Procoagulants and Osteonecrosis

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ABSTRACT. ***Objective***. To study the relationship between hypofibrinolysis, thrombophilia, and osteonecrosis. We evaluated the frequency of abnormal concentrations of 9 coagulation factors in patients diagnosed with osteonecrosis.

Methods. Blood samples were drawn from 45 patients diagnosed with osteonecrosis. Etiologic associations included systemic lupus erythematosus (n = 9), inflammatory bowel disease (n = 1), corticosteroid therapy (n = 20), or history of heavy alcohol (n = 4) or tobacco (n = 3) use. No associated risk factors were identified in 5 patients; these individuals were labeled "idiopathic." The patient cohort was matched to a similarly studied cohort of 40 healthy individuals without documented osteonecrosis. The following factors were analyzed: plasminogen activator inhibitor (PAI-Fx), stimulated tissue plasminogen activator, lipoprotein (a), resistance to activated protein C, anticardiolipin antibodies (aCL IgG, IgM), protein C, protein S (free), and homocysteine.

Results. Thirty-seven of the 45 patients (82.2%) with osteonecrosis were found to have at least one coagulopathy, versus 30% of controls ($p < 0.0001$). Twenty-one patients (46.7%) were identified with 2 or more abnormal test results versus 2.5% of controls ($p < 0.0001$). Patients were more likely than controls to have high levels of the hypofibrinolytic plasminogen activator inhibitor activity (42% vs 3%; $p < 0.0001$), and high anticardiolipin antibody IgG (34% vs 10%; $p = 0.008$). At least one coagulation factor abnormality was detected in all 5 idiopathic patients; elevated aCL IgG and PAI-Fx were evident in 4 patients.

Conclusion. This study revealed a high incidence of thrombophilic and hypofibrinolytic coagulation abnormalities in patients with osteonecrosis. These findings have major implications for the diagnosis as well as the treatment of this disease. Since some of these abnormalities may be the result of autosomal dominant disorders, it may be possible to detect individuals at risk for development of this disease. (J Rheumatol 2003:30:783–91)

Key Indexing Terms:
OSTEONECROSIS COAGULATION FACTORS THROMBOPHILIA
HYPOFIBRINOLYSIS AVASCULAR NECROSIS

In 1934, Phemister[1] proposed that vascular abnormalities that resulted in thrombosis and embolism contributed to the development of atraumatic osteonecrosis. This association has led others to use more descriptive terms for this disease including "avascular necrosis" or "ischemic necrosis." In 1961, Nilsson, *et al*[2] published a case report concerning a 28-year-old man with a history of thrombosis, high levels of the inhibitor to plasminogen activation, and skeletal abnormalities consistent with a diagnosis of osteonecrosis. Since then, several investigators have reported evidence of altered hemostasis in patients with osteonecrosis[3-6]. In 1974, Jones, *et al*[7] proposed that intravascular coagulation with fibrin thrombus propagation was a final common pathway for osteonecrosis associated with various etiologies. In 1994, Glueck, *et al*[8] developed this concept further to suggest that coagulation disorders actually promoted the development of osteonecrosis.

Over the past 2 decades, there has been an exponential increase in our knowledge of thrombotic and fibrinolytic pathways. Numerous factors have been identified and an integrated network of feedback mechanisms has been described. As a consequence, we now have a better understanding of thrombophilia — the increased tendency for thrombosis, and hypofibrinolysis — the impairment of the intravascular lysis of clots. With the advancement of laboratory techniques, several investigators have explored the possibility that these abnormalities may exist in patients with osteonecrosis. Many of these studies have focused on osteonecrosis of the jaw and on patients with Perthes' disease, a juvenile form of

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osteonecrosis[9-16]. These 2 entities are distinctly different from adult patients with osteonecrosis of the hip and other large joints. Investigators have reported an association between disseminated intravascular coagulation and osteonecrosis[17,18]. Although previous studies have noted a relationship between abnormalities in levels of coagulation factors and osteonecrosis of the adult femoral head, many of these studies are limited to case reports or series with small numbers of patients[8,19-28]. Recently, reports of larger, systematic studies of coagulation disorders in osteonecrosis of the hip have been published by our co-authors[29,30], as well as others[31].

We hypothesize that coagulation disorders play a role in the pathogenesis of osteonecrosis of the femoral head. The specific aim of our study was to assess associations between coagulation disorders and osteonecrosis.

MATERIALS AND METHODS

Patients. This study received institutional review, and informed consent was obtained for each participant. Forty-five consecutive patients presenting at our clinic with osteonecrosis of large joints (hip, knee, shoulder, and/or ankle) between July 1996 and October 1997 were identified. Patients were excluded if they were scheduled for one visit from out of town or for immediate surgery. This was because coagulation testing took 2 hours and required laboratory specimens to be drawn in the fasting state. Patients excluded represented a similar osteonecrosis population (age, sex, risk factors) than that included in the study. A definitive diagnosis of osteonecrosis was established by plain radiographs and confirmed by magnetic resonance imaging. Data collected from the patient interview included date of birth, gender, race, weight, height, family history of osteonecrosis, menopausal status, corticosteroid therapy, smoking habits, and alcohol use.

Patients had a mean age of 46 years (range, 28 to 78); there were 29 women and 16 men, of whom 36 were Caucasian, 7 African-American, and 2 Asian. Associated diseases that were identified included systemic lupus erythematosus (SLE) in 9 patients and inflammatory bowel disease in 1 patient.

