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CME ARTICLE

Coagulation Abnormalities in Osteonecrosis and Bone Marrow Edema Syndrome

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educational objectives

As a result of reading this article, physicians should be able to:

1. Identify relevant coagulation laboratory parameters at the site of osteonecrosis and bone marrow edema syndrome.

2. Understand the mechanisms of fibrinolysis and its possible role in the emergence of osteonecrosis and bone marrow edema syndrome.

3. Understand the mechanisms of thrombophilia and its possible role in the emergence of osteonecrosis and bone marrow edema syndrome.

4. Increase their knowledge about diagnostic and therapeutic measures at the site of osteonecrosis and bone marrow edema syndrome when disturbed coagulation laboratory parameters are present.

ABSTRACT

The aim of this review was to provide information about the variety of thrombophilic and hypofibrinolytic markers that are possible risk factors for the development of osteonecrosis and bone marrow edema syndrome. A total of 48 parameters were identified in 45 studies that included 2163 patients. The most frequently reported laboratory findings included altered serum concentrations of lipoproteins, decreased concentration and function of fibrinolytic agents, increased levels of thrombophilic markers, and sev-

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eral single nucleotide polymorphisms. Despite inhomogeneities in reported parameters, results, patients' collectives, and treatment strategies, these data suggest that coagulation abnormalities may play an important role in the emergence of osteonecrosis and bone marrow edema syndrome.

Osteonecrosis is an idiopathic, debilitating, and progressive disease with various causes, including disruption of the blood supply or venous occlusion, that results in increased intraosseous pressure. Etiologies include alcoholism, blood disorders, trauma, radiation therapy, corticosteroid administration, dysbaria, and autoimmune diseases.[1] Bone marrow edema syndrome is an uncommon, self-limiting skeletal disease. Curtiss and Kincaid[2] first reported the syndrome in 1959. They referred to it as transient osteoporosis because of the osteopenic appearance of the affected bone on plain radiographs. Since then, this entity has been described using other terms, such as transient marrow edema syndrome.[3] The natural course of this entity shows spontaneous remission after 6 to 12 months.[4]

The pathogenesis of osteonecrosis and bone marrow edema syndrome remains unknown, but several hypotheses have been proposed. For osteonecrosis, vascular disturbances with a mismatch between the arterial inflow and venous outflow, reduced vessel density, or thrombembolisms of the terminal vessels may play a role.[1,5] Traumatic injuries with an initial fracture of the subchondral bone that result in necrosis of the surrounding area may also cause osteonecrosis.[6] For bone marrow edema syndrome, thrombembolism, obstruction of arteriolar inflow or venous outflow, injury to the vessel wall secondary to vasculitis, altered lipid metabolism, and reduced fibrinolysis have been suggested as etiologic factors.[7] Some authors have postulated that bone marrow edema syndrome might be a reversible or a prestage form of osteonecrosis.[8,9]

For both entities, several studies have indicated that coagulation abnormalities causing thrombophilia or hypofibrinolysis might contribute to the emergence of these entities. However, inhomogeneities in the patients' collectives, the broad variety of determined parameters and differences in the diagnosis and therapy make a literature evaluation and comparison of data among these studies difficult. Hence, the purpose of the current work was to systemically review the literature reporting coagulation abnormalities at the site of osteonecrosis and bone marrow edema syndrome.

MATERIALS AND METHODS

Inclusion Criteria

A PubMed search was conducted to identify English-language articles published between 1968 and 2012. Search terms were *osteonecrosis*, *bone necrosis*, *transient osteoporosis*, and *(transient) bone marrow edema (syndrome) (*alone and in combination with *hypofibrinolysis* and/or *thrombophilia)*. An additional search was performed throughout the bibliography of the resulting studies to identify all possibly relevant articles. Only orthopedic studies were included, except for those originating from other facilities but contributing essential information. Reviews were excluded from the study.

Evaluation of Studies

All identified studies were evaluated with respect to publication year, number of patients, entity, etiology, diagnostic measurements, treatment procedures, and level of evidence.

RESULTS

Study Identification and Level of Evidence

A total of 45 studies with 2163 patients were identified (Table 1).[10-54] Of these, 13 were case reports and 32 were original manuscripts (those with a large series of patients). Eleven studies were published before 2000 and 34 after. Fourteen studies were level IV, 22 were level III, and 9 were level II studies; no level I study was identified (Table 1).

Etiology

The majority of studies focused on findings at the site of idiopathic or secondary osteonecrosis (41 studies; 1911 patients),[13-54] Three studies (60 patients) reported bone marrow edema syndrome.[10-12] One study reported a cohort of 145 patients with elevated lipoprotein(a) (Lp[a]) plasma values,[23] and another study described a cohort of 96 patients with *Morbus Perthes*[46] (Table 1). Thirteen etiologies were identified as possible factors for the emergence of secondary osteonecrosis, and 1 study describes cases of osteonecrosis and *M Perthes* (Table 2).[46]

Localization of Osteonecrosis and Bone Marrow Edema Syndrome

The hip joint was reported as the most commonly affected joint in 40 studies, followed by the knee and shoulder joint in 8 and 4 studies, respectively (Table 1).

Investigated Parameters

A total of 48 parameters were investigated (Table 3). The most frequently investigated parameter was Lp(a) (n=14), followed by plasminogen activator inhibitor (PAI), protein S (n=7) and C (n=5), tissue plasminogen activator (tPA) (n=5), factor VIII (n=5), apolipoproteins ApoA1 (n=5) and ApoB (n=4), and various genes or gene mutations.

The Lp(a) serum values were frequently elevated in cases of osteonecrosis and bone marrow edema syndrome (Table 3). However, some discrepancies are evident among the osteonecrosis studies. Glueck et al[24,27] reported that elevated Lp(a) values were evident in primary but not secondary osteonecrosis. Moreover, these findings only account for unifocal but not multifocal osteonecrosis.[27] Posan et al[46] reported elevated values in primary and secondary osteonecrosis. Jones et al[36] reported no differences in the serum Lp(a)

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Table 1

Reports of Coagulation Abnormalities in Patients With Osteonecrosis and Bone Marrow Edema Syndrome

