A prospective evaluation of atherosclerotic risk factors and hypercoagulability in young adults with premature lower extremity atherosclerosis

Pavel J. Levy, MD, M. Francisco Gonzalez, MD, Carlton A. Hornung, PhD, MPH, Wei W. Chang, MD, James L. Haynes, MD, and Daniel S. Rush, MD, *Columbia, S.C.*

Purpose: **Fifty-one consecutive patients with premature lower extremity atherosclerosis were prospectively evaluated for atherogenic risk factors and primary or acquired hypercoagulability, which might contribute to early ischemia and revascularization failure.** ***Methods:*** **Laboratory tests included plasma assays of (1) natural anticoagulants (NAC), lipoprotein (a) (Lp[a]), and anticardiolipin antibodies, and (2) fibrinolytic activators and inhibitors at baseline and stimulated after 20 minutes of upper extremity venous occlusion.** ***Results:*** **Forty-six (90%) of these 51 patients had laboratory abnormalities. One or more NAC deficiencies were found in 15 (30%) patients and included antithrombin III (n = 5), protein C (n = 8), protein S (n = 4), and heparin cofactor II (n = 2). Hypofibrinolysis was identified as a deficiency of stimulated tissue plasminogen activator in 22 (45%) patients and elevated plasminogen activator inhibitor-1 (PAI-1) in 29 (59%). Elevated Lp(a) was found in 43 (86%) patients. Five (10%) patients had anticardiolipin antibodies. Ten patients had combined NAC deficiency and hypofibrinolysis. Five (10%) patients had no abnormality. NAC deficiencies, especially protein C deficiency, were associated with acute ischemia ($p < 0.01$), prior vascular intervention ($p < 0.01$), an increasing number of total vascular procedures ($p < 0.01$), and major amputation ($p < 0.01$). PAI-1 was associated with a history of heart disease ($p < 0.05$) and prior vascular procedures ($p < 0.05$). Elevated Lp(a) was associated with elevated PAI-1 ($p < 0.05$). Retesting in 20 patients suggested that 80% of NAC deficiencies were acquired, but abnormalities persisted in 66% of patients with elevated PAI-1 and in 93% of those with elevated Lp(a).** ***Conclusions:*** **These data strongly support the hypothesis that the convergence of atherogenic risk factors and hypercoagulability play an important role in early ischemia and poor results reported for lower extremity vascular procedures in young adults. (J VASC SURG 1996;23:36-45.)**

Atherosclerotic arterial occlusive disease is recognized as the major cause of lower extremity ischemia in young adults.[1-5] Unlike the relatively slow progression of atherosclerosis in the elderly, premature atherosclerosis in young patients has been characterized as a "virulent" disease.[1] Recent studies agree that younger patients with lower extremity arterial occlusive disease have rapid deterioration to severe ischemia, an increased risk of early vascular reconstructive failure, and a high incidence of major limb amputation.[1-5] It has been proposed that unique hereditary or acquired risk factors, in addition to typical cardiovascular risk factors, distinguish the clinical course of atherosclerosis in the young from that seen in the elderly.

Among possible unique characteristics distinguishing atherosclerosis in younger and older patients are the so-called hypercoagulable states.[5-12] In the past, hypercoagulability was associated primarily with venous thrombosis but has become increasingly recognized in arterial thrombosis and embolism.[10-12] Hypercoagulability can exist in latent forms identifiable by various plasma markers or may under

From the Departments of Surgery and Medicine, University of South Carolina, School of Medicine, Columbia.

Presented at the Forty-third Scientific Meeting of the International Society for Cardiovascular Surgery, North American Chapter, New Orleans, La., June 13-14, 1995.

Reprint requests: Daniel S. Rush, MD, Department of Surgery, Two Medical Park, Suite no. 402, Columbia, South Carolina 29203.

0741-5214/96/$5.00 + 0 24/6/69537

ill-defined circumstances precipitate a clinical syndrome of episodic thrombotic events properly termed a *hypercoagulable state*. Such hypercoagulable states have been related to a number of primary or acquired deficiencies of regulatory anticoagulant plasma proteins (natural anticoagulants), defective or inhibited fibrinolytic activity (hypofibrinolysis), procoagulant autologous antibodies (anticardiolipin antibodies), and increased platelet aggregability.[10-12] Additionally, high levels of lipoprotein (a) (Lp[a]) may both be an independent risk factor for atherosclerosis and play a role in the inhibition of fibrinolysis.[13,14] In young patients with lower extremity ischemia, hypercoagulability has been shown to have a high predictive value for early bypass failure and major amputation.[5-8]

It was the possible association of hypercoagulability and the progression of lower extremity atherosclerosis that prompted this prospective laboratory evaluation. The goals of this study were to demonstrate the incidence of markers associated with atherogenesis and hypercoagulability in nonselected, young patients with symptoms of premature lower extremity atherosclerosis and to associate any abnormalities with clinical stages of peripheral arterial occlusive disease.