Corticosteroid-associated osteonecrosis (defined as greater than 2 grams of prednisone equivalent for a 3 month period)[21] was documented in 20 patients including all 9 patients with SLE. Twenty-six patients reported a history of alcohol use [4 reported heavy use (> 20 drinks per week), 22 moderate use (10 to 20 drinks per week)] and 14 patients had a history of tobacco use [3 reported heavy use (> 10 years of more than one pack per day), 11 moderate use > 10 years of 10–20 cigarettes per day)] with usage criteria defined previously[21].

Osteonecrosis was diagnosed in the hips in 41 patients (34 bilateral) and in the knees of 20 patients (13 bilateral). Eleven patients had involvement of joints other than the hip or knee (shoulder, ankle). There were 20 patients with only hip involvement, while 2 patients had only knee involvement. Eight patients were characterized as multifocal (more than 3 anatomical sites).

Controls. The patient's results were compared with those of a healthy cohort of 40 adult laboratory personnel (23 women, 17 men) who had a mean age of 37 years (range 24–54). Control coagulation assays were done in the same laboratory with the same methodology, reagents, and machinery as the patient's assays[29,30,32]. Controls were studied during September 1996. The 40 adults were selected to serve as a broad-based healthy control group for many studies of coagulation. They were without history of venous or arterial thrombosis; none were pregnant or took medication that could effect serologic measures of coagulation. They were not selected with attention to their alcohol and smoking history since these factors have little effect on the serologic coagulation measures made in the current report. They were not selected in regards to family history of thrombosis.

Although controls were not matched to patients by age, sex, or race, the controls' age, sex, and race were not correlated ($p > 0.09$)[30,32] with their 9 serologic coagulation measures (using stepwise multiple regression), making it unlikely that differences in measures of coagulation between controls and osteonecrosis patients represented age, sex, or race effects rather than a predisposition to thrombophilia or hypofibrinolysis.

Laboratory methods. Blood samples were obtained from individuals after a 12 hour fast, and after the patient was seated for 5 to 10 minutes. Venous blood was drawn between 7 and 12:00 AM. After discarding the first 3 ml, venous blood was collected in 5 ml sodium citrate (0.13 M; 3.8%) Vacutainer tubes (Becton and Dickinson, Franklin Lakes, NJ, USA). Blood samples to be analyzed for tissue plasminogen activator activity were collected in 4 ml Stabilyte® tubes (American Diagnostica, Greenwich, CT, USA) containing an acidified citrate anticoagulation solution. For determination of stimulated tissue plasminogen activator (tPA-Fx) levels, blood was collected in Stabilyte tubes following venous occlusion at 100 mm Hg with a blood pressure cuff for 10 minutes. The blood samples were centrifuged at 2000 × g for 20 minutes at 4°C. Platelet poor plasma was snap frozen and stored at –70°C until sent for analysis. All specimens were labeled with a patient study number and sent to The Cholesterol Center in Cincinnati, Ohio for analysis according to previously described techniques[8,13,20,29-40].

The serologic coagulation factors that were analyzed included assays to evaluate for thrombophilia and hypofibrinolysis. Resistance to activated protein C (RAPC) was measured by a functional clotting technique[39,40]. Anticardiolipin antibodies IgM and IgG, protein C, and free protein S were measured using ELISA[13]. Homocysteine was measured by gas chromatograph mass spectrometry[36]. Tissue plasminogen activator and plasminogen activator inhibitor activities were measured by chromogenic assays[13,37,38]. Lipoprotein (a) [Lp(a)] was measured by immunoprecipitin analysis[8].

The cutpoints for the 9 serologic measures were as follows: protein C < 70%, free protein S < 65%, resistance to activated protein C < 1.99, plasminogen activator inhibitor activity (≥ 21.1 U/ml), Lp(a) ≥ 35 mg/dl, anticardiolipin IgG antibody ≥ 22 GPL, anticardiolipin IgM antibody ≥ 10 MPL, homocysteine ≥ 14.1 µmol/l, and stimulated tissue plasminogen activator activity < 2.28 IU/ml.

Blood samples for our study were obtained in 1996–1997, before routine availability of the polymerase chain reaction (PCR) cDNA assays for thrombophilic [factor V Leiden, prothrombin, methylenetetrahydrofolate reductase (MTHFR)] and hypofibrinolytic (*4G/5G* polymorphism of the plasminogen activator inhibitor gene) gene mutations. These mutations have recently been shown to play an important role in osteonecrosis[29,30] and in venous and arterial thrombosis[32,35]. However, the serologic studies done provide gene markers for these gene mutations as follows: resistance to activated protein C — factor V Leiden; homocysteine — MTHFR; plasminogen activator inhibitor activity (PAI-Fx) — *4G/5G* polymorphism of the *PAI* gene. These gene products do not, however, provide entirely the same information as their parent gene mutations and optimal studies should, where possible, include both the serologic and PCR measurements[30,32,35].

