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Changes in coagulation and fibrinolysis of post-SARS osteonecrosis in a Chinese population

Abstract The purpose of this study was to detect changes in coagulation and fibrinolysis of post-severe acute respiratory syndrome (SARS) Chinese patients with osteonecrosis, investigate the aetiology of post-SARS osteonecrosis (ON), and select the sensitive molecular markers for identifying the susceptible population. For this study, blood samples were collected from 88 patients with post-SARS ON and 52 healthy people. Activated partial thromboplastin time (APTT), protein C (PC), antithrombin III (AT–III), plasminogen activator inhibitor (PAI), activated protein C resistance (APC–R), plasminogen (PLG), von Willebrand's factor(vWF), D–dimer (D–D), fibrinogen (Fib), and homocysteine (HCY) were examined by enzyme-linked immunosorbent assay (ELISA). We noted that blood agents of patients with ON changed obviously. APTT, PC, AT–III, PAI, APC–R, and PLG were significantly different between the two groups. Hypercoagulation and hypofibrinolysis were found in patients with post-SARS ON. Therefore, these examinations can be used to screen a population susceptible to ON. Measurements of APTT, PC, AT–III, PAI, APC–R, and PLG are sensitive blood tests for screening purposes.

Résumé Le but de cette étude est de détecter les troubles de la coagulation et de la fibrinolyse chez les patients ayant présenté, en Chine, un SRAS, avec ostéonécrose, d'étudier l'étiologie des ostéonécroses post-SRAS et de sérier la population susceptible de présenter de telles pathologies. Pour cette étude, des échantillons de sang ont été collectés chez 88 patients ayant présenté un SRAS avec ostéoné-chrose et, sur une population témoin de 52 autres patients. Le temps d'activation partielle de la thromboplastie (APT), la protéine C (PC), l'antithrombine III (AT III), l'activateur d'inhibition du plasminogène (PLG), l'activateur de la résistance de la protéine C (PAC R), le plasminogène (PLG), le facteur de Willebrand (vWF), les D–Dimmer (D–D), le fibrinogène (Fib) et l'homocystéine (HCY) ont été analysés par la technique d'Elisa. Nous avons noté que les facteurs sanguins de ces patients, présentant une ostéo-néchrose présentaient un changement significatif (APTT, PC, AT–III, PAI, APC–R, PLG). Cette différence étant significative également entre les deux groupes. Nous avons trouvé chez ces patients une hypercoagulation avec une hypofibrinolyse après post-SRAS. Ces examens, peuvent être utilisés en routine pour dépister les populations susceptibles de présenter une ostéonécrose après SRAS, les facteurs sensitifs du dépistage étant l'APTT, PC, A T–III, PAI, APC–R et le PLG.

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Introduction

In spring of 2003, severe acute respiratory syndrome (SARS) spread in China. To save the lives of these patients, high doses of corticosteroids were used, and consequently many patients with osteonecrosis (ON) were found in the period of rehabilitation. The aetiology and pathogenesis of nontraumatic ON were still not fully understood. The hypothesis that intravascular coagulation is likely the final common pathway for nontraumatic ON has recently gained support. ON is related to an underlying thrombophilia and/or hypofibrinolysis. Intravascular coagulation is only an intermediary event, which is always activated by some underlying risk factors [1]. In the past, most studies were limited to the Western population; we questioned whether or not a difference is present between Eastern and Western populations. Therefore, we studied the changes in coagulation and fibrinolysis of Chinese patients with post-SARS ON in order to compare the changes between the two ethnic groups.

Materials and methods

From December 2003 to March 2004, 88 patients demonstrated to have osteonecrosis (hip, knee, or other joints) by magnetic resonance imaging (MRI) were included in the study group (group I), and 52 healthy individuals served as the control group (group II). A definitive diagnosis of ON was established by both orthopaedic surgeons and radiologists. In the study group, there were 30 men and 58 women. The mean age was 32.8 years (range: 19–59), mean accumulated dose of corticosteroids (converting to methylprednisone) was 6614.1 mg (range: 750–30000), mean maximum daily dose was 404.4 mg (range: 80–1020), and mean duration of corticosteroid administration was 36.9 days (range: 14–110). ON was found only in the hips in 30 patients (24 bilateral) and only in the knees of nine patients (five bilateral); 48 patients were characterized as multifocal (more than three anatomical sites) and in one patient only bone infarction was detected in the shaft of the femur. The control group was composed of the healthy cohort of our medical staff (19 men, 33 women) who had a mean age of 35.1 years (range: 15–61).