PATIENTS AND METHODS

Patient selection. Between September 1991 and March 1995, 51 consecutive young patients referred with premature lower extremity atherosclerosis were prospectively evaluated for plasma markers associated with atherogenesis and hypercoagulability. All 51 patients included in the study had the onset of lower extremity ischemic symptoms before age 45 years. Patients with arterial trauma, thromboembolism, or other nonatherosclerotic causes of lower extremity arterial occlusive disease were excluded. Although patients were prospectively evaluated according to laboratory protocol, neither their past nor subsequent vascular surgical treatment followed a predetermined treatment protocol. Definitions, terminology, and reporting conventions conform to recommendations for reports dealing with lower extremity ischemia from the Ad Hoc Committee on Reporting Standards of the Joint Council of the Vascular Societies.[15] This study was conducted with informed consent and approved by Institutional Review Boards of the University of South Carolina School of Medicine and affiliated hospitals.

Data collection. Retrospectively collected clinical data included demographic characteristics; cardiovascular risk factors such as history of smoking, diabetes mellitus, hypertension, hyperlipidemia, renal insufficiency, family history, coronary artery disease, cardiac intervention(s), and stroke; history of deep vein thrombophlebitis or pulmonary embolism; clinical or vascular laboratory assessment of lower extremity ischemia; prior peripheral vascular intervention(s) or amputation(s); and routine laboratory studies of cholesterol, triglycerides, prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen, and platelets. Prospectively collected clinical data included clinical and vascular laboratory assessment of concurrent lower extremity ischemia and subsequent peripheral vascular interventions or amputations. Laboratory determination of plasma abnormalities associated with hypercoagulability included natural anticoagulants: antithrombin III, protein C, protein S, and heparin cofactor II; baseline and stimulated fibrinolytic activity: tissue plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1); Lp(a); and anticardiolipin antibodies. Determination of factor VII was performed for patients receiving warfarin. Efforts were made to repeat laboratory tests that had abnormal results at a later, more stable stage of each patient's vascular disease to determine whether observed abnormalities were primary or acquired. Retesting was preferably delayed at least 3 months in patients having vascular intervention, surgery, or acute arterial or venous thrombotic events. Normal controls were performed concurrently throughout the study. Normal ranges and number of patients tested for each laboratory determinant are shown in Table I.

Laboratory evaluation. The antithrombin III functional assay (American Dade, Miami, Fla.) was based on the principle that antithrombin III complexed with reagent heparin inhibits a known amount of thrombin. After incubation with a sample of patient plasma, residual (noninhibited) thrombin hydrolyzes a thrombin-specific chromogenic substrate, D-phenylalanine-proline-arginine-5-amidoisophthalic acid, to release a fluorescent ester that can be measured by spectrophotometry. The amidolytic activity of the residual thrombin is inversely proportional to the amount of antithrombin III in the patient sample, which can be quantitated by comparison to a standard.[16] Heparin cofactor II was determined by residual antithrombin activity in a sample of patient plasma after reagent thrombin/antithrombin incubation for 300 seconds.[17]

Protein C activity was determined by a coagulometric method based on the activation of protein C in a sample of patient plasma with a specific snake *(Agkistrodon contotrix)* venom activator (American

Table I. Laboratory evaluation of potential hypercoagulable states in 51 young patients with premature lower extremity atherosclerosis

	Normal range	*Patients tested (no.)*
Natural anticoagulants		
Antithrombin III (function)	70%-110%	51
Protein C (function)	70%-110%	51
Protein C/factor VII ratio	>0.69	
Protein S (function)	70%-110%	50
Protein S/factor VII ratio	>0.69	
Heparin cofactor II (function)	70%-110%	38
Fibrinolytic system		
Tissue plasminogen activator (antigen)		
Baseline t-PA	>1.2 IU/ml	49
20 min venous occlusion	4 X increase	49
Plasminogen activator inhibitor-1 (antigen)		
Baseline PAI-1	<16 IU/ml	49
20 min venous occlusion	<16 IU/ml	49
LP(a)		50
White	<110 mg/L	
Black	<200 mg/L	
Anticardiolipin antibodies	Negative	50

Bioproduct Co., Parsipanny, N.J.). Activated protein C inhibits factors V and VIII contained in protein C–deficient plasma. Inhibition of factors V and VIII prolongs the PTT, which is proportional to the amount of protein C in the patient sample.[18] Protein S activity is proportional to prolongation of the PT in a protein S–deficient plasma to which a diluted sample of patient plasma was added. In this determination, reagent bovine thromboplastin (American Dade) was used to measure the PT.[19] Protein C and protein S are vitamin K–dependent natural anticoagulants. Thus patients receiving warfarin therapy were also tested for factor VII levels. Factor VII activity was measured by nephelometric assay after incubation with tissue thromboplastin and quantitated by comparison of turbidity with controls (Sigma Chemical Co., St. Louis, Mo.).[20] Protein C/factor VII or protein S/factor VII ratios less than 0.69 support the diagnosis of protein C or protein S deficiency in patients receiving warfarin.

Assays of t-PA and PAI-1 activity were determined with chromogenic substrates by enzyme-linked immunosorbant assay (ELISA) (Sigma Chemical Co.).[21] These assays were performed preferably in the morning with the patient rested to avoid diurnal and exercise variations. Baseline samples of patient plasma were collected by a nontraumatic venipuncture, usually from the dorsum of the dominant hand. A sphygmomanometer was then applied to the upper arm and inflated to a pressure at midlevel between systolic and diastolic arterial pressures. After 20 minutes of venous occlusion, stimulated samples were collected from the same venipuncture site, which had been maintained patent with normal saline solution. Plasma t-PA activity was assayed by incubating samples with plasminogen activator reagent (American Diagnostics, New Haven, Conn.) for 15 minutes, at which time fibrin was added to stop t-PA reactivity. Absorbance differences between samples and controls were then measured by ELISA spectrophotometry at 480 nm. PAI-1 was determined by residual t-PA activity in patient plasma and controls after 135 minutes incubation with plasminogen activator reagent and PAI-1–depleted plasma.