Data analysis. The levels for each of the test results are summarized as the mean and standard deviation. As many of the data were not normally distributed, patients' results were compared to the normal values using the Wilcoxon nonparametric test for differences. In addition, the results from each assessment were compared to the values established as normal limits and were subsequently ranked as elevated (high) or depressed (low). Categorical data among groups of osteonecrosis patients as well as between the osteonecrosis patients and the controls were compared by the chi-square statistic, with Fisher's exact test for small sample size. Statistical significance was accepted at the $p < 0.05$ level. All statistical analysis was performed using SAS (SAS Institute, Cary, NC, USA) and the PEPI software package (USD, Inc., Stone Mountain, GA, USA).

RESULTS

Figure 1 displays variables that differed both quantitatively and categorically, comparing patients with osteonecrosis and healthy controls. Two thrombophilic abnormalities, high

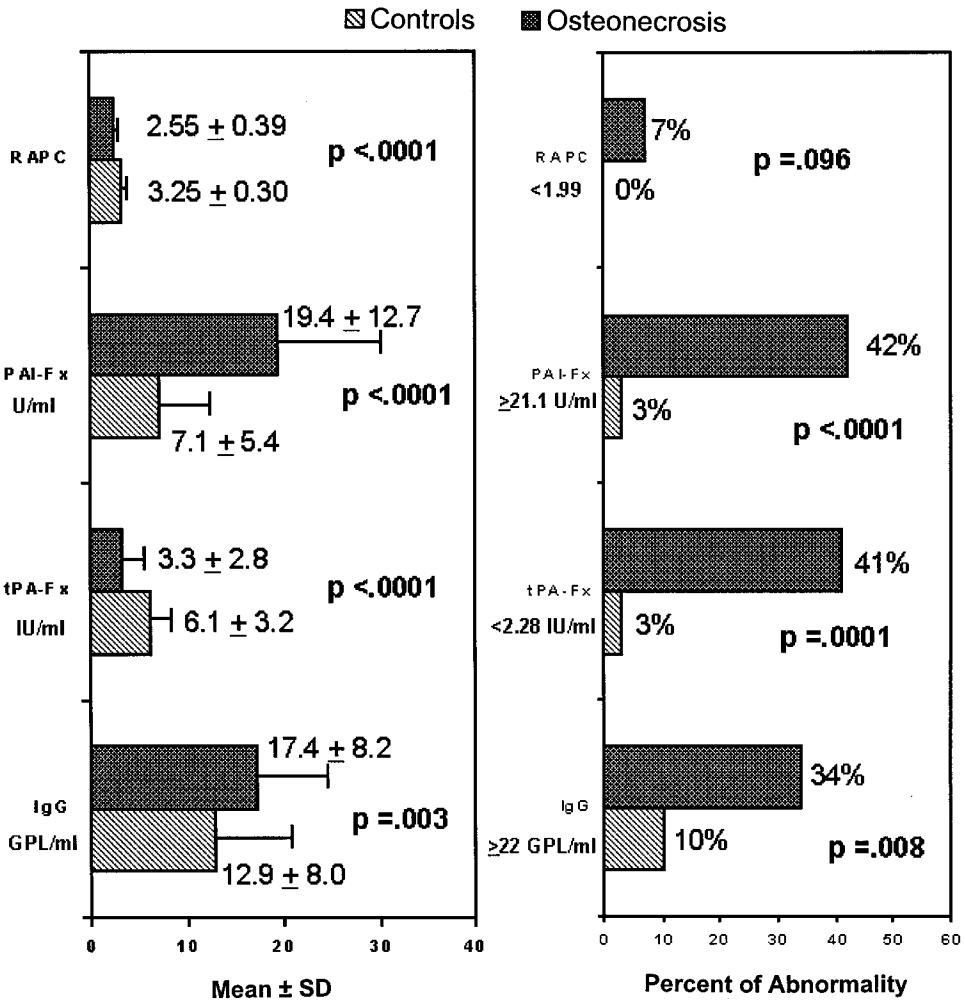

Figure 1. A numerical and categorical comparison of the levels and frequency of serologic coagulation factor abnormalities between osteonecrosis patients and controls.

levels of anticardiolipin antibody IgG and low levels of resistance to activated protein C, were much more common in patients than controls. Not displayed in Figure 1, mean serum homocysteine in patients (10.3 ± 8.4 µmol/l) was higher than in controls (8.3 ± 5), p = 0.032; however, the percentage of patients with homocysteine levels ≥ the normal 95th percentile (14.1 µmol/l) was not greater (p > 0.05) in patients than in controls.

Hypofibrinolysis, identified by low levels of stimulated tPA-Fx and high levels of its major inhibitor PAI-Fx, was much more common in patients than in controls (Figure 1).

Frequency of coagulopathies. Table 1 displays the demographics for the osteonecrosis group with and without detectable abnormalities. There were no apparent significant differences between these 2 groups with respect to etiologic associations, except that the group with no abnormalities was limited to only Caucasians.

As displayed in Figure 2, 8 of the osteonecrosis group (17.8%) had no detectable abnormality, while 28 of the control group had no abnormal results (70%) (p < 0.0001). Patients were more likely than controls to have one coagulation abnormality (35.5% vs 27.5%) and were much more likely to have 2 or more abnormalities (46.7% vs 2.5%) (Figure 2). Eight of the 45 patients (18%) had 3 coagulation abnormalities versus none of the 40 controls, and one patient (2%) had 4 abnormalities.

