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Significance of D-dimer assay in the diagnosis of acute pulmonary embolism after deep second-degree burn in rabbits

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Abstract

Objective: To explore the significance of D-dimer assay in the diagnosis of acute pulmonary embolism (APE) after deep second-degree burn by detecting the changes of plasma D-dimer in rabbits with deep second-degree burn and in different phases of burn with concomitant pulmonary embolism.
Methods: 48 healthy male or female Chinese white rabbits, were randomly divided into: control group, burn group, burn shock group, burn shock + pulmonary embolism group, burn infection group and burn infection + pulmonary embolism group, with 8 rabbits in each group. By use of the ELISA method, the plasma D-dimer was determined on 1 d, 3 d, 5 d, 7 d, 14 d and 21 d after modeling in the burn group and the control group, and the plasma D-dimer in other groups was detected in 30 min, 1 h, 3 h, 6 h, 24 h after modeling.
Results: The expression of D-dimer in the burn group was higher than that in the control group on 1 d, 3 d, 5 d and 7 d ($p < .05$). There were significant differences in the expression of D-dimer in the burn shock + pulmonary embolism group in comparison to the burn shock group ($p < .05$). There were also some significant differences in the expression of D-dimer in the burn infection + pulmonary embolism group in comparison with the burn shock + pulmonary embolism group ($p < .05$).
Conclusions: There were some differences in the expression of D-dimer in each group. D-dimer can be used as one of diagnostic indexes for deep second-degree burn with pulmonary embolism.

Key Words: D-dimer, Pulmonary embolism, Burn

Acute pulmonary embolism (APE) is an acute pulmonary circulation disorder syndrome caused by a sudden blockage of a pulmonary artery or its branch by thrombotic and/or non-thrombotic emboli that have moved from elsewhere in the body through the bloodstream. APE is a sudden-onset disease with critical and dangerous pathogenic conditions. The mortality is so high that about 11% of the population die of APE within one hour after the occurrence.[1] Risk factors for this disease include long-term bedridden condition, trauma, surgical operations and so on.

Pulmonary angiography is the "gold standard" for the diagnosis of PE, with a sensitivity of approximately 98% and a specificity of 95% to 98%. However, in the prospective study on the diagnosis of pulmonary embolism, it was found that 22.4% of patients had contraindications for pulmonary angiography.[2] Currently, to find a non-invasive and inexpensive inspection method has become an immediate area of research focus. D-dimer is considered to be a specific end product in the process of thrombosis, and its elevated level indicates that thrombi have been formed and degraded in

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the body. As one of the markers reflecting hypercoagulability and secondary increased fibrinolytic activity in the body, D-dimer has a high sensitivity in the diagnosis of pulmonary embolism. Therefore, the most important clinical value of D-dimer detection is to exclude venous thrombotic diseases. However, the specificity of D-dimer is low as pregnancy, malignant tumors and other factors will cause an increase in D-dimer. Deep vein thrombosis (DVT) is a common complication happening to burn patients,[3] a tendency towards hypercoagulation often appears in the early stage of burns, the burn shock phase and the infection phase. It is often found in clinical work that the level of D-dimer can also be elevated in the absence of DVT and pulmonary embolism. On that account, the guiding significance of D-dimer in the diagnosis of APE after burn remains to be explored.

1 Data and methods

1.1 Animals and main reagents

48 healthy male or female Chinese white rabbits were provided by Experimental Center of Inner Mongolia University, weighed 2.5-3.0 kg, 6-8 months old; ELISA kits for D-dimer were from Shanghai Jiemen Bio-Tech Co., Ltd.

1.2 Animal grouping

By use of the random number table method, 48 healthy male or female Chinese white rabbits, were randomly divided into: control group, burn group, burn shock group, burn shock + pulmonary embolism group, burn infection group and burn infection + pulmonary embolism group, with 8 rabbits in each group.

1.3 Modeling of APE in rabbits

Embolus preparation:[4] 1 ml of blood was drawn through the marginal ear vein before operation, and injected into a sterile vial to stand for 20 to 30 minutes. After autoagglutination, the vial was put into a water bath at 70°C for 10 minutes to make emboli. The emboli were guided in to a 5 ml syringe to mix with normal saline, and 2 ml to 3 ml of suspension was prepared consequently, containing 7 to 8 emboli.

