Therapeutic effect evaluation of reteplase on acute pulmonary embolism

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Abstract: Thrombolysis is the main therapeutic method of acute pulmonary embolism (APE). In order to investigate the efficacy of reteplase on APE and the changes of cytokines in the progression of APE, 72 patients with APE were randomized into reteplase group and urokinase group which received reteplase thrombolysis and urokinase thrombolysis, respectively. The clinical symptoms, blood pressure, heart rate (HR), blood gas index and cytokines of patients were observed before and after therapy for assessing the thrombolysis effect of each group; blood level of high sensitive C-reactive protein (hs-CRP), TNF-α, IL-1β, IL-6 and IL-10 was detected at 0h, 2h, 6h, 12h and 24h after thrombolysis. After treatment, the clinical symptoms of both groups were alleviated obviously; PaO_2, $PaCO_2$, blood pressure and HR in both groups were significantly improved than those before treatment (p<0.001), and reteplase group showed a more obvious improvement than urokinase group (p<0.001). Since 6h after therapy, the content of hs-CRP, IL-1β and IL-6 in patients of reteplase group declined significantly (p<0.05 or 0.01). In conclusion, therapeutic effect of reteplase is better than urokinase, hs-CRP, IL-1β and IL-6 can be used to monitor the thrombolysis efficacy of APE patients.

Keywords: Reteplase, acute pulmonary embolism, cytokine, inflammation.

INTRODUCTION

Pulmonary embolism (PE) is caused by endogenous or exogenous pulmonary artery blockage by thrombus embolus, characterized with disorders of pulmonary circulation and respiratory function (Daley *et al*., 2015). The symptom of PE is related to the size and quantity of embolus, embolism position and cardio-pulmonary function of patient. Acute pulmonary embolism (APE) caused by small embolus may manifest no clinical symptoms; if a bigger embolus blocks the left and right pulmonary artery, hemodynamic disturbance and even acute right ventricular failure will occur whose main clinical symptoms are cyanosis, faint, sudden death and so on (Go *et al*., 2013). However, PE may escape prompt diagnosis since clinical symptoms and signs are nonspecific, and the diagnosis of APE is amongst the most challenging problems encountered in clinical practice. The knowledge concerning the clinical presentation of APE is one of the key factors in ensuring an immediate diagnosis and adequate intervention (Wang *et al*., 2014).

The main therapeutic methods of APE are thrombolysis and anticoagulation, and the final objective of thrombolysis and anticoagulationis relieving pulmonary thrombosis, reducing pulmonary artery pressure and reversing right heart failure (Goldhaber *et al*., 1993), and ultimately alleviating clinical symptoms and reducing mortality. Thrombolytic therapy is a treatment to get rid of problems raised due to thrombus to renovate function to the affected area (Perler, 2005). For APE patient with hemodynamic change and without contraindication of thrombolysis, thrombolytic therapy is the first choice. Then, assisted with anticoagulant therapy, thrombus will be rapidly dissolved and pulmonary perfusion will achieve, thus reversing right heart failure and increasing the blood volume of pulmonary capillary. In the selection of thrombolytic drugs, urokinase is commonly used at present, but its thrombolytic effect is actually modest and with many complications. As the third generation of thrombolytic drug, reteplase is a new non-glycosylated activator of recombined plasminogen and has the advantages such as short half life, convenient administration and high safety. Now reteplase is widely used in thrombolytic therapy of myocardial infarction and hemodialysis (Baskin *et al*., 2012, Hilleman and Campbell, 2011, Panduranga *et al*., 2012).

Although in the last few years, reteplase is used for the treatment of APE and deep venous thrombosis (DVT) and for thrombosed catheters(Simpson *et al*., 2006, Bush *et al*., 2004), there are few studies on the thrombolysis scheme, clinical efficacy, safety and prognosis of reteplase for treating APE patients. In the study, through giving reteplase therapy to APE patients with urokinase as the control, the efficacy and safety of reteplase in treating APE was assessed.

MATERIALS AND METHODS

72 APE hospitalized in our hospital during January 2011 and June 2014were enrolled, including 47 males and 25

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females, with the age of 24.5~76.3 and the average age of 56.7±13.6. CT pneumoangiography was adopted for PE diagnosis, the diagnostic criteria was according to *Chinese Expert Consensus in Diagnosis and Treatment of Acute Pulmonary Embolism* formulated by Pulmonary Vascular Disease Group of Chinese Society of Cardiology in 2010. Inclusion criteria comprised: (1) those with massive PE in more than two pulmonary lobes; (2) those with changed hemodynamics, irrespective of the PE position and size; (3) those complicated by shock and hypoperfusion of systemic circulation; (4) patients with sinus tachycardia after PE; (5) PE patients with acute respiratory distress syndrome (including increase of respiratory rate, decline of arterial oxygen saturation, etc.). Exclusion criteria comprised: (1) those with hypertension, coronary heart disease, stroke, chronic obstructive pulmonary disease (COPD), diabetes, autoimmune disease and having history of malignant tumor; (2) those having a family history of venous thrombus embolism; (3) those having a history of similar attack; (4) those having contraindication of thrombolysis.