Lp(a) was assayed by use of microstrips coated with high-affinity purified anti-apo (a) antibodies (American Diagnostics), to which control and duplicate patient plasma samples were added and incubated for 60 minutes. Next, conjugated anti-Lp(a) immunoglobulin G antibody was added and incubated 15 min. Absorbance was then read by ELISA spectrophotometry at 492 nm in a microplate.[22] It is widely accepted that 300 mg/L is the upper limit of normal for Lp(a).[13,14] Controls in this laboratory suggested the need for the more stringent definitions and racial variations for Lp(a) levels shown in Table I.

Anticardiolipin antibodies were measured by ELISA (American Diagnostics). Samples of standard, control, and patient plasma were incubated with cardiolipin in microcuvettes for 60 min. Conjugated immunoglobulin G, A, and M were then added for colorimetric development at 405 nm by

ELISA spectrophotometry. Anticardiolipin antibodies were determined from the standard curve and controls.[23]

Statistical analysis. Data were entered into a case report file created on Epi-Info 6.0 (Centers for Disease Control, Atlanta) and verified for accuracy. These data were then transported to the mainframe computer at the University of South Carolina for analysis with SPSS-X (SPSS-X Inc., Chicago, Ill.). Categorical variables were assessed by chi-square or Fisher's exact test where expected cell frequencies were $n < 5$. Interval variables were assessed by one-way analysis of variance and t tests. Statistical significance was defined at $p < 0.05$.

RESULTS

Patient population. Of the 51 patients in this study, 30 (59%) were men, and 21 (41%) were women; 31 (61%) were white and 20 (39%) were black. The mean age at the onset of lower extremity ischemic symptoms was 41 years (range 29 to 45 years). The mean age at evaluation in this study was 45 years (range 29 to 53 years). Cardiovascular risk factors included current smoking in 34 (67%) and former smoking in 15 (29%) patients (96% total smokers with a mean 22.5 pack-years smoking history). Diabetes mellitus was found in 17 (33%) patients; nine were insulin dependent and eight were non–insulin dependent. Hypertension was present in 29 (56%), hyperlipidemia in 35 (80%), chronic kidney failure in 4 (8%), and a family history of cardiovascular disease in 31 (61%) patients. Eighteen (35%) patients had a prior myocardial infarction, and 13 underwent coronary artery revascularization. Four (8%) patients had a prior stroke. Additionally, six (12%) patients had prior deep vein thrombophlebitis (DVT), with pulmonary embolism in three. Of the 21 women 14 (67%) had premature menopause.

Symptoms indicating the need for vascular surgical evaluation and treatment included intermittent claudication in 47 (92%), ischemic rest pain in 29 (57%), and tissue loss in 16 (31%) patients. Mean ankle/brachial indexes in the symptomatic limbs were 0.59 for those with claudication, 0.47 for those with rest pain, and 0.34 for those with tissue loss. The distribution of arterial occlusive disease was aorto-iliac-femoral in 29 (57%), superficial femoral artery–popliteal in 33 (65%), and tibial-pedal in 16 (31%) patients. Thirteen (26%) had a clinical diagnosis of arterial hypoplasia (small-caliber arteries). Twenty (39%) patients had prior lower extremity vascular intervention(s) or amputation(s). At the time of laboratory evaluation, 13 (25.5%) patients were studied less than 3 months after acute lower extremity ischemia or vascular surgical procedure. The remaining 38 (74.5%) patients were evaluated for chronic lower extremity ischemia and either underwent operation more than 3 months before laboratory evaluation or had never undergone revascularization.

Acute ischemia and recent vascular surgery was associated with a history of myocardial infarction ($p < 0.05$) and DVT ($p < 0.05$). Thrombocytosis (platelet count > 450,000) was associated with acute ischemia ($p < 0.05$) and prior vascular surgical procedures ($p < 0.05$). Overall, 39 (77%) of these 51 young patients underwent lower extremity revascularization, and 28 (55%) have required more than one vascular procedure. Fourteen (27%) patients required major lower extremity amputations, nine above-knee and seven below-knee; two patients underwent bilateral amputation. Mean follow-up was 9.5 months (range 1 to 39 months). Seventeen patients were lost to follow-up after initial laboratory evaluation. Two other (4%) patients have since died.

Laboratory evaluation. Laboratory abnormalities were detected in 46 of the 51 patients (90%). At initial evaluation, one or more natural anticoagulant deficiencies were found in 15 (30%) patients. These included deficiencies of antithrombin III in five (10%), protein C in eight (16%), protein S in four (8%), and heparin cofactor II in two (5%) patients. Defective fibrinolytic activity was found in a total of 29 (59%) of 49 patients tested and included deficiency of t-PA after venous occlusion in 22 (44%), and elevated baseline and postvenous occlusion PAI-1 in 29 (59%). Ten (20%) patients had combined natural anticoagulant and fibrinolytic deficiencies. Elevated Lp(a) was found in 43 (86%) of 50 patients and was the only abnormality in 6; 37 (72%) patients had Lp(a) greater than 300 mg/L. Five (10%) patients had anticardiolipin antibodies. Five patients had no abnormalities.