The abnormal test results for each of the osteonecrosis patients are indicated in Table 2. The most frequently occurring abnormalities for the osteonecrosis group were high PAI-Fx (42.2%), low tPA-Fx (40.9%) (18/44, 1 missing), and high IgG anticardiolipin antibodies (34.1%) (15/44, 1 missing). For the control group, the most frequent abnormalities were Lp(a) (12.5%) and IgG anticardiolipin antibodies (10%). There were no abnormal levels of protein C or protein S (free) detected for either study group.

The increased frequency of abnormal test results in

Table 1. Demographics of patients with osteonecrosis.

	Patients with Osteonecrosis		
	Without Coagulopathy, n = 8	With Coagulopathy, n = 37	p_F
Age, yrs (range)	45 (28–78)	47 (29–74)	
Sex (%)			
Male	3 (38)	13 (35)	1.0
Female	5 (62)	24 (65)	
Race (%)			
Caucasian	8 (100)	28 (76)	
Afro-American	0	7 (19)	0.18
Asian	0	2 (5)	
Corticosteroid associated (%)			
Yes	4 (67)	16 (50)	
No	2 (33)	16 (50)	0.66
Not noted	2	5	
Alcohol associated (%)			
Yes	6 (86)	20 (63)	
No	1 (13)	12 (37)	0.39
Not noted	1	5	
Smoker (%)			
Yes	3 (43)	11 (33)	
No	4 (57)	22 (67)	0.68
Not noted	1	4	
Other risk factor (%)			
Yes	6 (75)	27 (77)	
No	2 (25)	8 (23)	1.0
Not noted	0	2	

p_F: Fisher's p value.

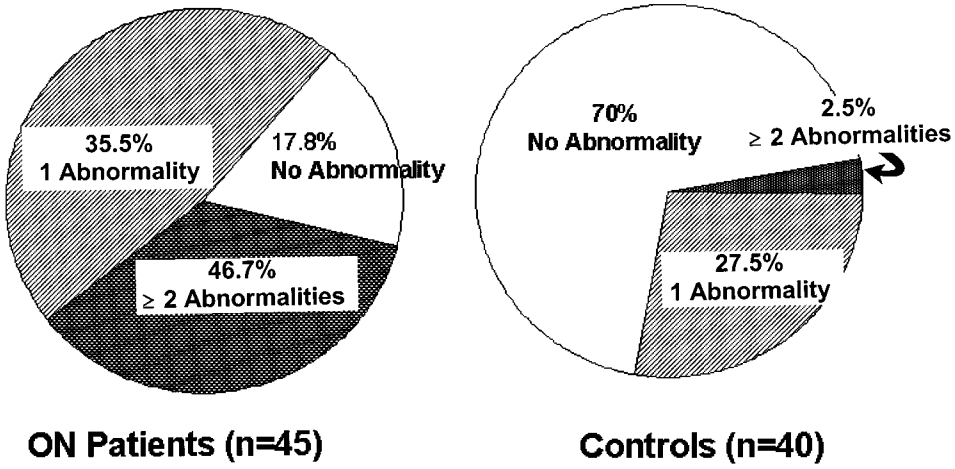

Figure 2. Comparison of the frequency of serologic coagulation factor abnormalities between osteonecrosis patients and controls. Fisher's test $p < 0.0001$. For definitions, see Table 2.

osteonecrosis patients as compared to controls was significantly different for PAI ($p < 0.0001$), IgG aCL ($p = 0.008$), and stimulated tPA ($p = 0.0001$). There was a boundary significance for increased frequency of abnormal activated protein C resistance ($p = 0.096$) and IgM aCL ($p = 0.093$).

The sensitivity of using ≥ 1 coagulation abnormality as a screen for pathoetiologies of osteonecrosis is 82% while the specificity is 70%. Specificity increases when using the criteria of more coagulation traits, 97.5% for using ≥ 2 abnormalities and 100% for using ≥ 3 abnormalities.

Table 2. Abnormal test results in patients with osteonecrosis.

Patient	s-tPA-Fx	PAI	Lp(a)	RAPC	aCL	ProC	ProS	HCY
1		x			G			
2	x		x		G			
3				x				
4		x			G			
5	x	x						
6		x	x					
7		x			G			
8					M			
9	x	x			G			
10	x				M			
11	x	x	x		G			
12	x							
13								
14			x					
15		x			G			
16				x				
17								
18								
19	x		x					
20	x							
21								x
22								
23	x	x			G			
24	x	x	x					
25					G			
26		x			G			
27					G			
28		x						
29				x				
30		x	x					
31	x	x			G			
32								
33	x				G			
34					M			
35	x							
36	x							
37	x							
38	x	x						x
39		x			G			
40								
41	x	x	x					
42								
43								
44	x	x			G			
45		x						

G: IgG; M: IgM; s-tPA-Fx: stimulated tissue plasminogen activator; PAI: plasminogen activator inhibitor; Lp(a): lipoprotein(a); RAPC: resistance to activated protein C; aCL: anticardiolipin antibodies; ProC: Protein C; ProS: Protein S; Hcy: homocysteine.

Etiologic associations. There was no correlation between the number of abnormal tests and the number of joints involved (r = 0.001).

Table 1 displays a comparison of the demographics between osteonecrosis patients with (n = 37) and without (n = 8) abnormal coagulation factor levels. In this study, there were fewer osteonecrosis patients with a history of smoking than those without. Three patients reported a history of heavy smoking. Of the osteonecrosis patients with at least one abnormal test result, there were fewer smokers (n = 11) than non-smokers (n = 22).