Study	Level of Evidence	No. of Patients	Diagnosis	Localization	Etiology
Berger et al[10]	IV	3	BMES	Hip	Idiopathic
Berger et al[11]	II	20	BMES	Hip	Idiopathic
Berger et al[12]	II	37	BMES	Hip	Idiopathic
Björkman et al[13]	III	38	ON	Knee	32 idiopathic, 6 secondary
Cenni et al[14]	II	36	ON	Hip	18 idiopathic, 18 secondary
Chang et al[15]	III	71	ON	Hip	18 idiopathic, 53 secondary
Chotanaphuti et al[16]	III	55	ON	Hip	40 idiopathic, 15 secondary
Dai et al[17]	III	474	ON	Hip	140 idiopathic, 334 secondary
de Larranaga et al[18]	IV	19	ON	Hip	Secondary
Ekmekci et al[19]	III	19	ON	Hip	Secondary
Elishkewich et al[20]	IV	1	ON	Hip	Idiopathic
Glueck et al[21]	III	30	ON	Hip	12 idiopathic, 18 secondary
Glueck et al[22]	II	5	ON	Hip	Idiopathic
Glueck et al[23]	II	145	Elevated Lp(a)	NR	NR
Glueck et al[24]	III	31	ON	Hip, knee, shoulder	18 idiopathic, 13 secondary
Glueck et al[25]	III	59	ON	Hip	31 idiopathic, 28 secondary
Glueck et al[26]	IV	1	ON	Hip	idiopathic
Glueck et al[27]	III	36	ON	Hip	20 idiopathic, 15 secondary
Glueck et al[28]	III	95	ON	Hip	36 idiopathic, 59 secondary
Glueck et al[29]	II	133	ON	Hip	71 idiopathic, 62 secondary
Glueck et al[30]	II	26	ON	Multifocal	13 idiopathic, 13 secondary
Glueck et al[31]	IV	2	ON	Hip	Secondary
He and Li[32]	III	31	ON	Hip	Secondary
Hirata et al[33]	III	34	ON	Hip	Secondary
Hirata et al[34]	III	20	ON	Hip	Secondary
Jones[35]	IV	3	ON	Hip	1 idiopathic, 2 secondary
Jones et al[36]	III	45	ON	Hip, shoulder, knee, ankle	Secondary
Kechli et al[37]	III	24	ON	Hip, knee, shoulder, ankle, hand	Secondary
Kim et al[38]	III	206	ON	Hip	98 idiopathic, 108 secondary
Kubo et al[39]	IV	1	ON	Multifocal	Idiopathic
Kutlar et al[40]	III	45	ON	Hip, shoulder	Secondary
Mehsen et al[41]	III	39	ON	Hip, knee, ankle	11 idiopathic, 28 secondary
Miyanishi et al[42]	III	59	ON	Hip	12 idiopathic, 47 secondary
Moore et al[43]	IV	1	ON	Knee	Secondary
Oinuma et al[44]	II	32	ON	Hip, knee	Secondary
Pierre-Jacques et al[45]	IV	1	ON	Multifocal	Idiopathic
Posan et al[46]	III	96	ON *Morbus Perthes*	Hip	21 idiopathic ON, 28 secondary ON
Seguin et al[47]	II	49	ON	Hip	5 idiopathic, 44 secondary
Shahin[48]	IV	1	ON arthritis	Patella calcaneus	Idiopathic
Üreten et al[49]	IV	1	ON	Hip	Idiopathic
Vairaktaris et al[50]	IV	1	ON	Mandible	Secondary
Wermes et al[51]	IV	1	ON	Hip	Idiopathic
Zalavras et al[52]	III	68	ON	Hip	17 idiopathic, 51 secondary
Zalavras et al[53]	III	66	ON	Hip	23 idiopathic, 43 secondary
Zhang et al[54]	IV	3	ON	Hip	Idiopathic

Abbreviations: BMES, bone marrow edema syndrome; Lp(a), lipoprotein(a); NR, not reported; ON, osteonecrosis.

values in patients with osteonecrosis compared with a control group.

The PAI has been investigated at the site of bone marrow edema syndrome and osteonecrosis. Berger et al[10] reported that decreased PAI levels were seen in 1 patient with bone marrow edema syndrome, whereas the levels were normal in 2 other cases. Jones et al[36] reported a significant difference in PAI activity between patients with osteonecrosis and healthy controls. Most of the studies reporting PAI involvement in the pathophysiology of osteonecrosis have been reported by Glueck et al[21,22,27]: high serum levels of PAI combined with increased protein function and antigen activity were found in unifocal idiopathic but not unifocal secondary osteonecrosis. However, in multifocal osteonecrosis, an elevated PAI activity was observed in cases of secondary osteonecrosis but not for idiopathic osteonecrosis.[30] Regardless of the etiology, a higher frequency of the 4G/4G polymorphism of the PAI-1 gene has also been identified as an osteonecrosis risk factor.[25,27,38]

Chotanaphuti et al[16] reported a high prevalence of protein S deficiency in patients with idiopathic osteonecrosis. Elishkewich et al[20] made similar observations in a 36-year-old man. Familial protein S deficiency, causing a low level of free protein S, was also identified as a risk factor for idiopathic osteonecrosis by Glueck et al.[26,27,30] Studies by Pierre-Jacques et al[45] and Üreten et al[49] confirmed the contribution of low protein S levels to the development of idiopathic multifocal or unifocal osteonecrosis, respectively. Low protein C concentration levels also reportedly contributed to the emergence and maintenance of idiopathic osteonecrosis by Glueck et al,[24] Mehsen et al,[41] and Wermes et al.[51]

Consistent data were found regarding the tPA in osteonecrosis: Glueck et al[22] reported a decrease of stimulated tPA function in 4 of 5 patients with idiopathic osteonecrosis. They also confirmed these findings for secondary osteonecrosis in larger patient cohorts.[24,25] Jones et al[36] also reported a significant decrease in tPA function among 45 patients with secondary osteonecrosis. Glueck et al[21] reported a higher stimulated tPA function in secondary osteonecrosis compared with idiopathic osteonecrosis.

Blood coagulation factor VIII was reported to be significantly elevated in patients with osteonecrosis.[47] Chotanaphuti et al[16] and Glueck et al[29,30] identified this parameter as a risk factor for idiopathic osteonecrosis. Coagulation factor VIII was also elevated in 1 patient with steroid-induced secondary bilateral femoral head osteonecrosis.[31]

Controversial data have been reported for serum ApoA1 and ApoB levels. In patients with idiopathic osteonecrosis, serum ApoA1 levels were elevated in 5 patients and decreased in 1 patient, whereas elevated serum ApoB levels were observed in 2 patients.[22] Hirata et al[34] reported that the serum ApoA1 and ApoB levels were not associated with the development of osteonecrosis. Glueck et al[21] reported higher serum ApoB levels in idiopathic than in secondary osteonecrosis. Zalavras et al[52] reported significantly higher serum ApoB levels in patients with idiopathic osteonecrosis compared with a control group without osteonecrosis, but this effect was not evident for the serum ApoA1 levels. Miyanishi et al[42] reported that a high serum ApoB:ApoA1 ratio was a risk factor for the development of nontraumatic osteonecrosis compared with traumatic osteonecrosis; similar findings were reported by Hirata et al.[33]