Natural anticoagulant deficiencies found during initial evaluation were associated with acute ischemia ($p < 0.01$) and recent or remote vascular surgical procedures ($p < 0.01$) (Table II). All patients with protein C deficiency had previously undergone vascular intervention ($p < 0.01$). Natural anticoagulant deficiencies, generally, and protein C deficiency, in particular, were associated with an increasing number of total vascular procedures per patient ($p < 0.01$).

Table II. Hypercoagulable abnormalities in relation to ischemia and prior vascular surgical interventions in 51 young patients with premature lower extremity atherosclerosis

	Lower extremity ischemia			*Prior vascular intervention*		
	Acute (n = 13)	*Chronic (n = 38)*	*p*	*Yes (n = 20)*	*No (n = 31)*	*p*
Natural anticoagulant deficiencies	61.5%	18.4%	<0.01	57.1%	10.0%	<0.01
Antithrombin III (*n* = 5)	23.1%	5.3%	NS	14.3%	6.7%	NS
Protein C (*n* = 8)	23.1%	13.2%	NS	38.1%	0%	<0.01
Protein S (*n* = 4)	15.4%	5.4%	NS	9.5%	6.9%	NS
Heparin cofactor II (*n* = 2)	9.1%	3.7%	NS	11.1%	0%	NS
Fibrinolytic abnormalities	61.5%	68.4%	NS	85.7%	53.3%	<0.05
Tissue plasminogen activatory (t-PA)						
Baseline t-PA (*n* = 14)	25.0%	29.7%	NS	33.3%	25.0%	NS
20 min venous occlusion (*n* = 22)	50.0%	43.2%	NS	52.4%	39.3%	NS
Plasminogen activator inhibitor-1 (PAI-1)						
Baseline PAI-1 (*n* = 29)	58.3%	59.5%	NS	76.2%	46.4%	NS
20 min venous occlusion (*n* = 29)	66.7%	56.8%	NS	76.2%	43.4%	NS
Elevated Lp (a) (*n* = 43)	84.6%	75.7%	NS	85.7%	72.4%	NS
Anticardiolipin antibodies (*n* = 5)	15.5%	8.1%	NS	0%	17.2%	NS

Chi-square analysis for trend confirmed the association of natural anticoagulant deficiencies and protein C deficiency with an increasing number of total vascular procedures per patient ($p < 0.03$). Both natural anticoagulant deficiencies and protein C deficiency were also associated with major lower extremity amputations ($p < 0.01$), especially above-knee amputation ($p < 0.01$). Additionally, protein C deficiency was associated with a history of DVT ($p < 0.01$). All four patients with protein S deficiency were black ($p < 0.05$). Fibrinolytic abnormalities were associated with a history of coronary artery disease ($p < 0.05$) and prior vascular surgical procedures ($p < 0.05$). Elevated Lp(a) was associated with both elevated baseline and postvenous occlusion PAI-1 ($p < 0.05$). Lp(a) was unrelated to cholesterol and triglyceride levels. Fibrinogen level was available in 20 patients and was unrelated to fibrinolytic activity.

Twenty patients underwent repeat testing after their vascular status stabilized to distinguish between primary and acquired laboratory abnormalities. Two patients were retested for antithrombin III deficiency; levels in both patients returned to the normal range in 3 months. Five patients with protein C deficiency were retested; levels in four patients normalized in 1 to 36 months. The remaining patient had protein C deficiency on three separate occasions over 3 years; this patient later died. Two patients were retested for protein S deficiency; the level in one patient normalized at 1 month, but the level in the other remained deficient repeatedly over 3 years. One patient with heparin cofactor II deficiency was retested and had normalization at 1 month. No association between resolution of acquired natural anticoagulant deficiencies and acute, postoperative, or chronic stages of lower extremity ischemia was established. Of 13 patients retested for elevated PAI-1, only four (34%) were later found to have had normalization. Of 13 patients with elevated Lp(a), the levels in 12 (93%) remained elevated at later testing. Anticardiolipin antibodies remained present in one patient retested at 4 months.

DISCUSSION

In terms of demographics, cardiovascular risk factors, severity of lower extremity ischemia, and response to surgical treatment, the 51 young patients prospectively evaluated in this study were similar to the earlier retrospectively studied cohort of 109 patients from this community.[5] Notably, this prospective study documented a high incidence of cardiovascular risk factors, especially smoking and hyperlipidemia, and identified specific laboratory markers associated with atherosclerosis and thrombosis. These factors may offer some explanation for the aggressive nature and poor response to conventional vascular surgical procedures seen in young adults with premature lower extremity ischemia.[1-5] The implication of these data is that the poor vascular

surgical results reported in young patients may be attributable to a convergence of accelerated atherosclerotic and prothrombotic processes in the lower extremity arterial bed, which is disproportionate to that typically seen in older patients.