Fasting blood samples from cubital veins were collected from both groups after the subject was seated for 5–10 min; 3 ml venous blood was collected in a Vacutainer tube containing sodium citrate (VACUETTE, 3.2%, China), while another 2 ml was collected in a Vacutainer tube containing ethylenediaminetetraacetate (EDTA) (VACUETTE, China) for analysing homocysteine (HCY), which must be centrifuged within 0.5 h. The volume ratio of anticoagulation solution and blood was 1:9. The blood samples were centrifuged at 2000 g for 15 min at room temperature; platelet-poor plasma was snap-frozen and stored at −80°C. A coagulometer (STACompact, France) was used for analysing all of the factors. According to the kit instructions (STA–LIATEST, Diagnostica STAGO, France), protein C (PC), antithrombin III (AT–III), plasminogen activator inhibitor (PAI), activated protein C resistance (APC–R), plasminogen (PLG), von Willebrand's factor (vWF), D–dimer (D–D), and homocysteine (HCY) were examined by enzyme-linked immunosorbent assay (ELISA). Activated partial thromboplastin time (APTT) was analysed by a two-step technique, and fibrinogen (Fib) was analysed according to the method of Clauss.

The levels for all of the test results are shown as mean and standard deviation, as many data were not normally distributed; comparison between the two groups was performed with Wilcoxon's nonparametric test and a chi-square test was used to analyze the members of abnormality. Statistical significance was accepted at the $p < 0.05$ level. SAS 6.12 and SPSS 11.0 were used for statistics.

Results

The blood agents of post-SARS patients changed obviously. Of 88 patients with post-SARS, 78 (88.64%) were found to have at least one coagulopathy versus 36.54% of controls ($p < 0.01$); PC, APC–R, AT–III, PAI, and PLG were significantly different between the two groups (Tables 1 and 2).

Discussion

In post-SARS patients, ON may be caused by corticosteroids; however, whether the SARS coronary virus has effects on ON or not is resolved. Recently, many papers have described the relationship between ON and virus in patients with human immunodeficiency virus (HIV) infection. Miller [2] studied 339 asymptomatic HIV-infected adults and 118 age- and sex-matched HIV-negative controls: 15% of the HIV-positive group was diagnosed to have ON as detected by MRI. Whereas, there were no cases of ON in the HIV-negative group. The risk factors included lipid-lowering agents, testosterone, and detectable level of anticardiolipin in the HIV-infected patients. Most recently, Bottaro [3] postulated that the risk factors peculiar to HIV-infected individuals that might play a role in the pathogenesis of ON include the introduction of protease inhibitors and resulting hyperlipidemia, the presence of anticardiolipin antibodies in serum leading to a hypercoagulable state, immune recovery, and vasculitis. We are studying the damage from the SARS coronary virus caused to blood vessel endothelial cells.

In this study, most patients have both an external contributor to ON (corticosteroids, SARS) and thrombophilic and/or hypofibrinolytic coagulation disorder. The importance of coagulation abnormalities in the pathogenesis of ON has been noted for a long time. In 1974, Jones [4, 5] postulated that intravascular coagulation, activated by a variety of underlying diseases, is the likely final common pathway producing intraosseous thrombosis and necrosis. Intravascular coagulation with fibrin-platelet thrombosis begins in the vulnerable subchondral bone microcirculation (the capillary and sinusoidal bed) and is associated with

Table 1 Comparison of coagulation and fibrinolysis values of the two groups (mean±SD)

Group	APTT	PC	APC–R	AT–III	PAI	PLG	Fib	D–D	vWF	HCY
I	35.63± 7.02	88.91± 32.40**	183.07± 58.57	86.62± 27.38**	17.89± 14.83**	72.57± 23.60**	232.15± 104.27	0.27± 0.22	91.38± 43.51	10.30± 5.73
II	33.59± 3.64	109.04± 19.50	197.31± 46.00	103.69± 14.51	7.96 ± 4.27	94.52± 15.21	233.04± 69.65	0.28± 0.18	94.59± 29.53	9.62± 3.49

**$p < 0.01$ APTT: 26~38 s, PC: 70~130%, APC–R: 120–300 s, AT–III: 80~120%, PAI: 0~10 U/ml, PLG: 80~120%, Fib: 100–400 mg/dl, D–D: <0.5 mg/l, vWF: 50–160%, HCY: 5–15 μmol/l

Table 2 Comparison of frequency of coagulation and fibrinolysis abnormality between the two groups (n/N, %)

Group	APTT	PC	APCR	AT–III	PAI	PLG	FIB	vWF	D–D	HCY	Total
I	12/86	25/88	11/87	39/87	46/88	52/88	7/86	6/88	7/86	8/75	78/88
	13.95%	28.41%**	12.64%*	44.82%**	52.27%**	59.09%**	8.14%	6.82%	8.14%	10.67%	88.64%**
II	4/52	2/52	1/52	2/52	5/52	3/52	4/52	1/52	4/52	3/52	19/52
	7.69%	3.85%	1.92%	3.85%	9.62%	5.77%	13.5%	1.92%	7.69%	5.77%	36.54%

*$p<0.05$, **$p<0.01$

vasoconstriction and impaired secondary fibrinolysis. Starklint [6] found microvascular thrombus in avascular necrosis; this provides evidence for the hypothesis.