Ten genes or gene mutations were investigated in 18 studies (Table 3). Several studies demonstrated a homozygosity or heterozygosity of methylenetetrahydrofolate reductase (MTHFR) polymorphisms,[15,20,26,40,53] Factor V Leiden,[13,19,29,30,46] and prothrombin 20210A mutation[13,19,50] in association with osteonecrosis; Kechli et al[37] reported no association of the aforementioned mutations with osteonecrosis during or after treatment for malignancy in a pediatric population. Dai et al[17] reported that various haplotypes of the tissue factor pathway inhibitor gene were associated with the emergence of idiopathic or alcohol-induced osteonecrosis. He and Li[32] reported significant differences for the P-glycoprotein gene ABCB1 in patients with steroid-induced osteonecrosis compared with a control group. Glueck et al[28,30] described the T786C polymorphism of eNOS in patients with idiopathic osteonecrosis. Hirata et al[33] reported a higher frequency of the T7623T and CT alleles of the ApoB gene in 34 patients with osteonecrosis than in control patients, resulting in a statistically significant elevated odds ratio. Zhang et al[54] reported an underexpression of the CHST2 and the GPCR26 gene in 3 patients with femoral head osteonecrosis.

In the majority of studies, coagulation parameters were determined in the peripheral blood. One study compared the serum laboratory findings with those locally determined in the affected bone and showed an increase of those in the bone marrow.[12] An-

Table 2

Etiologic Factors for Secondary Osteonecrosis

Etiologic Factors for Secondary Osteonecrosis
Corticosteroids
Alcohol
Trauma
Sickle cell disease
Disseminated intravascular coagulation
Tobacco
Thrombotic thrombocytopenic purpura
Biphosphonates
Autoimmune diseases (eg, systemic lupus erythematodes)
Chemotherapy
Acquired immune deficiency syndrome
Malignancy
Caisson disease

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Table 3

Investigated Coagulation Parameters as Potential Etiologic Factors

Coagulation Parameter	Study
Lp(a)	Berger et al,[10] Berger et al,[11] Glueck et al,[21-24,26,27,29,30] Jones et al,[36] Mehsen et al,[41] Posan et al,[46] Zalavras et al[52]
PAI (antigen and function)	Berger et al,[10] Cenni et al,[14] Glueck et al,[21,22,25,27,30] Jones et al,[36] Kim et al[38]
Protein S	Chotanaphuti et al,[16] Elishkewich et al,[20] Glueck et al,[26,27,30] Pierre-Jacques et al,[45] Üreten et al[49]
MTHFR mutation C677T	Chang et al,[15] Elishkewich et al,[20] Glueck et al,[26,31] Kechli et al,[37] Kutlar et al,[40] Zalavras et al[53]
Factor V Leiden	Björkman et al,[13] Ekmekci et al,[19] Glueck et al,[29-31] Kechli et al,[37] Posan et al[46]
tPA (antigen and function)	Glueck et al,[21,22,24,25] Jones et al[36]
Prothrombine mutation 20210A	Björkman et al,[13] Cenni et al,[14] Ekmekci et al,[19] Kechli et al,[37] Vairaktaris et al[50]
Protein C	Cenni et al,[14] Chotanaphuti et al,[16] Glueck et al,[24] Mehsen et al,[41] Wermes et al[51]
Factor VIII	Chotanaphuti et al,[16] Glueck et al,[29-31] Seguin et al[47]
ApoA1	Glueck et al,[22] Hirata et al,[33] Miyanishi et al[42]
ApoB	Glueck et al,[21,22] Miyanishi et al,[42] Zalavras et al[52]
homocysteine	Elishkewich et al,[20] Glueck et al,[27,30,31]
RAPC	Glueck et al,[23,28] Jones et al[36]
Triglycerides	Berger et al,[10] Glueck et al,[21] Jones et al[35]
AT III (antigen and function)	Cenni et al,[14] Kubo et al,[39] Mehsen et al[41]
eNOS mutation T786C	Glueck et al,[28,30]
beta-globulin	Posan et al,[46] Zalavras et al[52]
ApoB:ApoA1 ratio	Hirata et al,[33] Miyanishi et al[42]
Cholesterol	Berger et al,[10] Jones et al[35]
Plasminogen	Cenni et al,[14] Posan et al[46]
ApoB gene, low molecular weight ApoA isoforms	Hirara et al[33]
ALP, OC, procollagen type I N-terminal propeptide, C-terminal cross-linking telopeptide, thrombocyte count	Berger et al[12]
TFPI gene	Dai et al[17]
CD4+ cells	de Larranaga et al[18]
P-glycoprotein gene ABCB1	He & Li[32]
Anticardiolipin antibody IgG	Jones et al[36]
TTP	Moore et al[43]
TAT, PIC	Oinuma et al[44]
vWF antigen, vWF CoRistocetin	Seguin et al[47]
α1-globulin, α2-globulin	Zalavras et al[52]
CHST2, GPCR26	Zhang et al[54]
Clot lysis speed	Posan et al[46]
Fibrin degradation products	Kubo et al[39]
Iron deficiency	Üreten et al[49]
Platelet glycoprotein IIIa	Glueck et al[26]
Platelet factor 4, PDGF-BB, TGF- β1, VEGF	Cenni et al[14]

Abbreviations: ALP, bone-specific alkaline phosphatase; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; AT III, antithrombin III; CHST2, carbohydrate sulfotransferase 2; eNOS, endothelial nitric oxide synthase; GPCR26, G protein coupled receptor; Lp(a), lipoprotein(a); MTHFR, methylentetrahydrofolate reductase; OC, osteocalcin; PAI, plasminogen activator inhibitor; PIC, plasmin-alpha2-plasmin inhibitor complex; PDGF-BB, platelet-derived growth factor-BB; RAPC, resistance to activated protein C; TAT, thrombin-antithrombin III-complex; TFPI, tissue factor pathway inhibitor; TGF-β1, transforming growth factor-β 1; tPA, tissue plasminogen activator; TTP, thrombotic thrombocytopenic purpura; vWF, von-Willebrand-Factor; VEGF, vascular endothelial growth factor.

other study solely investigated the gene expression in osteonecrotic femoral heads.[54]

Treatment Procedures

Twenty-seven (60%) of 45 studies reported no data on the treatment procedures of osteonecrosis or bone marrow edema syndrome. In 9 (20%) studies, anticoagulation therapy was administered and various substances have been used (eg, warfarin, phenprocoumon, enoxaparine, fondaparinux, heparin, ticlopidin, and acetylsalicylic acid) (Table 4). A core decompression of the affected region was reported in 4 (9%) studies. Total hip arthroplasty was performed in 4 (9%) studies (Table 4).