Past studies have demonstrated that patients with defects in regulatory anticoagulant plasma proteins are at increased risk for spontaneous arterial, venous, and vascular graft thrombosis.[6-14] Unfortunately, the current understanding of hypercoagulable states in patients with arterial disease, especially studies involving young patients, remains incomplete and worthy of further investigation. In this study of 51 young patients with premature atherosclerosis, 15 (30%) were found by prospective laboratory evaluation to have natural anticoagulant deficiencies. In the earlier retrospective survey from this institution, only 15% of 109 young patients with lower extremity ischemia had a clinical diagnosis of hypercoagulability.[5] Despite generally poor vascular surgical results in young patients, the clinical possibility of an underlying hypercoagulable state appears to be largely underappreciated.

Other prospective studies have also indicated that young patients have a higher incidence of hypercoagulability than the general vascular population. Eldrup-Jorgensen et al.[6] found one or more natural anticoagulant deficiencies in six (33%) of 20 patients less than 50 years old with vascular disease. In that study 20% were protein S deficiencies, and 15% were protein C deficiencies; hypercoagulability was found in all four patients with early graft failures.[6] Aronson et al.[7] found coagulation abnormalities in 11 (33%) of 37 patients less than 45 years old with vascular disease. Only four (9%) of these patients, however, had natural anticoagulant deficiencies (three patients with protein S and one with antithrombin III deficiency).[7] Valentine et al.[8] recently reported natural anticoagulant deficiencies in 15 (30%) of 50 young male patients less than 45 years old with vascular disease. Protein C deficiency has been found in less than 2% of the general population but is three to four times more frequent in young patients with unexplained vascular thrombosis.[24] Hereditary protein S deficiency was recently reported in 8% of young patients with arterial disease.[25] On the basis of these surprisingly similar observations, it can be expected that natural anticoagulant deficiencies will be found in approximately one third of young patients evaluated for premature lower extremity atherosclerosis.

In contrast, recent series from other vascular surgical centers imply that natural anticoagulant deficiencies in typical patients with peripheral arterial disease are uncommon.[9-11] Donaldson et al.[9] prospectively studied hypercoagulability in 158 patients drawn from the general vascular population with a mean age of 65.4 years (range 30 to 91 years). Fifteen (9.5%) of these patients had laboratory abnormalities, but only seven (4%) had natural anticoagulant deficiencies (four patients with protein C, two with antithrombin III, and one with protein S deficiency).[9] Tollefson et al.[10] reported four patients with protein C deficiency and spontaneous arterial thrombosis. Eason et al.[11] also treated four patients with natural anticoagulant deficiencies (two with protein S, one with protein C, and one with combined protein S and C deficiency). Overall, these data suggest that protein C deficiency may be the most frequent abnormality in patients with vascular disease and natural anticoagulant deficiencies and especially those patients with thrombotic events.[6-12]

Information regarding the role of fibrinolytic activity in patients undergoing peripheral vascular surgery is unfortunately lacking. In this study, 44% of the 49 patients tested had a deficiency of stimulated t-PA, and 59% had abnormally elevated baseline and stimulated PAI-1. The association of impaired fibrinolysis and thrombotic complications caused by elevated PAI-1 has been reported.[26] It has been previously shown that fibrinolytic deficiencies can occur with arterial and venous thrombosis and early lower extremity bypass failures.[27] Elevated PAI-1 has been related to other cardiovascular risk factors such as diabetes, hypertension, and hyperlipidemia.[26,27] Laboratory models suggest the presence of an intravascular profibrinolytic response to conditions of ischemia or hypoxia.[28] This protective mechanism may be impaired in many young patients with premature lower extremity ischemia. At least 10% of the patients in this study had combined natural coagulant deficiencies and inhibition of fibrinolysis. Further studies are needed to define better the role of fibrinolytic activation and inhibition in patients of all ages with peripheral arterial occlusive disease.

Of the 51 young patients in this study, 86% had elevated Lp(a). Also note that 72% still had elevated Lp(a) with the less stringent but more widely accepted value of 300 mg/L as the upper limit of normal. Valentine et al.[14] recently demonstrated that elevated Lp(a) was an independent risk factor for premature atherosclerosis in young white men.

High-levels of Lp(a) have been shown previously to be an independent risk factor for coronary artery disease and early aortocoronary vein graft occlusion.[13] Additionally, elevated Lp(a) in this study was related to baseline and stimulated elevations in PAI-1. Cellular and molecular mechanisms linking Lp(a) and competitive inhibition of fibrinolysis have been proposed because of structural homology between the apolipoprotein side-chains of Lp(a) and plasminogen.[13]

Earlier studies in patients with vascular disease have not attempted the difficult task of distinguishing between primary (congenital or familial) and acquired (secondary) natural anticoagulant deficiencies. In this study, 20% of natural anticoagulant deficiencies retested were persistent on repeated tests over several years under stable conditions suggesting that these two were primary deficiencies. It was found that the remaining 80% that were retested later normalized, indicating that they were acquired deficiencies. It has been previously shown that plasma levels of natural anticoagulants can be adversely affected by clinical circumstances, especially after acute arterial or venous thrombotic events and major vascular surgical procedures.[29] Acquired natural anticoagulant deficiencies may be clinically relevant, but caution must be exercised when interpreting such abnormalities as primary without retesting under stable conditions. In contrast, abnormalities of fibrinolysis, Lp(a), and anticardiolipin antibodies were more persistent over time. No association between acquired hypercoagulability and acute ischemia or recent surgery was established. There were too few patients in this study to draw conclusions with regard to thrombotic implications of anticardiolipin antibodies.