Thrombotic/fibrinolytic pathways are a complicated process; the coagulopathy is not the cause of ON but is only an intermediary event initiated by an underlying aetiological factor, which may be reversible (treatable) or irreversible, subthreshold or suprathreshold, additive or multiplicative, and environmental or genetic [7]. With the increasing knowledge of the thrombotic/fibrinolytic pathways and with more advanced laboratory testing, numerous factors have been identified that exist in patients with ON [8, 9]. The association of thrombophilia (increased tendency for thrombosis) and/or hypofibrinolysis (reduced ability to lyse thrombi) with ON in adults has been reported in studies spanning more than 40 years [10–21]. In 1961, Nilsson [10] described ON associated with hypofibrinolysis. In 1993–1994, Glueck [11–13] and Van Veldhuizen [14] described the association of a familial high level of PAI with ON. In 2001, Glueck [15] evaluated coagulation disorders in 36 adults with ON and found the cohort had enrichment in both thrombotic and fibrinolytic risk factors. A majority of post-SARS ON patients have the tendency to develop thrombophilia and hypofibrinolysis. These may predispose to thrombotic venous occlusion in bone, which leads to intramedullary hypertension, anoxia, and ischaemic bone death characteristic of ON [7]. Cosgriff [22] reported that steroid treatment places some patients in a "prethrombotic state." The patients with a coagulation abnormality are at a higher risk to the effects of steroid treatment. This has been termed "the second hit" theory for the initiation of thrombosis in ON [23].

In this research, 88.64% of post-SARS ON patients were found to have at least one abnormal coagulation and fibrinolysis factor. PC, APC–R, AT–III, PAI, and PLG were significantly different between the two groups. This is similar to the studies of Jones et al. [8] and Glueck et al. [9]. The effects on the thrombotic/fibrinolytic system of corticosteroid treatment are transient [8, 22] and the blood samples of those patients were collected more than six months after stopping corticosteroid therapy; therefore, most of these coagulation disorders must be heritable, not acquired. The study of genetic mutations in thrombosis and hypofibrinolysis which are unaffected by environmental vectors will prove this [16, 24, 25]. Moreover, research into SARS patients without ON will enrich our understanding about the aetiology and pathogenesis of nontraumatic ON; however, for objective reasons, blood samples of post-SARS non-osteonecrosis patients were difficult to obtain. We are trying our best to advance our study.

In the relationship of coagulopathy and ON, there are some controversies. There may be differences between Western and Eastern populations. Lee [26] from Korea investigated 24 consecutive patients who had been diagnosed as having nontraumatic ON of the femoral head, matched with 24 control subjects. The data do not confirm an aetiological role for thrombotic and fibrinolytic disorders in East Asian patients with nontraumatic ON of the femoral head, and it was thought that the difference may be explained by ethnic differences. Our research does not support this opinion. Zheng et al. [27] studied early and late stage Chinese ON patients and found blood changes in these patients and thought thrombophilia and hypofibrinolysis was present at every stage of ON patients in China.

There were some coagulation abnormalities in the control group (36.54% had at least one abnormality). Using ten tests to assess thrombophilia and hypofibrinolysis, with the cutoff based on the 5th percentile of a healthy population, this is not such a surprisingly high percent of abnormal findings for controls. Recently, Mont et al. [28] studied risk factors for pulmonary emboli after total hip or knee arthroplasty, 21 serological measures and five genes associated with thrombophilia and/or hypofibrinolysis were assessed: 13 of 27 (48%) in the control group also had at least one abnormality.

In this study, we did not detect any coagulation abnormalities in a small proportion of the patients; this may reflect limitations in our understanding of the causes of thrombosis and hypofibrinolysis. These patients categorized as "normal" may be shown to be abnormal if more tests of coagulation are carried out. It is also possible that in some cases the coagulation factor abnormality was transient, making it difficult to detect.

In conclusion, we did find hypercoagulation and hypofibrinolysis in post-SARS Chinese patients with osteonecrosis. Hypercoagulation and hypofibrinolysis examinations can be used to screen the population at high risk for osteonecrosis. Measurements of APTT, PC, AT–III, PAI, APC–R, and PLG are sensitive blood tests for screening a high-risk population. Presuming thrombophilia and/or hypofibrinolysis to be causative for ON, it should be possible to correct patients' coagulation disorders and thus retard the progression or reverse the ON process. Preliminary screening of patients requiring corticosteroid treatment (systemic lupus erythematosus, organ transplantation, nephropathy, etc.) may increase patient and physician awareness and detect the population susceptible to

ON. Regular MRI/bone scan screening of these patients and earlier surgical management will improve prognosis. Anticoagulant medications and lipid-clearing agents in patients receiving corticosteroids will prevent the onset of ON.

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