Table 4

Reported Treatment Procedures[a]

Study	Treatment
Berger et al[10,11]	Partial weight bearing for 6-8 wk; core decompression after treatment failure
Berger et al[12]	Core decompression
Elishkewich et al[20]	Warfarin, target INR 3.0-3.5; enoxaparin, 1 mg/kg/d; core decompression after treatment failure
Glueck et al[21]	Stanozolol, 6 mg/d for 12 wk
Glueck et al[22]	Stanozolol, 6 mg/d
Glueck et al[24]	Stanozolol 6 mg/d for 68 wk warfarin, target INR 2.5 for 20 wk
Glueck et al[26]	Hip alloarthroplasty
Glueck et al[27]	Enoxaparin, 60 mg/d,
Glueck et al[31]	Enoxaparin, 120 mg/d; fondaparinux, 2.5 mg/d; warfarin, target INR 2.5-3.5; hip alloarthroplasty
Jones et al[35]	Hip alloarthoplasty
Kechli et al[37]	Bilateral hip alloarthroplasty hip fusion
Kubo et al[39]	Warfarin, 5 mg/d
Moore et al[43]	At initial symptoms: plasmapheresis, 150 mg/d acetylsalicylic acid, 40 mg/d prednisone, transfusion therapy; at ON onset: ticlodipine for 3 mo
Pierre-Jacques et al[45]	physiotherapy; heparin; warfarin; core decompression
Shahin[48]	Warfarin, 5 mg/d, with target INR 1.5; acetylsalicylic acid for synovitis
Vairaktaris et al[50]	Bone resection and coverage of bony defect with platelet rich plasma
Wermes et al[51]	Since neonatal period: phenprocoumon, target INR 3.5-4.5; ON onset: enoxaparin 1 mg/kg/d; 4×1000 U protein C concentrate/d

Abbreviations: d, day; INR, international normalized ratio; NR, not reported; ON, osteonecrosis; U, units.
[a]All studies not listed did not report the treatment procedures used.

DISCUSSION

The current report systematically reviewed the literature for a possible involvement of thrombophilia and hypofibrinolysis in the etiology of osteonecrosis and bone marrow edema syndrome. Forty-eight thrombophilic and hypofibrinolytic parameters were identified in 45 studies with a total of 2163 patients. The most frequently reported laboratory findings included altered serum concentrations of Lp(a), ApoA1, and ApoB, decreased concentration and function of fibrinolytic agents (tPA, protein C, and protein S) and increased levels of thrombophilic markers (PAI and coagulation factor VIII). Furthermore, several single nucleotide polymorphisms (Factor V Leiden, methylene tetrahydrofolate reductase C677T, and prothrombin 20210A mutations) were identified in the molecular biological pathogenesis of osteonecrosis and bone marrow edema syndrome. Despite inhomogeneities in the reported results, patients' collectives, and determined parameters, these data strongly suggest that coagulation abnormalities may play an important role in the emergence of both diseases.

Lp(a) was first reported in 1963 by Berg.[55] Lp(a) is a low-density lipoprotein-like particle in which ApoB-100 is bound with a disulfide bridge to ApoA.[56] This unique structural feature accounts for the potential atherogenic and thrombophilic activity of Lp(a).[10] In plasma, Lp(a) exists as peaks in the low-density lipoprotein range; a form of density intermediate between low- and high-density lipoproteins and another ApoE-rich fraction closer to the density of high-density lipoproteins.[56] Lp(a) is made by the low-density lipoprotein synthesis machinery in the endoplasmic reticulum and the ApoA moiety is added on the surface of hepatocytes.[56] Plasma concentrations of Lp(a) correlate inversely with the size of ApoA isoproteins.[57] Lp(a) levels are higher in women than in men; they do not appear to be affected by physical exercise.[56] Moderate alcohol consumption might lower Lp(a) concentration.[56] With regard to the pathogenesis of osteonecrosis and bone marrow edema syndrome, Lp(a) reduces fibrinolytic activity by competing with plasminogen at the fibrin surface for the common lysine binding domains, causing increased susceptibility to arterial and venous thrombotic events.[10] In accordance with this pathophysiological background,

Berger et al[10-12] identified elevated levels of Lp(a) in patients with bone marrow edema syndrome. Although data reported in the literature are inconsistent regarding elevated Lp(a) levels in osteonecrosis, with some authors reporting a lack of significance of this parameter,[36,52] most studies reported increased Lp(a) concentrations in this patient cohort.[22,26,27,41,46] The majority of data for the increase of Lp(a) levels in the course of idiopathic[23,29] and secondary[21,24] osteonecrosis were reported by Glueck et al.

ApoB, the structural protein for the atherogenic lipoproteins (low and intermediate-density lipoprotein and large, buoyant, low-density lipoprotein and small, dense, low-density lipoprotein), is responsible for transporting lipids from the liver and gut to the peripheral tissues.[58] Each lipoprotein particle contains 1 ApoB molecule. Plasma ApoB levels increase with age[59,60] and are higher in men than in women.[59] In contrast, ApoA1 is the major structural protein for high-density lipoproteins and reflects the atheroprotective side of lipid metabolism.[58] ApoA1 is produced in the liver and intestine and is responsible for initiating reverse cholesterol transport, whereby excess cholesterol in peripheral tissues is carried back to the liver for excretion.[58] ApoA1 levels are reportedly higher in women than men.[61] An association of elevated serum low-density lipoprotein and ApoB, as well as decreased serum high-density lipoproteins and ApoA1, has been reported in coronary artery disease.[62] Against this background, ApoA1 may be regarded as protective for vascular diseases, whereas ApoB might have a deleterious effect. An elevated ApoB:ApoA1 ratio was reported to predispose individuals to the emergence of osteonecrosis and, therefore, should be ruled out before steroid administration.[33,42]

Following the conversion of plasminogen into the active enzyme plasmin, fibrinolysis of blood clots is mediated by the degradation of matrix components and activation of procollagenases.[63] However, plasminogen activation may be hampered by PAI. In this respect, PAI-1 is the major fibrinolysis inhibitor.[64] Increased concentration of PAI-1, as well as enhanced PAI function, may cause arterial occlusion and ultimately lead to myocardial infarction.[65,66] In addtion, upregulation of this fast-acting inhibitor of fibrinolysis[38] is associated with an increased incidence of thrombophilia.[67] Furthermore, as early as in 1961, elevated PAI levels have been found to be involved in the pathogenesis of osteonecrosis,[68] possibly mediated by an increased intraosseous venous pressure that restricts blood flow to the subchondral bone regions and may culminate in osteonecrosis.[25,69] Over the past 3 decades, this finding has been confirmed by Glueck et al.[21,22,27,30] Single nucleotide insertion or deletion polymorphisms of the PAI-1 gene with prevalence of the 4G allele seems to be a risk factor for hypofibrinolysis and, consequently, osteonecrosis.[25,27,38]