These data bring us closer to an understanding of the virulence associated with premature atherosclerosis. In this study, observed laboratory abnormalities were strongly associated with severe ischemia, early bypass failure, multiple revascularization attempts, and amputation. It was shown that patients with premature atherosclerosis are at an exceptional risk because of a high incidence of cardiovascular risk factors, especially elevated Lp(a). Additionally, they are at an increased risk of thrombotic complications because of hypercoagulability associated with natural anticoagulant deficiencies and hypofibrinolysis. Future clinical efforts should be directed toward aggressive risk factor modification and reversal of observed laboratory abnormalities by appropriate use of anticoagulants, antiplatelet agents, semisynthetic estrogens and androgens, and corticosteroids. Clinical trials are currently in progress testing the effectiveness of these therapies as adjuncts to standard vascular surgical options.

REFERENCES

1. McCready RA, Vincent AE, Schwartz RW, Hyde GL, Mattingly SS, Griffen WO Jr. Atherosclerosis in the young: a virulent disease. Surgery 1984;96:863-8.
2. Pairolero BC, Joyce JW, Skinner CR, Hollier LH, Cherry KJ. Lower limb ischemia in young adults: prognostic implications. J VASC SURG 1984;1:459-64.
3. Hallett JW, Greenwood LH, Robison JG. Lower extremity arterial disease in young adults: a systemic approach to early diagnosis. Ann Surg 1985;202:647-52.
4. Valentine RJ, MacGillivray DC, DeNobile JW, Snyder DA, Rich NM. Intermittent claudication caused by atherosclerosis in patients aged forty years and younger. Surgery 1990;107: 560-5.
5. Levy PJ, Hornung CA, Haynes JL, Rush DS. Lower extremity ischemia in adults younger than 40 years of age: a community-wide survey of premature atherosclerotic arterial disease. J VASC SURG 1994;19:873-81.
6. Eldrup-Jorgensen EJ, Flanigan P, Brace L, et al. Hypercoagulable states and lower limb ischemia in young adults. J VASC SURG 1989;9:334-41.
7. Aronson DC, Ruys T, van Bockel JH, et al. A prospective survey of risk factors in young adults with arterial occlusive disease. Eur J Vasc Surg 1989;3:227-32.
8. Valentine RJ, Kaplan HS, Green R, Jacobsen DW, Myers SI, Clagett GP. Lipoprotein (a), homocystine, and hypercoagulable states in young men with premature peripheral atherosclerosis: a prospective, controlled analysis. J VASC SURG 1996;23:53-63.
9. Donaldson MC, Weinburg DS, Belkin M, Whittemore AD, Mannick JA. Screening for hypercoagulable states in vascular surgical practice. J VASC SURG 1990;11:825-31.
10. Tollefson DJF, Friedman KD, Marlar RA, Bandyk DF, Towne JB. Protein C deficiency: a cause of unusual or unexplained thrombosis. Arch Surg 1988;123:881-4.
11. Eason JD, Mills JL, Beckett WC. Hypercoagulable states in arterial thromboembolism. Surg Gynecol Obstet 1992;174: 211-5.
12. Levy PJ, Gonzalez FM, Rush DS, Haynes JL. Hypercoagulable states as an evolving risk for spontaneous venous and arterial thrombosis. J Amer Coll Surg 1994;178:266-70.
13. Rader DJ, Brewer HB. Lipoprotein (a): clinical approach to a unique atherogenic lipoprotein. JAMA 1992;267:1109-12.
14. Valentine RJ, Grayburn PA, Vega GL, Grundy SM. Lp(a) lipoprotein is an independent discriminating risk factor for premature peripheral atherosclerosis among white men. Arch Intern Med 1994;154:801-6.
15. Ad Hoc Committee on Reporting Standards, Society for Vascular Surgery and North American Chapter, International Society for Cardiovascular Surgery. Suggested standards for reports dealing with lower extremity ischemia. J VASC SURG 1986;4:80-4.
16. Abildgaard U, Lie M, Odegard OR. Antithrombin assay with new chromogenic substrates (S-2238 and Chromozyn TH). Thromb Res 1977;11:549-53.
17. Tollefsen DM, Majerus DW, Blank MK. Heparin cofactor II: purification and properties of a heparin dependent inhibitor of thrombin in human plasma. J Biol Chem 1982;257:2162-9.
18. Esmon CT. Protein C: biochemistry, physiology and clinical implications. Blood 1983;62:1155-8.