In the total patient cohort, there were 26 patients reporting occasional to heavy consumption of alcohol, while 13 patients reported no alcohol consumption. Only 4 patients reported

heavy alcohol use. Both groups of osteonecrosis patients (with and without coagulopathy) had a greater percentage of alcohol consumers than abstainers.

In the 37 osteonecrosis patients with a coagulopathy, 32 of whom had data on corticosteroid use, the frequency of patients with corticosteroid associated disease (n = 16) was the same as those without corticosteroid treatment (n = 16). Further, no increase in the number of abnormal tests per patient was noted for the corticosteroid group (1.4, range 0–3) versus the non-steroid group (1.7, range 0–4). There were no significant differences in the frequency of abnormal results for any specific test between the corticosteroid and non-corticosteroid groups ($p > 0.05$). The most frequent abnormalities of the corticosteroid group were PAI-Fx (n = 10), IgG aCL (n = 8), and tPA-Fx (n = 7).

There were 5 patients with no known external risk factors (corticosteroids, alcohol, comorbid conditions, etc.) (Table 3). Four of the 5 patients had 2 or more coagulation abnormalities. Four patients (80%) had abnormal levels of IgG aCL and 4 (80%) had high PAI-Fx, while 2 patients (40%) had abnormal stimulated tPA-Fx levels and 1 patient (20%) had abnormal Lp(a) levels.

Of the 20 patients with a diagnosis of hip involvement only, 16 patients were detected with a coagulation abnormality. The most frequent abnormalities were high PAI-Fx (n = 9), low stimulated tPA-Fx (n = 8), and elevated IgG aCL (n = 7).

There were 2 patients with involvement limited to the knee. Both patients had abnormal stimulated tPA-Fx; one patient had 4 coagulation abnormalities. There were 11 patients with joint involvement other than the hip or knee. The most frequent abnormality in this group was high PAI-Fx (n = 6).

Of the 8 patients with multifocal disease, 6 patients had at least one abnormal test result, while 3 patients had 2 or more abnormalities. There was no apparent pattern in the type of abnormal test result noted. Elevated PAI-Fx was the most frequent abnormality noted (n = 4).

DISCUSSION

The importance of coagulation abnormalities in the pathogenesis of osteonecrosis has been noted since the disease was first described[1]. With increasing knowledge of the thrombotic/fibrinolytic pathways and with more advanced laboratory testing, the role of coagulation disorders has been revisited. Jones proposed that intravascular coagulation with fibrin-platelet thrombosis begins in the vulnerable subchondral microcirculation (the capillary and sinusoidal bed) and is associated with vasoconstruction and impaired secondary fibrinolysis (reperfusion of necrotic vessels with peripheral marrow hemorrhages)[5,7,41,42]. Glueck, *et al* suggested that thrombotic occlusion of venous outflow may lead to intramedullary hypertension, reduced arterial perfusion, hypoxia-anoxia, and bone infarction[20,29,30]. There is some evidence of fibrin thrombi within the transitional zone of the osteonecrotic lesion[41], but this is not a universal finding[43]. Other pathologic features have been noted such as increased intraosseous pressure[44-46], bone marrow edema[43], hemorrhage[47], and abnormal venograms[44]. Additional basic science and clinical studies are needed to further understand the role of coagulation disorders in the disease process as the associations found in this article may lead to osteonecrosis or inversely, osteonecrosis might indeed produce changes in coagulation variables.

Table 3. Patients with idiopathic osteonecrosis.

Patient	Race	Sex	Age, yrs	Joint	Abnormal Tests
1	White	F	47	Hip	Lp(a), IgG aCL, PAI-Fx
2	White	M	36	MCP, Talus	IgG aCL, PAI-Fx
3	White	F	78	Bil hips	IgG aCL, PAI-Fx
4	Asian	F	34	Bil hips	IgG aCL, s-tPA-Fx, PAI-Fx
5	White	M	41	Mutifocal	s-tPA-Fx

MCP: metacarpophalangeal; Bil hips: bilateral hips. For additional definitions see Table 2.

In our study, most patients had both an external contributor to osteonecrosis (corticosteroids, alcoholism, SLE) and a thrombophilic and/or hypofibrinolytic coagulation disorder. In our patient cohort, 82.2% of the patients were identified with at least one coagulation abnormality, while 46.7% had more than one abnormality. Significant differences were found in the levels of specific coagulation factors (RAPC, PAI-Fx, tPA-Fx, IgG), as well as the percentages of patients with these defects when comparing the osteonecrosis patients to our controls. This is similar to the results reported by Glueck, *et al* in studies of the osteonecrosis population[8,20,29,30]. They found that 74% of patients with osteonecrosis have one or more primary coagulation disorders[20]. Similar findings have been reported for other osteonecrosis populations including Legg-Perthes' disease and osteonecrosis of the jaw[11,13]. Unlike the previous study of Glueck, *et al*[30], however, we did not find significant differences in the percentage of patients with low free protein S or high Lp(a).