The tPA is another key player in the plasminogen activation system; reciprocally to the plasminogen activator inhibitor, tPA is considered the major stimulator of fibrinolysis.[24] This serine protease catalyzes the conversion of plasminogen to plasmin by cleavage of plasminogen at its arginine-valine peptide bond.[70] Clinically, recombinant tPA such as alteplase is approved by the US Food and Drug Administration for the treatment of myocardial infarction,[71,72] ischemic stroke,[73] or pulmonary embolism.[74] For the field of orthopedic research, decreased tPA function was found in idiopathic and secondary osteonecrosis.[21,22,24,25]

Blood coagulation factor VIII is a glycoprotein released by the vascular, glomerular, and tubular endothelium and the sinusoidal cells of the liver.[75] Defects in its gene result in hemophilia A, a recessive X-linked coagulation disorder.[76] Patients with elevated levels of factor VIII are at increased risk for deep venous thrombosis and thromboembolism.[77] This thrombophilic potential has also been reported as a potential risk factor for the development of osteonecrosis in 5 studies included in the current article.[16,29-31,47]

The anticoagulative effect of protein C was first reported by Mammen et al.[78] The activated form of this serine protease (activated protein C)[79] is capable of inactivating the coagulation factors Va and VIIIa, which are part of the prothrombinase complex, and, thus, are crucially involved in the generation of thrombin and blood clotting.[80] Protein S is an important cofactor of protein C in the inactivation of both coagulation factors.[81] Patients with protein C or S deficiency have a significantly higher risk of developing deep venous thrombosis or thromboembolism and disseminated intravascular coagulation,[82,83] and a high prevalence of protein S[16,26,27,30,45,49] and protein C[24,41,51] deficiency was detected in patients with osteonecrosis.

Besides a decreased concentration or function of protein C, the heritable resistance to activated protein C was reported by Dahlbäck et al[84] and is associated with familial thrombophilia. Most commonly, resistance to activated protein C is caused by a genetic mutation (replacement of arginine with glutamine at nucleotide position 506), resulting in a loss of the cleavage site of coagulation factor V and producing Factor V Leiden, a severe hypercoagulability disorder.[84,85] This disease is characterized by an elevated risk for venous and arterial thromboembolism.[86] However, several other genetic traits affect the anticoagulant response to activated protein C, but none cause the same severe resistance to activated protein C phenotype as Factor V Leiden, and their importance as risk factors for thrombosis is unclear.[79] A poor activated protein C response may also result from acquired conditions.[79] In the current review, resistance to activated protein C and the Factor V Leiden mutation were identified as potential risk factors for the development of osteonecrosis in numerous studies.[13,19,29,30,46]

Another important gene mutation involved in the pathogenesis of osteonecrosis is the C677T polymorphism of the MTHFR. Replacement of cytosine with thymine at the nucleotide position 677 decreases the activity of this enzyme, interferes with the intracellular metabolism of homocysteine, and thereby mildly elevates the plasma homocysteine level.[15] Because hyperhomocysteinemia is an established risk factor for thrombotic events,[87] the increased incidence of osteonecrosis in patients with the C677T MTHFR mutation is coherent in this regard.[15,20,26,40,53]

Mutation in the prothrombin gene (substitution of guanine for arginine at nucleotide position 20210) results in increased plasma prothrombin levels and is associated with venous thrombosis.[88] The frequency of the prothrombin 20210A gene mutation ranges from 6% to 12% in patients with deep venous thrombosis compared with a range of 1% to 4% in the general population.[89] The current authors believe that this specific prothrombin mutation is associated with the emergence of osteonecrosis.[13,19,50]

The treatment strategy for osteonecrosis and bone marrow edema syndrome has been reported in 18 (40%) of 45 studies. With regard to painful bone marrow edema syndrome, Berger et al[10,11] reported the ineffectiveness of partial weight bearing for 6 to 8 weeks with regard to pain reduction and the necessity for surgical core decompression. To minimize bone loss during the acute episodes, calcitonin has been used.[90] Furthermore, the intravenous administration of the prostacyclin analogue iloprost yielded clinical success in patients with painful bone marrow edema syndrome of the knee.[4] Sowers et al[91] reported that the presence of subchondral bone marrow edema does not satisfactorily correlate with the presence or absence of knee pain. However, concomitant subchondral cortical bone defects in patients with bone marrow edema seem to have a stronger effect on the susceptibility to knee pain.[91] Therefore, the causes of pain in patients with bone marrow edema syndrome and a possible relation with laboratory or MRI findings will have to be elucidated in more detail.

For patients with osteonecrosis with coexistent thrombophilic or hypofibrinolytic disorders, no standardized treatment plan is available. Because the supposed pathogenesis with venous occlusion of the bone by fibrin clots, hypertension in the affected cancellous bone, and cell death by hypoxia[24] is similar to Legg-Calvé-Perthes disease,[92-94] several pharmacological substances have been tested to improve osseous perfusion and normalize laboratory disorders. Any conservative pharmacological treatment must be applied before irreversible collapse of the respective bone region (eg, Ficat stages I and II at the femoral head).[22] The most commonly applied treatments included oral anticoagulants, such as warfarin[20,21,31,39,45,48] and phenprocoumon,[51] with target international normalized ratio values ranging between 1.5[48] and 4.5.[51] Enoxaparine,[20,27,51] fondaparinux,[31] heparin,[45] ticlopidin,[43] and acetylsalicylic acid[43,48] were also given for anticoagulative therapy. Stanozolol, an anabolic androgenic steroid, potentially normalizes PAI- and tPA-function and Lp(a) levels[22] and has been reported to inhibit the progress of osteonecrosis at different anatomical sites by Glueck et al.[21,22,24] Surgical treatment options include core decompression,[20,45] bone resection and coverage of the osteonecrotic defect with platelet rich plasma,[50] alloarthroplasty,[26,31,35,37] and joint fusion.[37]

When a systematic literature review is performed, some parameters have to be critically reconsidered. For example, besides its idiopathic form, bone marrow edema can arise in young, healthy, athletic patients after surgery or severe blunt injuries. However, this does not mean that all of these patients have coagulopathies that will cause the emergence of bone marrow edema. To clarify this topic, a multicenter, prospective, combined hematological-orthopedic and clinical-genetical study is strongly recommended.

