Clinical study on the related markers of blood coagulation in the patients with ANFH after SARS

WU Lianhua (✉)¹, BM, GAO Chunjin¹, WANG Guozhong¹, MD, YANG Lin¹, MS, HOU Xiaomin¹, MS, GE Huan¹, BM, XIA Chengqing², MD, QI Man²

¹ Department of Hyperbaric Oxygen, Chaoyang Hospital, Capital University of Medical Sciences, Beijing 100020, China

² Department of Pathology, Chaoyang Hospital, Capital University of Medical Sciences, Beijing 100020, China

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Abstract The aim of this research was to investigate the blood coagulation function in the patients with avascular necrosis of the femoral head (ANFH) after severe acute respiratory syndrome (SARS). The expression of CD31, CD61, CD62p, CD63 and PAC-1 on platelet membrane was measured respectively by flowcytometry, and the plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) and fibrinogen (Fbg) were measured by blood clotting instrument in 26 patients with ANFH after SARS and in 17 healthy adults. The expression of CD31, CD61, CD 62p, CD63 and PAC-1 on platelet membrane in 26 patients was all lower than that in 17 healthy subjects ($P < 0.01$). The levels of PT, APTT, TT and Fbg in 26 patients were all normal. There is no significant difference ($P > 0.05$) in those markers between patients and 17 healthy adults. The blood may not be in hypercoagulable state in patients with ANFH after SARS.

Keywords severe acute respiratory syndrome; femur head necrosis; blood coagulation

1 Introduction

Twenty-six patients with avascular necrosis of the femoral head (ANFH) after severe acute respiratory syndrome (SARS) were treated in our department during September, 2003 to April, 2004. The markers related to blood coagulation including CD31, CD61, CD 62P, CD63 PAC-1 on platelet membrane, and the plasma prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), and fibrinogen (Fbg) were measured respectively to investigate

the blood coagulation function and the etiology of ANFH after SARS.

2 Methods

2.1 General materials

Twenty-six patients (aged 23–49 years; mean, (34.42 ± 7.22) years) with ANFH after SARS were enrolled in our study. Of these patients (eight males; 18 females), seventeen cases were medical staffs. During episode of this disease, they were all administered radiosone at the dose of 1 200–8 000 mg for 14–28 days. They all experienced slight or serious discomfort or pain at the site of hip-joint four to six months after administration of hormone, and were all confirmed to be avascular necrosis of the femoral head by magnetic resonance imaging (MRI) examination. These diseases belonged to stages I–II according to classification of the Association Research Circulation Osseous (ARCO) [1]. These patients returned to hospital for visiting doctor three to four months after being discharged from hospital. In addition, 17 healthy volunteers were collected and served as the control group. Of these subjects, six cases were male, 11 cases were female. Their age range was 22–50 years old (median age, (31.44 ± 8.12) years). There was no significant statistical difference in gender or age between the two groups ($P > 0.05$).

2.2 Methods

2.2.1 Measurement of markers

When patients experienced discomfort or pain at the site of hip-joint four to six months after administration of hormone, their blood samples were taken intravenously under the condition of fasting to measure CD31, CD61, CD62P, CD63, PAC-1 on platelet membrane and coagulation four indices

including PT, APTT, Fbg and TT. Before their blood samples were taken, they did not take any anticoagulant drug and platelet suppressant drug.

2.2.2 Reagent, instrument and equipment

All these reagents, including FITC-labeled human CD31 antibody, Percp-labeled human CD61 antibody, PE-labeled human CD62P antibody, PE-labeled human CD63 antibody and FITC-labeled human PAC-1 antibody, were provided by American B. Dpbarm. Ingen Corporation. Coagulation four indices reagents were provided by Japanese East-Asia Corporation. Calibur flow cytometer was provided by American BD Corporation. Blood coagulation instrument (model number: CA-1500) was provided by Japanese East-Asia Corporation.

2.2.3 Statistical treatment

Statistics software was used, and all of data were expressed as $\bar{x} \pm s$, and were analyzed with the method of *t*-test.

3 Results

The expression of CD31, CD61, CD62p, CD63 and PAC-1 on platelet membrane in the necrosis group was all lower than that in the control group ($P < 0.01$). There was no significant difference ($P > 0.05$) in coagulation four indices including PT, APTT, Fbg and TT between patients and healthy subjects.

It is demonstrated in Tables 1 and 2.

4 Discussion

According to the relevant data, it was found that half of the patients would suffer from ANFH when recovered from SARS [2]. Chen [3] reported that its incidence was 53.5%. Previously, the research on ANFH showed that administration of hormone was the most common reason for ANFH. Soucacos [4] found that 84 in 187 cases of ANFH (44%)

resulted from the use of corticoids. It was reported that the maximal dose administered on SARS patients was 1 200 mg/d. In addition, the duration of administration was almost within one month. The incidence of ANFH was direct correlated with the use of hormone [3]. There was no doubt that hormone was an important reason for this disease.