Modeling: The experimental rabbits were injected with 3% pentobarbital sodium solution through the marginal ear vein at a dose of 30 mg/kg. After achieving satisfactory anesthesia outcomes, the rabbits were placed on the operation table in the supine position, 20 G vein detained needle was inserted with the femoral vein freed. In the control group, the rabbits were injected with 3 ml of normal saline through the femoral vein detained catheter, and rabbits in the other group were injected with the ready column-shaped autologous blood clots and treated with bolus injection of 3 ml of normal saline to prevent those blood clots from staying in the catheter or the femoral vein and avoid the blood clot being incarcerated in the pulmonary artery along with the blood circulation to establish an APE model. Rabbits dying from anesthesia accidents and modeling were removed and excluded from this experiment. In the burn shock group, burn shock + pulmonary embolism group, burn infection group, burn infection + pulmonary embolism group, venous blood was collected at 30 min, 1 h, 3 h, 6 h, 24 h after modeling, centrifuged and separated, with the suspension placed in the -70°C refrigerator, for the purpose of testing. In the control group and the burn group, venous blood was collected at 1 d, 2 d, 3 d, 5 d, 7 d and 14 d after modeling, centrifuged and separated, with the suspension placed in the -70°C refrigerator, for the purpose of testing. The detection of D-dimer was made by use of the ELISA method.

Burn modeling: The experimental rabbits were injected with 3% pentobarbital sodium solution through the marginal ear vein at a dose of 30 mg/kg. After achieving satisfactory anesthesia outcomes, the rabbits, with the back hair removed, were placed in a self-made scalding mold in the supine position, immersed in 85°C water for 15 s, resulting in the production of deep second-degree burn wounds. Lactated Ringer's solution was injected into the abdominal cavity for resuscitation.

1.4 Statistical analysis

The data were represented by $\bar{x} \pm s$. SPSS 12.0 statistical software kit was applied to this research, and the comparison between groups was made by means of *t*-test. The difference $p < .05$ was of statistical significance.

2 Results

2.1 Model establishment

General observation: One case of anesthetic-related death was in 48 rabbits, and 2 rabbits died during the process of embolus injection. 14 models of pulmonary embolism were established, with a success rate of 87.5%. The rest models were established later. All rabbits showed shortness of breath, purple sputum and increased heart rate after embolus injection.

2.2 Expression of D-dimer

There was no significant change in the expression of D-dimer at each time point in the control group. The difference was not statistically significant.

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The expression of D-dimer in the burn group was the highest at 1 d. Meanwhile, the expression at 1 d, 3 d, 5 d and 7 d were higher in the burn group than those in the control group ($p < .05$, see Table 1).

The expression of D-dimer in the burn shock group was increased significantly at 3 h, with the peak at 6 h, and then gradually decreased. The expression of D-dimer peaked at 1 h in the burn shock + pulmonary embolism group, and there was no significant change from 1 h to 6 h. After 6h, the expression of D-dimer was gradually decreased. There was a significant difference in the expression of D-dimer between the burn shock group and the burn shock + pulmonary embolism group ($p < .05$, see Table 2).

The expression of D-dimer in the burn infection group was increased significantly at 3 h, and then increased at a slow rate, with the peak at 24 h. The expression of D-dimer peaked at 1 h in the burn infection + pulmonary embolism group, and there was no significant change from 1 h to 6 h. After 6 h, the expression of D-dimer was gradually decreased. There was a significant difference in the expression of D-dimer between the burn infection group and the burn infection + pulmonary embolism group ($p < .05$, see Table 3).

Table 1: The expression of D-dimer in the burn group and the control group (mg/L) (n, $\bar{x} \pm s$)

Group	1 d	3 d	5 d	7 d	14 d
Control Group (n = 8)	0.2262 ± 0.0482	0.2222 ± 0.0472	0.2251 ± 0.0481	0.2231 ± 0.0463	0.2246 ± 0.0478
Burn Group (n = 8)	4.3138 ± 2.0279	3.9087 ± 1.7382	3.1563 ± 1.4395	2.8875 ± 1.3103	0.2225 ± 0.0498

Note. In comparison to the burn shock group, $p < .05$

Table 2: The expression of D-dimer in the burn shock group and the burn shock + pulmonary embolism group (mg/L) (n, $\bar{x} \pm s$)

Group	30 min	1 h	3 h	6 h	24 h
Burn Shock Group (n = 8)	3.4388 ± 0.7591	4.0875 ± 0.6694	4.4375 ± 0.5378	5.1263 ± 1.4707	4.4438 ± 1.1523
Burn Shock + Pulmonary Embolism Group (n = 8)	3.7963 ± 0.4090	5.4475 ± 1.2073	5.4638 ± 1.2209	5.4613 ± 1.1349	4.9063 ± 1.0675

Note. In comparison to the burn shock group, $p < .05$

Table 3: The expression of D-dimer in the burn infection group and the burn infection + pulmonary embolism group (mg/L) (n, $\bar{x} \pm s$)

Group	30 min	1 h	3 h	6 h	24 h
Burn Infection Group (n = 8)	3.1238 ± 0.7645	4.4938 ± 0.7282	5.7288 ± 0.3303	6.2188 ± 0.3987	6.4887 ± 0.4853
Burn Infection + Pulmonary Embolism Group (n = 8)	3.4975 ± 0.7189	5.3638 ± 0.6695	5.3637 ± 0.6458	5.3587 ± 0.7969	3.9137 ± 0.6626