CONCLUSION

The current review describes a broad variety of thrombophilic and hypofibrinolytic parameters that contribute to the emergence of osteonecrosis and bone marrow edema syndrome. The data indicate that conditions of coagulative disorders may play a key role in the pathogenesis of both diseases. Determining lipoprotein concentrations and coagulation markers (PAI, tPA, protein C and S, factor VIII) is recommended in patients with idiopathic etiologies of osteonecrosis and bone marrow edema syndrome and prior to prolonged corticosteroid use or chemotherapy.

REFERENCES

1. Tektonidou MG, Moutsopoulos HM. Immunologic factors in the pathogenesis of osteonecrosis. *Orthop Clin North Am*. 2004; 35(3):259-263.
2. Curtiss PH Jr, Kincaid WE. Transitory demineralization of the hip in pregnancy. A report of three cases. *J Bone Joint Surg Am*. 1959; 41:1327-1333.
3. Wilson AJ, Murphy WA, Hardy DC, Totty WG. Transient osteoporosis: transient bone marrow edema? *Radiology*. 1988; 167(3):757-760.
4. Aigner N, Petje G, Steinboeck G, Schneider W, Krasny C, Landsiedl F. Treatment of bone-marrow oedema of the talus with the prostacyclin analogue iloprost. An MRI-controlled investigation of a new method. *J Bone Joint Surg Br*. 2001; 83(6):855-858.
5. Lankes M, Petersen W, Hassenpflug J. Arterial supply of the femoral condyles [in German]. *Z Orthop Ihre Grenzgeb*. 2000; 138(2):174-180.
6. Lotke PA, Abend JA, Ecker ML. The treatment of osteonecrosis of the medial femoral condyle. *Clin Orthop Relat Res*. 1982; (171):109-116.
7. Aigner N, Meizer R, Petje G, Meizer E, Abdelkafy A, Landsiedl F. Natural course of intra-articular shifting bone marrow edema syndrome of the knee. *BMC Musculoskelet Disord*. 2008; 9:45.
8. Radke S, Kenn W, Eulert J. Transient bone marrow edema syndrome progressing to avascular necrosis of the hip: a case report and review of the literature. *Clin Rheumatol*. 2004; 23(1):83-88.
9. Hofmann S, Schneider W, Breitenseher M, Urban M, Plenk H Jr. "Transient osteopo-

rosis" as a special reversible form of femur head necrosis [in German]. *Orthopade*. 2000; 29(5):411-419.