There was no agreement among many pathogenesis that explained how hormone resulted in ANFH, including the theory of lipin metabolic disturbance, the intravascular coagulation theory, the osteoporosis theory, the theory of sediment of immune complex that resulted in arterial vasculitis, the theory of venostasis that resulted in high-pressure in bone, the theory of hormone that had toxicant effect on bone cell. Wang [5] performed a study and found that administration of large doses of hormone in short time could make blood be in hyper-coagulable state. Liu [6] thought that, during the episode of ANFH, abnormal blood coagulation was present. Zheng [7] found that hormone could result in high coagulation and early activation of platelet. But up to date, there was no report that discussed platelet membrane glycoprotein and tetrachoric coagulation. It was found that, in these patients with ANFH after SARS, the expression of glycoprotein CD31, CD61, CD62P, CD63 and PAC-1 on platelet membrane decreased; coagulation four indices including PT, APTT and TT time were all normal. The content of Fbg was also normal. It indicated that there was no high blood coagulation when our patients were found to be ANFH, which was consistent with the results acquired by Wang [8]. They conducted a study on blood coagulation function of SARS patients and found that PT, APTT prolonged remarkably and Fbg had a significant rise during the acute stage of episode, but became normal three months after the disease was cured.

Glycoprotein CD31, CD61, CD62P, CD63 and PAC-1 on platelet membrane played a part in adhesion, release and aggregation of platelet, and were the markers of activation of platelet. In our study, the expression of glycoprotein on platelet membrane decreased, which suggested that platelet did not be activated. The reasons might be as follows. (1) The expression of glycoprotein on platelet membrane was impaired because its structure was destroyed by SARS virus. (2) Large doses of hormone were administrated. DU [9] reported that

Table 1 Expression of CD31, CD61, CD62p, CD63 and PAC-1 on platelet membrane ($\bar{x} \pm s$)

Group	Number	CD31 fluorescence intensity	CD61 fluorescence intensity	CD62p/%	CD63/%	PAC-1/%
Necrosis group	26	22.35 $\pm$ 4.90*	31.25 $\pm$ 8.43*	28.80 $\pm$ 47.40*	1.49 $\pm$ 0.82*	0.20 $\pm$ 0.11*
Control group	17	49.20 $\pm$ 4.47	53.10 $\pm$ 7.32	36.20 $\pm$ 5.30	3.17 $\pm$ 1.42	0.59 $\pm$ 0.63

Note: Compared with control group, * $P < 0.01$.

Table 2 Coagulation four indices: PT, APTT, Fbg, TT measurement ($\bar{x} \pm s$)

Group	Number	PT/s	APTT/s	Fbg/mg $\cdot$ dL ⁻¹	TT/s
Necrosis group	26	11.15 $\pm$ 0.67*	24.85 $\pm$ 2.07*	289.6 $\pm$ 50.23*	15.86 $\pm$ 1.17*
Control group	17	11.40 $\pm$ 0.91	25.10 $\pm$ 2.85	279.60 $\pm$ 36.58	15.71 $\pm$ 1.36

Note: Compared with control group, * $P > 0.05$.

the levels of glycoprotein GPIIb/IIIa, GMP-140 on platelet membrane in asthmatic patients at remission stage were higher than those in normal patients. The activation rate of platelet increased, but decreased after glucocorticoid was inhaled. (3) The immunologic function was inhibited. Administration of large quantities of glucocorticoid resulted in the damage of immunologic function. Autoantibody was produced after patients were infected by virus. Epitope family were mostly located in glycoprotein GPIIb/IIIa on platelet membrane, in the meantime, glycoprotein on platelet membrane was impacted by enclosure of antibody and lost the function of adhesion and aggregation. (4) Large quantities of antibiotics were applied. Coagulation four indices could reflect the blood coagulation function of human body, among which PT mostly reflected the content and activity of coagulation factors in the route of extrinsic coagulation; APTT mostly reflected the content and activity of coagulation factors in the route of intrinsic coagulation; TT reflected whether blood antithrombotics increased. Fbg was not only a principal coagulation factor in the blood coagulation system, but also an accessory factor for platelet aggregation. Only if platelet was activated, its spatial configuration changed, and fibrinogen receptor was exposed, identification and incorporation between fibrinogen and platelet could be facilitated and thus platelet aggregated. That coagulation four indices were normal indicated that the contents of internal and extrinsic coagulation factors in ANFH patients after SARS were normal, and that their blood was not hypercoagulable, at the meantime indirectly proved that platelet did not be activated, and blood coagulation system did not be initiated, which was in accordance with decreasing expression of glycoprotein on platelet membrane.

Based on the comprehensive analysis, it was found that their blood was not hypercoagulable when patients suffered from ANFH after SARS. The possible reasons include: (1) hormone was administered at large doses in a short time; (2) SARS virus had impinge on platelet activation; (3) immunosuppression; (4) the application of antibiotics; (5) abnormal antithrombotics in blood were present. Consequently, it is inferred that ANFH after SARS may not develop in the mechanism of abnormality of blood coagulation. However,

only several markers related to blood coagulation were measured. It is possible that there are other special blood coagulation substances in the blood of patients with ANFH after SARS, which needs to be studied further.

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