Our control group of healthy individuals had a percentage of coagulation abnormalities [27.5% with one abnormality, only one patient (2.5%) with 2 abnormalities, none with ≥ 3 abnormalities]. Using 9 serologic tests to assess thrombophilia and hypofibrinolysis, with cutpoints based on the upper 5th percentile of a healthy population (homocysteine, anticardiolipin antibody IgG, IgM, Lp(a), PAI-Fx) or lower 5th percentile (resistance to activated protein C, protein C, free protein S, tPA-Fx), it is not surprising that of 9 tests in aggregate, 27.5% of healthy controls have one coagulation abnormality, or that 30% had ≥ 1 abnormality. Our control group is not, moreover, unrepresentative in this regard. In a recent study of 95 healthy Israeli women with ≥ 1 normal pregnancy, 63% had no coagulation abnormalities, 33% had one, 5% had 2, and 0% had 3 or 4, consistent with the control group in our study[35]. As noted by Rosendaal[48], venous thrombosis (the postulated etiology in osteonecrosis of the hip), is a multicausal disease; usually more than one coagulation abnormality needs

to be present before thrombosis occurs. As further noted by Rosendaal[48], the younger an individual, the more risk factors are required to precipitate thrombosis. It should be noted that our control group is composed of healthy patients, and different controls with more comorbidities might produce different results.

In 1970, Boettcher, *et al*[3] tested 30 osteonecrosis patients with a battery of coagulation tests including bleeding time, clotting time, fibrinolysis, thromboplastin, factor VIII, and others. There was at least one abnormal test for each patient. Of the 30 patients, 5 were classified as hypercoagulable and 4 were classified as hypocoagulable. Four of 5 hypercoagulable patients had elevated factor VIII. In a study evaluating osteonecrosis patients with and without SLE, Nagasawa, *et al*[6] reported that 29% (7/24) of SLE patients with osteonecrosis had prolonged activated partial thromboplastin time as compared to 11% (5/44) of SLE patients without osteonecrosis. They also noted similar findings for the presence of lupus anticoagulant (25% with osteonecrosis, 11% without osteonecrosis).

Asherson, *et al*[19] reported an association of anticardiolipin antibodies and osteonecrosis. While most of the reports[13,31,46,47] have been in support of this association, Alarcon-Segovia, *et al*[49] found no such relationship in a study of patients with SLE. In our study, elevated anticardiolipin antibodies (IgG) were present in 34% of the osteonecrosis patients as compared to only 10% of the controls ($p = 0.008$).

In 1993, Van Veldhuzen, *et al*[27] reported that in a study of 5 patients with avascular necrosis, all had an abnormality of fibrinolytic potential. Further, elevated protein C ($n = 2$), protein S ($n = 3$), and free protein S ($n = 1$) were observed. Pierre-Jacques, *et al*[50] published a case report in which an adult female diagnosed with osteonecrosis was identified with a protein S deficiency. The total antigenic, free, and functional protein S concentrations were significantly lower than normal. In this study, no patients were identified with abnormal protein C or protein S levels.

Glueck, *et al*[8] reported that 9 of 12 patients with idiopathic osteonecrosis had high heritable PAI-Fx and low tPA-Fx. We found that 42% of our osteonecrosis patients had high PAI-Fx, while 41% of osteonecrosis patients had low stimulated tPA-Fx. Both were significantly different than for the control group (3% for both tests; $p = 0.0001$). In our idiopathic group (no known risk factors), 2 patients had low tPA-Fx, 4 had high PAI-Fx, and one patient had both abnormalities (Table 3). Four of the 5 patients had abnormal levels of IgG aCL.

Thrombophilic and hypofibrinolytic disorders may be inherited or acquired. Defects in genes for each of these factors have been identified. However, it is also possible for the serologic measures to be affected by other comorbidities. PCR-DNA analysis of coagulation abnormalities optimally distinguishes whether an inherited defect is responsible for the abnormal serologic levels observed in the osteonecrosis patients[29,30]. Recent studies relying on PCR-DNA measurements of genetic mutations unaffected by environmental vectors suggest that the great majority of coagulation disorders in osteonecrosis are heritable, not acquired[29,30,32], comparable to findings in obstetric complications[35]. However, additional studies are needed to examine this diverse patient cohort more thoroughly.

In our study as well as in previous reports[20,24], coagulation abnormalities were not detected in a small subset of the osteonecrosis patient cohort. This may reflect limitations in our understanding of the causes of thrombosis and hypofibrinolysis. In the last 5 years, for example, many very important coagulation factors have been rendered diagnosable by advances in laboratory methodology including the factor V Leiden mutation, the prothrombin gene mutation, the MTHFR gene mutation, and the *4G/5G* polymorphism of the *PAI-1* gene[32,35]. Recognition of factors VIII and XI as thrombophilic risk factors has been better appreciated in the last 2 to 3 years. We speculate that those patients categorized as "normal" by currently existing coagulation tests will be shown in the future to have currently unrecognized coagulation abnormalities. It is also possible that in some cases the coagulation factor abnormality is not inherited but related to the patient's comorbidities or therapies and thereby may be transient, making it difficult to detect. For example, there is a documented association between steroid therapy and hypercoagulability. Cosgriff[51] hypothesizes that steroid treatment places some patients in a "prethrombotic state." It may be that the patients with a coagulation abnormality are at higher risk to the effects of steroid treatment.