10. Berger CE, Kluger R, Urban M, Kowalski J, Haas OA, Engel A. Elevated levels of lipoprotein(a) in familial bone marrow edema syndrome of the hip. *Clin Orthop Relat Res*. 2000; (377):126-131.
11. Berger CE, Kroner A, Stiegler H, Erdel M, Haas OA, Engel A. Hypofibrinolysis, lipoprotein(a), and plasminogen activator inhibitor. *Clin Orthop Relat Res*. 2002; (397):342-349.
12. Berger CE, Kroner AH, Minai-Pour MB, Ogris E, Engel A. Biochemical markers of bone metabolism in bone marrow edema syndrome of the hip. *Bone*. 2003; 33(3):346-351.
13. Bjorkman A, Burtscher IM, Svensson PJ, Hillarp A, Besjakov J, Benoni G. Factor V Leiden and the prothrombin 20210A gene mutation and osteonecrosis of the knee. *Arch Orthop Trauma Surg*. 2005; 125(1):51-55.
14. Cenni E, Fotia C, Rustemi E, et al. Idiopathic and secondary osteonecrosis of the femoral head show different thrombophilic changes and normal or higher levels of platelet growth factors. *Acta Orthop*. 2011; 82(1):42-49.
15. Chang JD, Hur M, Lee SS, Yoo JH, Lee KM. Genetic background of nontraumatic osteonecrosis of the femoral head in the Korean population. *Clin Orthop Relat Res*. 2008; 466(5):1041-1046.
16. Chotanaphuti T, Heebthamai D, Chuwong M, Kanchanaroek K. The prevalence of thrombophilia in idiopathic osteonecrosis of the hip. *J Med Assoc Thai*. 2009; 92(suppl 6):S141-S146.
17. Dai XL, Hong JM, Oh B, et al. Association analysis of tissue factor pathway inhibitor polymorphisms and haplotypes with osteonecrosis of the femoral head in the Korean population. *Mol Cells*. 2008; 26(5):490-495.
18. de Larranaga G, Bottaro E, Martinuzzo M, et al. Thrombophilia in human immunodeficiency virus-infected patients with osteonecrosis: is there a real connection? The first case-control study. *Clin Appl Thromb Hemost*. 2009; 15(3):340-347.
19. Ekmekci Y, Keven K, Akar N, et al. Thrombophilia and avascular necrosis of femoral head in kidney allograft recipients. *Nephrol Dial Transplant*. 2006; 21(12):3555-3558.
20. Elishkewich K, Kaspi D, Shapira I, Meites D, Berliner S. Idiopathic osteonecrosis in an adult with familial protein S deficiency and hyperhomocysteinemia. *Blood Coagul Fibrinolysis*. 2001; 12(7):547-550.
21. Glueck CJ, Freiberg R, Glueck HI, et al. Hypofibrinolysis: a common, major cause of osteonecrosis. *Am J Hematol*. 1994; 45(2):156-166.
22. Glueck CJ, Freiberg R, Glueck HI, Tracy T, Stroop D, Wang Y. Idiopathic osteonecrosis, hypofibrinolysis, high plasminogen activator inhibitor, high lipoprotein(a), and therapy with Stanozolol. *Am J Hematol*. 1995; 48(4):213-220.
23. Glueck CJ, Tracy T, Sieve-Smith L, Wang P. Whether, to what degree, and why lipoprotein(a) levels change over time. *Clin Chim Acta*. 1995; 238(1):11-19.
24. Glueck CJ, Freiberg R, Tracy T, Stroop D, Wang P. Thrombophilia and hypofibrinolysis: pathophysiologies of osteonecrosis. *Clin Orthop Relat Res*. 1997; (334):43-56.
25. Glueck CJ, Fontaine RN, Gruppo R, et al. The plasminogen activator inhibitor-1 gene, hypofibrinolysis, and osteonecrosis. *Clin Orthop Relat Res*. 1999; (366):133-146.
26. Glueck CJ, Phillips HG, Cameron D, Wang P. Estrogen replacement in a protein S deficient patient leads to diarrhea, hyperglucagonemia, and osteonecrosis. *JOP*. 2001; 2(5):323-329.
27. Glueck CJ, Freiberg RA, Fontaine RN, Tracy T, Wang P. Hypofibrinolysis, thrombophilia, osteonecrosis. *Clin Orthop Relat Res*. 2001; (386):19-33.
28. Glueck CJ, Freiberg RA, Oghene J, Fontaine RN, Wang P. Association between the T-786C eNOS polymorphism and idiopathic osteonecrosis of the head of the femur. *J Bone Joint Surg Am*. 2007; 89(11):2460-2468.
29. Glueck CJ, Freiberg RA, Wang P. Heritable thrombophilia-hypofibrinolysis and osteonecrosis of the femoral head. *Clin Orthop Relat Res*. 2008; 466(5):1034-1040.
30. Glueck CJ, Freiberg RA, Boppana S, Wang P. Thrombophilia, hypofibrinolysis, the eNOS T-786C polymorphism, and multifocal osteonecrosis. *J Bone Joint Surg Am*. 2008; 90(10):2220-2229.
31. Glueck CJ, Goldenberg N, Budhani S, et al. Thrombotic events after starting exogenous testosterone in men with previously undiagnosed familial thrombophilia. *Transl Res*. 2011; 158(4):225-234.
32. He W, Li K. Incidence of genetic polymorphisms involved in lipid metabolism among Chinese patients with osteonecrosis of the femoral head. *Acta Orthop*. 2009; 80(3):325-329.
33. Hirata T, Fujioka M, Takahashi KA, et al. ApoB C7623T polymorphism predicts risk for steroid-induced osteonecrosis of the femoral head after renal transplantation. *J Orthop Sci*. 2007; 12(3):199-206.
34. Hirata T, Fujioka M, Takahashi KA, et al. Low molecular weight phenotype of Apo(a) is a risk factor of corticosteroid-induced osteonecrosis of the femoral head after renal transplant. *J Rheumatol*. 2007; 34(3):516-522.
35. Jones JP Jr. Fat embolism, intravascular coagulation, and osteonecrosis. *Clin Orthop Relat Res*. 1993; (292):294-308.
36. Jones LC, Mont MA, Le TB, et al. Procoagulants and osteonecrosis. *J Rheumatol*. 2003; 30(4):783-791.
37. Kechli AM, Wilimas JA, Pui CH, Park VM, Tonkel S, Deitcher SR. Factor V Leiden and other hypercoagulable state mutations are not associated with osteonecrosis during or after treatment for pediatric malignancy. *J Pediatr*. 1999; 134(3):310-314.
38. Kim H, Cho C, Cho Y, Cho S, Yoon K, Kim K. Significant associations of PAI-1 genetic polymorphisms with osteonecrosis of the femoral head. *BMC Musculoskelet Disord*. 2011; 12:160.
39. Kubo T, Tsuji H, Yamamoto T, Nakahara H, Nakagawa M, Hirasawa Y. Antithrombin III deficiency in a patient with multifocal osteonecrosis. *Clin Orthop Relat Res*. 2000; (378):306-311.
40. Kutlar A, Kutlar F, Turker I, Tural C. The methylene tetrahydrofolate reductase (C677T) mutation as a potential risk factor for avascular necrosis in sickle cell disease. *Hemoglobin*. 2001; 25(2):213-217.
41. Mehsen N, Barnetche T, Redonnet-Vernhet I, et al. Coagulopathies frequency in aseptic osteonecrosis patients. *Joint Bone Spine*. 2009; 76(2):166-169.
42. Miyanishi K, Yamamoto T, Irisa T, Noguchi Y, Sugioka Y, Iwamoto Y. Increased level of apolipoprotein B/apolipoprotein A1 ratio as a potential risk for osteonecrosis. *Ann Rheum Dis*. 1999; 58(8):514-516.
43. Moore J, Coyle L, Isbister J, Roche J. Bilateral knee osteonecrosis in a patient with thrombotic thrombocytopenic purpura. *Aust N Z J Med*. 1999; 29(1):88-89.
44. Oinuma K, Harada Y, Nawata Y, et al. Sustained hemostatic abnormality in patients with steroid-induced osteonecrosis in the early period after high-dose corticosteroid therapy. *J Orthop Sci. 2000*; 5(4):374-379.
45. 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(7):1079-1084.
46. Posan E, Szepesi K, Gaspar L, et al. Thrombotic and fibrinolytic alterations in the aseptic necrosis of femoral head. *Blood Coagul Fibrinolysis*. 2003; 14(3):243-248.
47. Seguin C, Kassis J, Busque L, et al. Nontraumatic necrosis of bone (osteonecrosis) is associated with endothelial cell activation but not thrombophilia. *Rheumatology (Oxford)*. 2008; 47(8):1151-1155.
48. Shahin AA. Arthritis and osteonecrosis in a patient with thrombophilia. *Rheumatol Int*. 2001; 20(6):243-245.
49. Ureten K, Ozturk MA, Bostanci A, Ceneli O, Ozbek M, Haznedaroglu IC. Atraumatic osteonecrosis after estrogen replacement therapy associated with low protein S level

in a patient with Turner syndrome. *Clin Appl Thromb Hemost*. 2010; 16(5):599-601.

