Ann Intern Med 1990;112:682–98.
39. Matsuura E, Igarashi Y, Fujimoto M, et al. Anticardiolipin cofactors and differential diagnosis of autoimmune disease [letter]. Lancet 1990;336:177–8.
40. Schultz DR. Antiphospholipid antibodies: basic immunology and assays. Semin Arthritis Rheum 1997;26:724–39.
41. Labarca JA, Rabaggliati RM, Radrigan FJ, et al. Antiphospholipid syndrome associated with cytomegalovirus infection: case report and review. Clin Infect Dis 1997;24:197–200.
42. Suarez Ortega S, Artiles Vizcaino J, Balda Aguirre Y, et al. Tuberculosis as risk factor for venous thrombosis. Ann Med Int 1993;10:398–400.
43. Macko RF, Ameriso SF, Gruber A, et al. Impairments of the protein C system and fibrinolysis in infection-associated stroke. Stroke 1996;27:2005–11.
44. De Groot PG, Horbach DA, Derksen RHWM. Protein C and other cofactors involved in the binding of antiphospholipid antibodies: relation to the pathogenesis of thrombosis. Lupus 1996;5:488–93.
45. Nihoyannopoulos P, Gomez PM, Joshi J, et al. Cardiac abnormalities in systemic lupus erythematosus: association with raised anticardiolipin antibodies. Circulation 1990;82:369–75.
46. Brenner B, Blumenfeld Z, Markiewicz W, Reisner SA. Cardiac involvement in patients with primary antiphospholipid syndrome. J Am Coll Cardiol 1991;18:931–6.
47. Lins M, Zurborn KH, Pries B, Bruhn HD. The thrombotic status of patients with inflammatory diseases. Dtsch Med Wochenschr 1996;121:855–9.
48. Colucci M, Paramo JA, Collen D. Generation in plasma of a fast-acting inhibitor of plasminogen activator in response to endotoxin stimulation. J Clin Invest 1985;75:818–24.
49. McGill SN, Ahmed NA, Christou NV. Increased plasma von Willebrand factor in the systemic inflammatory response syndrome is derived from generalized endothelial cell activation. Crit Care Med 1998;26:296–300.
50. Falciani M, Gori AM, Fedi S, et al. Elevated tissue factor and tissue factor pathway inhibitor circulating levels in ischaemic heart disease patients. Thromb Haemost 1998;79:495–9.
51. De Stefano V, Leone G, Mastrangelo S, et al. Clinical manifestations and management of inherited thrombophilia: retrospective analysis and follow-up after diagnosis of 238 patients with congenital deficiency of antithrombin III, protein C, protein S. Thromb Haemost 1994;72:352–8.
52. Pabinger I, Kyrle PA, Heistinger M, et al. The risk of thromboembolism in asymptomatic patients with protein C and protein S deficiency: a prospective cohort study. Thromb Haemost 1994;71:441–5.