19. Suzuki K, Nishioka J. Plasma protein S activity measurement using Protac, a snake venom derived activator of protein C. Thromb Res 1988;49:241-51.
20. Avvisati G, ten Cate JW, van Wijk EM, Kahle LH, Mariani G. Evaluation of a new chromogenic assay for factor VII and its application in patients on oral anticoagulant treatment. Br J Hematol 1980;45:343-52.
21. Falkon L, Gari IM,Borrel M, Fontcuberta J. The release of plasminogen activators (t-PA and U-PA) and plasminogen activator inhibitor (PAI-1) after venous stasis. Blood Coag Fibrinolysis 1992;3:33-8.
22. Edelberg JM, Gonzalez GM, Pizzo SU. Lipoprotein (a) inhibits streptokinase mediated activation of human plasminogen. Biochemistry 1989;28:2370-4.
23. Harris EN, Boeg ML, MacKnorth-Young CG. Anticardiolipin antibodies: detection by ELISA and association with thrombosis in systemic lupus erythematosus. Lancet 1983;2: 1211-3.
24. Gladson CL, Schaarer I, Hach V, Beck KH, Griffin JH. The frequency of type I heterozygous protein S and protein C deficiency in 141 unrelated young adults with venous thrombosis. Thromb Haemost 1988;59:18-22.
25. Allaart CF, Aronson DC, Ruys T, et al. Hereditary protein S deficiency in young adults with arterial occlusive disease. Thromb Haemost 1990;64:206-10.
26. Dawson S, Henny A. The status of PAI-1 as a risk factor for arterial and thrombotic disease: a review. Atherosclerosis 1992;95:105-7.
27. Earnshaw JJ, Westby JC, Makin GS, Hopkinson BR. Defective fibrinolysis: a risk factor in early femoropopliteal graft thrombosis. Br J Surg 1987;4:932-5.
28. Schneiderman J, Eguchi Y, Adar R, Sawdey M. Modulation of fibrinolytic system by major peripheral ischemia. J VASC SURG 1994;19:516-24.
29. McDaniel MD, Pearce WH, Yao JST, et al. Sequential changes in coagulation and platelet function following femorotibial bypass. J VASC SURG 1984;1:261-8.

Submitted June 15, 1995; accepted Sept. 24, 1995.

DISCUSSION

Dr. Magruder C. Donaldson (Boston, Mass.). We are all aware that patients under the age of 50 with manifestations of peripheral arterial occlusive disease are among the most challenging to treat. Among others grappling with this fact over the years, Levy et al.[5] presented a series of 109 young patients studied retrospectively, documenting at best a 14% incidence of hypercoagulable states. Undaunted, they have pushed ahead since then with this prospective study, taking advantage of the increasing variety of useful laboratory probes into the multiple facets of hypercoagulability. With this more systematic and sensitive approach, they have uncovered specific prothrombotic abnormalities in no less than 46 of 51 consecutive patients. Most novel is their finding of impaired fibrinolysis in the form of inadequate stimulation of tissue plasminogen activator secretion in 45% of patients and elevated baseline or stimulated levels of plasminogen activator inhibitor in 59% of subjects. Elevated levels of Lp(a) were present in fully 86% of patients, albeit with 11 or 20 mg/dl rather than the more standard 30 mg/dl mark as the upper limit of normal. Interestingly, in addition to contributing to atherosclerotic plaque, Lp(a) has been found by others to retard t-PA activation of plasminogen, and the authors confirmed an association between elevated Lp(a) and increased PAI-1 activity. These findings strongly suggest that the most critical problem in many of these young people is hypofibrinolysis rather than hypercoagulability.

What proportion of your patients with elevated Lp(a) levels had values greater than 30 mg/dl, which is the upper limit of normal in other laboratories, including our own?

And given their overall experience with this complicated group, many members of which had multiple markers of hypercoagulability, would the authors care to elaborate on a possible unifying pathologic mechanism behind the fundamental abnormality at the arterial wall and blood interface?

Though incomplete, these data comparing patients with acute and chronic conditions and follow-up assays in 20 of the original 51 subjects suggests that fibrinolytic abnormalities and Lp(a) elevation remain present with time, perhaps in association with other chronic stimuli such as smoking and genetics. On the other hand, efficiency in natural anticoagulants such as antithrombin III and protein C appear relatively evanescent, possibly in association with acute disease or surgical trauma. Despite this fact, the authors found an unusually strong correlation between natural anticoagulant deficiency and clinical thrombosis.

Did the timing of the thrombotic events in these patients fit convincingly with the presumed flux in natural anticoagulants? And if so, is the association strong enough to support a strategy of preoperative screening and targeted perioperative factor repletion or anticoagulation to protect this subgroup?

In a more general, practical vein, given these new insights into possible mechanisms of disaster, can you speculate on any adjunctive therapies, perhaps directed at impaired fibrinolysis, which might supplement sole reliance on antiplatelet and anticoagulant agents?

It has been fascinating to see our understanding of hypercoagulability emerge over the last 15 years, and we are indebted to Dr. Levy and his colleagues for this unique prospective study of a very complicated but fundamental problem facing us all.

Dr. Pavel J. Levy. With regard to the first question of Lp(a), normal values were > 30 mg/dl. The reason we used the lower values was because, while performing the study

and analyzing the data, our hematology laboratory was performing normal controls and found that the normal levels should be lower. That was why we reported 86%. However, when we initially evaluated the data, using 30 mg/dl and up, we had 75% of hyperlipoproteinemia, which indicates that the increase in lowering the normal value was not significant.

With regard to the second question of unifying hypercoagulable mechanisms in patients with premature atherosclerosis, the only thing we can extrapolate from our data and previously published series is that there indeed is correlation between atherosclerosis and thrombosis, therefore I used the term *atherothrombosis*. We believe that markers of endothelial cell perturbation are the key factor in atherothrombogenesis and atherothrombosis itself.