50. Vairaktaris E, Vassiliou S, Avgoustidis D, Stathopoulos P, Toyoshima T, Yapijakis C. Bisphosphonate-induced avascular osteonecrosis of the mandible associated with a common thrombophilic mutation in the prothrombin gene. *J Oral Maxillofac Surg*. 2009; 67(9):2009-2012.
51. Wermes C, Bergmann F, Reller B, Sykora KW. Severe protein C deficiency and aseptic osteonecrosis of the hip joint: a case report. *Eur J Pediatr*. 1999; 158(suppl 3):S159-S161.
52. Zalavras C, Dailiana Z, Elisaf M, et al. Potential aetiological factors concerning the development of osteonecrosis of the femoral head. *Eur J Clin Invest*. 2000; 30(3):215-221.
53. Zalavras CG, Malizos KN, Dokou E, Vartholomatos G. The 677C-->T mutation of the methylene-tetrahydrofolate reductase gene in the pathogenesis of osteonecrosis of the femoral head. *Haematologica*. 2002; 87(1):111-112.
54. Zhang H, Zhang L, Wang J, et al. Proteomic analysis of bone tissues of patients with osteonecrosis of the femoral head. *OMICS*. 2009; 13(6):453-466.
55. Berg K. A new serum type system in man: the Lp system. *Acta Pathol Microbiol Scand*. 1963; 59:369-382.
56. Tziomalos K, Athyros VG, Wierzbicki AS, Mikhailidis DP. Lipoprotein a: where are we now? *Curr Opin Cardiol*. 2009; 24(4):351-357.
57. Gavish D, Azrolan N, Breslow JL. Plasma Ip(a) concentration is inversely correlated with the ratio of Kringle IV/Kringle V encoding domains in the apo(a) gene. *J Clin Invest*. 1989; 84(6):2021-2027.
58. Davidson MH. Apolipoprotein measurements: is more widespread use clinically indicated? *Clin Cardiol*. 2009; 32(9):482-486.
59. Schaefer EJ, Lamon-Fava S, Cohn SD, et al. Effects of age, gender, and menopausal status on plasma low density lipoprotein cholesterol and apolipoprotein B levels in the Framingham Offspring Study. *J Lipid Res*. 1994; 35(5):779-792.
60. Millar JS, Lichtenstein AH, Cuchel M, et al. Impact of age on the metabolism of VLDL, IDL, and LDL apolipoprotein B-100 in men. *J Lipid Res*. 1995; 36(6):1155-1167.
61. Schaefer EJ, Lamon-Fava S, Ordovas JM, et al. Factors associated with low and elevated plasma high density lipoprotein cholesterol and apolipoprotein A-I levels in the Framingham Offspring Study. *J Lipid Res*. 1994; 35(5):871-882.
62. Pulkkinen A, Viitanen L, Kareinen A, Lehto S, Laakso M. MspI polymorphism at +83 bp in intron 1 of the human apolipoprotein A1 gene is associated with elevated levels of HDL cholesterol and apolipoprotein A1 in nondiabetic subjects but not in type 2 diabetic patients with coronary heart disease. *Diabetes Care*. 2000; 23(6):791-795.
63. Lang IM, Moser KM, Schleef RR. Elevated expression of urokinase-like plasminogen activator and plasminogen activator inhibitor type 1 during the vascular remodeling associated with pulmonary thromboembolism. *Arterioscler Thromb Vasc Biol*. 1998; 18(5):808-815.
64. Sprengers ED, Kluft C. Plasminogen activator inhibitors. *Blood*. 1987; 69(2):381-387.
65. Eriksson P, Kallin B, van 't Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci U S A*. 1995; 92(6):1851-1855.
66. Ossei-Gerning N, Mansfield MW, Stickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. *Arterioscler Thromb Vasc Biol*. 1997; 17(1):33-37.
67. Juhan-Vague I, Valadier J, Alessi MC, et al. Deficient t-PA release and elevated PA inhibitor levels in patients with spontaneous or recurrent deep venous thrombosis. *Thromb Haemost*. 1987; 57(1):67-72.
68. 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):323-337.
69. Van Veldhuizen 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(4):243-248.
70. Bode W, Renatus M. Tissue-type plasminogen activator: variants and crystal/solution structures demarcate structural determinants of function. *Curr Opin Struct Biol*. 1997; 7(6):865-872.
71. Mueller HS, Rao AK, Forman SA. Thrombolysis in myocardial infarction (TIMI): comparative studies of coronary reperfusion and systemic fibrinogenolysis with two forms of recombinant tissue-type plasminogen activator. *J Am Coll Cardiol*. 1987; 10(3):479-490.
72. Topol EJ, Morris DC, Smalling RW, et al. A multicenter, randomized, placebo-controlled trial of a new form of intravenous recombinant tissue-type plasminogen activator (activase) in acute myocardial infarction. *J Am Coll Cardiol*. 1987; 9(6):1205-1213.
73. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N Engl J Med*. 1995; 333(24):1581-1587.
74. Goldhaber SZ, Kessler CM, Heit J, et al. Randomised controlled trial of recombinant tissue plasminogen activator versus urokinase in the treatment of acute pulmonary embolism. *Lancet*. 1988; 2(8606):293-298.
75. Bhopale GM, Nanda RK. Blood coagulation factor VIII: An overview. *J Biosci*. 2003; 28(6):783-789.
76. Antonarakis SE. Molecular genetics of coagulation factor VIII gene and hemophilia A. *Thromb Haemost*. 1995; 74(1):322-328.
77. Bank I, Libourel EJ, Middeldorp S, et al. Elevated levels of FVIII:C within families are associated with an increased risk for venous and arterial thrombosis. *J Thromb Haemost*. 2005; 3(1):79-84.
78. Mammen EF, Thomas WR, Seegers WH. Activation of purified prothrombin to autoprothrombin I or autoprothrombin II (platelet cofactor II or autoprothrombin II-A). *Thromb Diath Haemorrh*. 1960; 5:218-249.
79. Nicolaes GA, Dahlback B. Congenital and acquired activated protein C resistance. *Semin Vasc Med*. 2003; 3(1):33-46.
80. Esmon CT. The protein C pathway. *Chest*. 2003; 124(suppl 3):26S-32S.
81. Castoldi E, Hackeng TM. Regulation of coagulation by protein S. *Curr Opin Hematol*. 2008; 15(5):529-536.
82. Goldenberg NA, Manco-Johnson MJ. Protein C deficiency. *Haemophilia*. 2008; 14(6):1214-1221.
83. Garcia de Frutos P, Fuentes-Prior P, Hurtado B, Sala N. Molecular basis of protein S deficiency. *Thromb Haemost*. 2007; 98(3):543-556.
84. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A*. 1993; 90(3):1004-1008.
85. De Stefano V, Leone G. Resistance to activated protein C due to mutated factor V as a novel cause of inherited thrombophilia. *Haematologica*. 1995; 80(4):344-356.
86. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med*. 1994; 330(8):517-522.
87. den Heijer M, Koster T, Blom HJ, et al. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med*. 1996; 334(12):759-762.
88. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood*. 1996; 88(10):3698-3703.
89. Emmerich J, Rosendaal FR, Cattaneo M, et al. Combined effect of factor V Leiden

and prothrombin 20210A on the risk of venous thromboembolism: pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. *Thromb Haemost*. 2001; 86(3):809-816.

90. Varenna M, Zucchi F, Binelli L, Failoni S, Gallazzi M, Sinigaglia L. Intravenous pamidronate in the treatment of transient osteoporosis of the hip. *Bone*. 2002; 31(1):96-101.
91. Sowers MF, Hayes C, Jamadar D, et al. Magnetic resonance-detected subchondral bone marrow and cartilage defect characteristics associated with pain and X-ray-defined knee osteoarthritis. *Osteoarthritis Cartilage*. 2003; 11(6):387-393.
92. Balasa VV, Gruppo RA, Glueck CJ, et al. Legg-Calve-Perthes disease and thrombophilia. *J Bone Joint Surg Am*. 2004; 86(12):2642-2647.
93. 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(1):3-13.
94. Gruppo R, Glueck CJ, Wall E, Roy D, Wang P. Legg-Perthes disease in three siblings, two heterozygous and one homozygous for the factor V Leiden mutation. *J Pediatr*. 1998; 132(5):885-888.