Note. In comparison to the burn infection group, $p < .05$

3 Discussion

In the treatment of severe large-area deep burn, a tendency towards hypercoagulation often appears due to hemoconcentration, the damage of vascular endothelial cells and reduced abilities of anticoagulation and anti-thrombosis in endothelial cells in the early stage of burns, burn shock phase and burn infection phase. Therefore, patients with severe large-area deep burn become a high-risk group for venous thrombosis, some of whom suffer from pulmonary embolism caused by a blockage of a pulmonary artery by venous emboli that have moved from elsewhere in the body. As a serious disease that impairs human health, pulmonary embolism has a low specificity in clinical manifestations, resulting in a high rate of misdiagnoses and missed diagnoses but a low detection rate and early diagnosis rate. The current primary method of diagnosing pulmonary embolism is imageological examinations, such as spiral CT, EBT and radionuclide ventilation-perfusion lung scans. These examinations are expensive and difficult to be spread in primary hospitals. It is also difficult to apply them to critically ill patients who are not suitable for moving.

D-dimer is considered to be a specific end product in the process of thrombosis, and its elevated level indicates that thrombi have been formed and degraded in the body. It is one of the markers reflecting hypercoagulability and secondary increased fibrinolytic activity in the body. There-

fore, the most important clinical value of D-dimer detection is to exclude venous thrombotic diseases. A tendency towards hypercoagulation often appears in the early stage of burns, the burn shock phase and the infection phase. It is often found that the level of D-dimer can also be elevated in the absence of venous thrombosis and pulmonary embolism. On that account, the guiding significance of D-dimer in the diagnosis of APE after burn remains to be explored. In this experiment, rabbits with deep second-degree burn were selected as research objects. With the influence of other clinical complications excluded, the interval between burn shock and burn infection, which shows an obvious hypercoagulability state, is considered as the observational focus to study the expression of D-dimer in concomitant pulmonary embolism.

In this experiment, it was found that the expression of D-dimer was the highest in the burn group at 1 d. Meanwhile, the expression at the 1 d, 3 d, 5 d and 7 d were higher than those in the control group, indicating there was an activated thrombosis and fibrinolytic activity, which suggested that DIC may occur in the early stage of severe burn. It is similar to recent results reported by Nakae et al.[5]

In this experiment, the expression of D-dimer in the burn shock group was increased significantly at 3 h, with the peak at 6 h, and then gradually decreased. The expression of D-dimer peaked at 1 h in the burn shock + pulmonary embolism group, and there was no significant change from 1 h to 6 h. After 6 h, the expression of D-dimer was gradually decreased. There was a significant difference in the expression of D-dimer between the burn shock group and the burn shock + pulmonary embolism group. Such results may be mainly related to hypercoagulability caused by decreased circulating blood volume and plasma volume in the phase of burn shock. It was reported that the plasma volume in the phase of burn shock was reduced by 50% at 2 h after burn, and the whole blood volume was reduced to the lowest value at 4-6 h after injury.[6] It is consistent with the pattern of D-dimer expression in this experiment.

In this experiment, the expression of D-dimer in the burn infection group was increased significantly at 3 h, and then increased at a slow rate, with the peak at 24 h. The expression of D-dimer peaked at 1 h in the burn infection + pulmonary embolism group, and there was no significant change from 1 h to 6 h. After 6 h, the expression of D-dimer was gradually decreased. There was a significant difference in the expression of D-dimer between the burn infection group and the burn infection + pulmonary embolism group. The analysis results showed infection could cause the inflammatory response, damage vascular endothelial cells and increase the level of endothelin, resulting in the consumption of coagulation factors, increased fibrinolytic activity and increased D-dimer level.[7,8] It was reported that the expression of vascular endothelin was significantly higher at 1 h, 2 h and 4 h after embolism than that before embolism.[9,10] When vascular endothelial cells were damaged, the level of vascular endothelin was increased along with the increase of D-dimer. It may be the reason for the increase of D-dimer at the above time points in the present experiment.

In view of the above results, we make the following preliminary conclusion: After deep second-degree severe burn, a variety of factors eventually lead to vascular endothelial damage, the activation of the coagulation system and the increase of D-dimer, which is especially obvious in the phases of shock and infection. This type of increase is associated with the enhancement of coagulation-fibrinolysis activity in the shock and the infection phases. After burns complicated with pulmonary embolism, in comparison with simple burn shock and infection, the expression of D-dimer is significantly increased due to the dual factors of burn and pulmonary embolism. Hence, it suggests that D-dimer has an important guiding significance in the diagnosis of burns with pulmonary embolism. However, it is necessary to raise the positive value standard for D-dimer, which still remains to be further studied.

Conflicts of Interest Disclosure

The authors have no conflicts of interest related to this article.

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