To the third question about the flux of natural anticoagulants, we showed that the levels of about 80% of patients who were studied serially with elevated natural anticoagulant became normal during the follow-up period. Unfortunately, one of the limitations of our study is that we could not perform serial studies on all patients as planned initially. Of two patients who continued to have a deficiency of protein C, one of them remained in the so-called "acute stage" and eventually died. The other patient had chronic ischemia during the follow-up because of deficient anticoagulants.

With regard to the final question about what would be our proposal for screening of premature atherosclerosis in young patients, we believe that screening for natural anticoagulants may be very important in particular groups of patients, especially those who had had prior history of venous thromboembolism, as did six patients in our group who had a family history of venous thromboembolism or repeated thrombotic events. We definitely would study those patients for natural anticoagulants. From our study and from the literature regarding heparin cofactor II, it probably will be unnecessary.

We don't believe that anticoagulant antibodies have any scientific importance in evaluating the patients, although there are several series that indicate an extremely high prevalence of patients with venous thrombosis receiving anticoagulants. However, we definitely would advise screening the patient for Lp(a).

Dr. Charles O. Brantigan (Denver, Colo.). It seems that a significant number of patients in this series had acute arterial ischemia, and, in the presence of major vessel thrombosis, many coagulation tests, as a result are disorder of the thrombosis. How do we know in this study that we're studying the chicken instead of the egg?

Dr. Levy. Twenty-five percent of patients in our study indeed had either acute arterial ischemia or were studied within less than 3 months after surgery, being operated sometimes for chronic ischemia. So those were only one fourth of the patients. These patients did not differ statistically from patients with chronic ischemia, with regard to decreased fibrinolytic activity and the prevalence of Lp(a) and anticoagulant antibodies. Some of these hypofibrinolytic factors, such as PAI-1, could be acute phase reactants; however, we didn't find any difference between patients with acute and chronic ischemia. I would like to stress the importance of studying patients for possible hypercoagulability divided into acute and chronic stages.

Mr. H. H. G. Eastcott (London, United Kingdom). That was a very interesting survey of a problem that we are going to see more of. We have seen something like this in Europe, particularly with Arab patients. Colleagues in Lisbon and in Porto have described a similar condition, which has one particular feature on investigation that is not in your report, and that is the red blood cell sedimentation rate. The patients we have seen mostly had raised sedimentation rate. And in association with this, a peculiarity of the lesion itself, which although it was clearly atheromatous and although this is the age group for Buerger's disease, your patient in no way resembled that condition except for their age and history of smoking. But the chief feature of this lesion in the Portuguese and the Arab patients was its adherence. The adventitia was strongly adherent to the surrounding tissues and the endarterectomy, or the grafting procedure was unusually difficult.

I was very interested that you had five cases of the antiphospholipid syndrome. Medicolegally vascular surgeons may plead this deficiency as a cause for unexpected failure after vascular reconstruction. We are told that it's rather uncommon in arterial cases and is seen more often with venous thrombosis. I'm interested to see that no fewer than five of your cases had this.

Dr. Erwin A. Cohen (Cheltenham, Pa.). Over the years, I've operated on some young women in their 20s. The historical background was birth control pills and heavy smoking. I have not seen it recently. I don't know whether it's because they've changed the birth control pill dosage. We had not, at that time, studied them for hypercoagulable states. I wonder if you have any experience with this group of patients?

Dr. Levy. We did find a very high prevalence of young women; however, it appears that most of the women had premature menopause at the time that they were tested. We don't have clear medical history of use of birth control pills; however, recently, there were several clinical studies about association between birth control pills and antithrombin III deficiency. So, if a relatively high prevalence of natural anticoagulants is found at initial study and almost half of our patients are women, this brings us to an interesting point that we should more thoroughly ask about birth control pills, because then we may find unexpectedly more arterial diseases, not only venous.

Dr. Bo Risberg (Malmö, Sweden). We studied patients with acute embolic or thrombotic ischemia within 3 days of onset. When those patients arrived at the hospital, we found that if they had reduced single-chain urokinase plasminogen activator levels or if they had high Lp(a), those were the patients that eventually died. That points out important connections between fibrinolysis and death.

Now, Lp(a) competitively inhibits the plasminogen binding to fibrin and that's why the fibrinolytic system can be inhibited by high Lp(a).

We know that there are at least 15 isoforms of Lp(a) with different fibrin binding. So if we have isoforms that bind very much to fibrin, we may have a more pronounced effect on the fibrinolytic system. Do you have any information on those isoforms?

Dr. Levy. Two of our patients died; however, clearly there was an association between hyperfibrinolysis and recurrent myocardial infarction in patients younger than age 45. And that's exactly the group of our patients, bearing in mind that two thirds of our patients had a history of coronary artery disease.

With regard to the question about 15 isoforms and Lp(a), I do not have information. We will need to evaluate more thoroughly the association of Lp(a) and different findings from the basic sciences about association with premature atherosclerosis.

Dr. Peter M. Kasprzan (Regensburg, Germany). Can you comment on any possible influence of medical treatment on your data, especially in patients with arterialization before your initial investigation?

Dr. Levy. Regarding the medical treatment, most of the patients who came to our attention were not treated medically except with aspirin. However, because we started to notice a high prevalence of hypercoagulability markers in our patients, we started with noncontrolled, nonrandomized treatment of some of our patients with low-dose or full-dose warfarin (Coumadin).