Coagulation disorders may play a role early or late in the pathogenesis of the disease. It is possible that the specific coagulopathy is the etiology, or causative agent, of osteonecrosis. The intravascular coagulation that results would then be the initial event in the pathogenic cascade that leads to clinical disease[42]. One way to examine this issue would be to perform coagulation profile testing in patient groups at risk for corticosteroid-associated osteonecrosis (e.g., patients with SLE, organ and bone marrow transplant patients) before and after initiating corticosteroid treatment. It should be noted that in our study, whether the hypercoagulable state exists prior to the initiation of osteonecrosis is still conjectural.

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REFERENCES

1. Phemister DB. Fractures of the neck of the femur, dislocation of hip, and obscure vascular disturbances producing aseptic necrosis of the head of the femur. Surg Gynec Obstet 1934;59:415-40.
2. Nilsson IM, Krook H, Sternby NH, Soderberg E, Soderstrom N. Severe thrombotic disease in a young man with bone marrow and skeletal changes and with a high content of an inhibitor in the

fibrinolytic system. Acta Med Scand 1961;169:323-7.
3. Boettcher WG, Bonfiglio M, Hamilton HH, Sheets RF, Smith K. Non-traumatic necrosis of the femoral head. I. Relation of altered hemostasis to etiology. J Bone Joint Surg Am 1970;52:312-21.
4. Egan R, Munn RK. Catastrophic antiphospholipid antibody syndrome presenting with multiple thromboses and sites of avascular necrosis. J Rheumatol 1994;21:2376-9.
5. Jones JP Jr. Intravascular coagulation and necrosis. Clin Orthop 1992;277:41-53.
6. Nagasawa K, Ishii Y, Mayumi T, et al. Avascular necrosis of bone in systemic lupus erythematosus: possible role of haemostatic abnormalities. Ann Rheum Dis 1989;48:672-6.
7. Jones JP Jr, Sakovich L, Anderson CE. Experimentally produced osteonecrosis as a result of fat embolism. In: Beckman EL, Elliott DH, Smith EM, editors. Dysbarism-related osteonecrosis. HEW pub (NIOSH) 75-153, Washington, DC: US Government Printing Office; 1974:117-32.
8. Glueck CJ, Freiberg R, Glueck HE, et al. Hypofibrinolysis: A common, major cause of osteonecrosis. Am J Hematol 1994;45:156-66.
9. Gallistl S, Reitinger T, Linhart W, Muntean W. The role of inherited thrombotic disorders in the etiology of Legg-Calve-Perthes disease. J Pediatr Orthop 1999;19:82-3.
10. Glueck CJ, Brandt G, Gruppo R, et al. Resistance to activated protein C and Legg-Perthes disease. Clin Orthop 1997;338:139-52.
11. Glueck CJ, Crawford A, Roy D, Freiberg R, Glueck H, Stroop D. Association of antithrombotic factor deficiencies and hypofibrinolysis with Legg-Perthes disease. J Bone Joint Surg Am 1996;78:3-13.
12. Glueck CJ, McMahon RE, Bouquot J, Stroop D, Tracey T, Wang P. Pathophysiology of osteonecrosis of the jaw: Thrombophilia and hypofibrinolysis. Oral Surg Oral Med Oral Pathol 1996;81:557-66.
13. Gruppo R, Glueck CJ, McMahon RE, et al. The pathophysiology of alveolar osteonecrosis of the jaw: anticardiolipin antibodies, thrombophilia, and hypofibrinolysis. J Lab Clin Med 1996;127:481-8.
14. Herndon WA. Association of antithrombotic factor deficiencies and hypofibrinolysis with Legg-Perthes disease. J Bone Joint Surg Am 1997;79:1114-5.
15. Hunt DM, Holmes Z, Pickering W, Cohen H. Association of antithrombotic factor deficiencies and hypofibrinolysis with Legg-Perthes disease. J Bone Joint Surg Am 1998;80:604-6.
16. Kealey WDC, Mayne EE, McDonald W, Murray P, Cosgrove AP. The role of coagulation abnormalities in the development of Perthes' disease. J Bone Joint Surg Br 2000;82:744-6.
17. Campbell WN, Joshi M, Sileo D. Osteonecrosis following meningococcemia and disseminated intravascular coagulation in an adult: case report and review. Clin Infect Dis 1997;24:452-5.
18. Damry N, Schurmans T, Perlmutter N. MRI evaluation and follow-up of bone necrosis after meningococcal infection and disseminated intravascular coagulation. Pediatr Radiol 1993;23:429-31.
19. Ashershon RA, Khamashta MA, Ordi-Ros J, et al. The "primary" antiphospholipid syndrome: major clinical and serological features. Medicine 1989;68:366-74.
20. Glueck CJ, Freiberg R, Tracey T, Stoop D, Wang P. Thrombophilia and hypofibrinolysis. Pathophysiologies of osteonecrosis. Clin Orthop 1997;334:43-56.
21. Mont MA, Baumgarten KM, Rifai A, Bluemke D, Jones LC, Hungerford DS. Atraumatic osteonecrosis of the knee. J Bone Joint Surg Am 2000;82:1279-90.
22. Mont MA, Glueck CJ, Pacheco IH, Wang P, Hungerford DS, Petri M. Risk factors for osteonecrosis in systemic lupus erythematosus. J Rheumatol 1997;24:654-62.
23. Mont MA, Jones LC. Management of osteonecrosis in systemic lupus erythematosus. Rheum Dis Clin North Am 2000;26:279-309.
24. Mont MA, Jones LC, Petri M, et al. Coagulopathies associated with osteonecrosis of the femoral head [abstract]. Arthritis Rheum 1997;40 Suppl:S264.
25. Mont MA, Payman RK, LaPorte DM, Petri M, Jones LC, Hungerford DS. Atraumatic osteonecrosis of the humeral head. J Rheumatol 2000;27:1766-73.
26. Sheikh JS, Retzinger GS, Hess EV. Association of osteonecrosis in systemic lupus erythematosus with abnormalities of fibrinolysis. Lupus 1998;7:42-8.
27. Van Veldhuzen PJ, Neff J, Murphey MD, Bodensteiner D, Skikne BS. Decreased fibrinolytic potential in patients with idiopathic avascular necrosis and transient osteoporosis of the hip. Am J Hematol 1993;44:243-8.
28. Vela P, Batlle E, Salas E, Marco P. Primary antiphospholipid syndrome and osteonecrosis. Clin Exp Rheumatol 1991;9:545-9.
29. Glueck CJ, Fontaine RN, Gruppo R, et al. The plasminogen activator inhibitor-1 gene, hypofibrinolysis, and osteonecrosis. Clin Orthop 1999;366:133-46.
30. Glueck CJ, Freiberg RA, Fontaine RN, Tracy T, Wang P. Hypofibrinolysis, thrombophilia, osteonecrosis. Clin Orthop 2001;386:19-33.
31. Korompilias AV, Gilkeson GS, Ortel TL, Seaber AV, Urbaniak JR. Anticardiolipin antibodies and osteonecrosis of the femoral head. Clin Orthop 1997;345:174-80.
32. Amiral J, Boyer C, Rothchild C, Wolf M. Determination of protein C by an immunoenzymatic assay. In: Witt I, editor. Protein C — Biochemical and medical aspects. Berlin; New York: Walter de Gruyter; 1983:67-9.
33. Amiral J, Adam M, Plassart V, Minard F, Vissac AM. Immunoassays for the measurement of protein S. In: Gaffney PJ, editor. Fibrinolysis: current prospects. London: John Libbey; 1988:125-8.
34. Glueck CJ, Bell H, Vadlamani L, et al. Heritable thrombophilia and hypofibrinolysis. Arch Ophthalmol 1999;117:43-9.
35. Glueck CJ, Kupferminc MJ, Fontaine RN, Wang P, Weksler BB, Eldor A. Genetic hypofibrinolysis in complicated pregnancies. Obstet Gynecol 2001;97:44-8.
36. Glueck CJ, Shaw P, Lang J, et al. Evidence that homocysteine is an independent risk factor for atherosclerosis in hyperlipidemic patients. Am J Cardiol 1995;75:132-6.
37. Ranby M, Norrman B, Wallen P. A sensitive assay for tissue plasminogen activator. Thromb Res 1982;27:743-9.
38. Ranby M, Sundell IB, Nilsson TK. Blood collection in strong acid citrate anticoagulant used in a study of dietary influence on basal tPA activity. Thromb Haemost 1989;62:917-22.
39. Sochynsky CL, Joyner KA, Ortel TI. A method for testing APC resistance in thrombotic patients. Am Clin Lab 1994;13:26-7.
40. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994;300:517-22.
41. Jones JP Jr. Risk factors potentially activating intravascular coagulation and causing nontraumatic osteonecrosis. Ch. 12. In: Urbaniak JR, Jones JP Jr, editors. Osteonecrosis. Rosemont, IL: AAOS; 1997.
42. Jones JP Jr. Fat embolism intravascular coagulation, and osteonecrosis. Clin Orthop 1993;292:294-308.
43. Chernetsky SG, Mont MA, LaPorte DM, Jones LC, Hungerford DS, McCarthy EF. Pathologic features in steroid and nonsteroid associated osteonecrosis. Clin Orthop 1999;368:149-61.
44. Hungerford DS. Pathogenic considerations in ischemic necrosis of the bone. Can J Surg 1981;24:583-90.
45. Mont MA, Hungerford DS. Current concepts review. Non-traumatic avascular necrosis of the femoral head. J Bone Joint Surg Am 1995;77:459-74.
46. Mont MA, Jones LC, Sotereanos DG, Amstutz HC, Hungerford DS. Understanding and treating osteonecrosis of the femoral head. Inst Course Lect 2000;49:169-85.

47. Saito S, Ohzono K, Ono K. Early arteriopathy and postulated pathogenesis of osteonecrosis of the femoral head. The intracapital anterioles. Clin Orthop 1992;227:98-110.
48. Rosendaal FR. Thrombosis in the young; epidemiology and risk factors; a focus on venous thrombosis. Thromb Haemost 1997;78:1-6.
49. Alarcon-Segovia D, Deleze M, Oria CV, et al. Antiphospholipid antibodies and the antiphospholipid syndrome in systemic lupus erythematosus. A prospective analysis of 500 consecutive patients. Medicine 1989;68:353-65.
50. Pierre-Jacques H, Glueck CJ, Mont MA, Hungerford DS. Familial heterozygous protein-S deficiency in a patient who had multifocal osteonecrosis. A case report. J Bone Joint Surg Am 1997; 79:1079-84.
51. Cosgriff SW. Thromboembolic complications associated with ACTH and cortisone therapy. JAMA 1951;147:924-6.