36 healthy participants who conducted physical examination in our hospital during the same period were enrolled as the control group, including 23 males and 13 females, with the age of 25~74 years old and the average age of 55.1±14.2.

In the study, all the patients and all the healthy control participators gave consent to publish this paper, and this study was approved by the Ethics Committee of our hospital.

General treatment was given to all patients after admission, including nasal oxygen breath or facemask oxygen breath. As appropriate, drugs were given to expand pulmonary vessels; comprehensive treatment including sedation, relieving cough, reducing sputum, antipyresis, analgesia and antiphlogosis, was given depending on the condition of patient. Patients with drop of blood pressure or shock immediately received antihypotensive therapy or antishock therapy. Patients were carefully monitored, and the vital signs, such as respiratory rate, heart rate, blood pressure and pulmonary arterial pressure (PAP), were recorded.

After the relevant exams were completed, patients were randomly divided into reteplase group and urokinase group, and venous thrombolytic therapy was given to patient within 24h after grouping. Reteplase group was given intravenous drip of 100ml 0.9% sodium chloride injection containing 50mg reteplase, and the intravenous drip was completed in 2h. Urokinase group was given intravenous drip of urokinase 20000IU/kg/2h, which was also completed in 2h. After the thrombolysis therapy, subcutaneous injection of dalteparin sodium was conducted immediately for anticoagulation, 5000IU/12h. Meanwhile, warfarin (3mg/d) was taken orally for 3~5days.When the international normalized ratio (INR) reached 2.0~3.0, heparin sodium was stopped, and warfarin treatment was continued. The dosage of warfarin was timely adjusted according to INR value. The treatment lasted 3~6 months.

Observational indices

The clinical manifestation and vital signs of all patients before thrombolysis and 4h after thrombolysis were observed. Heart rate (HR), pulmonary arterial pressure (PAP) and systolic blood pressure (SBP) were recorded. Routine urianlysis was conducted, and the lower limbs arterial-venous color Doppler ultrasound was conducted to detect the thrombus level in deep vein. The arterial partial pressure of oxygen (PaO_2) and arterial partial pressure of carbon dioxide ($PaCO_2$) were recorded before oxygen uptake and 30min after oxygen uptake. Moreover, the incidence of bleeding after thrombolytic therapy was also observed.

Determination of plasma cytokines: 3ml elbow vein blood of patients in control group and reteplase group was collected before thrombolytic therapy and after 0h, 2h, 6h, 12h and 24h of thrombolytic therapy. The content of plasma hs-CRP was determined by fully automatic biochemical analyzer, level of TNF-α, IL-1β, IL-6 and IL-10 in serum was detected by ELISA Kit according to the instruction of kit. All these observational indices were listed in table 1.

Therapeutic effect evaluation of thrombolytic therapy

On the next day after thrombolysis, reexamination was conducted to evaluate the efficacy of thrombolytic therapy. Recovery: Symptoms such as dyspnea disappeared; CT pulmonary angiography or catheter pulmonary angiography indicated that the defect lung segments completely disappeared. Excellence: Symptoms such as dyspnea were obviously relieved; CT pulmonary angiography or catheter pulmonary angiography indicated that 7~9defect lung segments reduced or the area of defect lung reduced 75%. Improvement: Symptoms such as dyspnea were decreased, CT pulmonary angiography or catheter pulmonary angiography indicated that 1~6 defect lung segments reduced or the area of defect lung reduced 50%. Ineffectiveness: Symptoms did not show any obvious change, CT pulmonary angiography or catheter pulmonary angiography indicated no obvious change. Exacerbation: Symptoms such as dyspnea worsened, CT pulmonary angiography or catheter pulmonary angiography indicated that the defect lung segments increased; Death.

$$\text{Effective rate} = \frac{(\text{Recovery} + \text{Excellence} + \text{Improvement})}{\text{Total number of people}} \times 100$$

Table 1: Observational indices before and after thrombolysis

Observational indices	Observation time
Heart rate (HR)	Before thrombolysis and 4h after thrombolysis
Pulmonary arterial pressure (PAP)	
Systolic blood pressure (SBP)	
Routine urianlysis	
Thrombus in deep vein	
Arterial partial pressure of oxygen (PaO_2)	Before oxygen uptake and 30 min after oxygen uptake
Arterial pressure of carbon dioxide ($PaCO_2$)	
Incidence of bleeding	After thrombolysis
Plasma cytokines (includinghs-CRP, TNF-α, IL-1β, IL-6 and IL-10)	Before thrombolysis and 0 h, 2 h, 6 h, 12 h, 24 h after thrombolysis

Table 2: Base characteristics of patients

Groups	Reteplase group	Urokinase group	*t* or χ^2 value	*p* value
N	36	36	-----	-----
Gender (male/female)	25/11	22/14	0.551	0.458
Smoking status				
Never	11	13	0.285	0.867
Former	6	5		
Current	19	18		
Current alcohol drinking (Yes/No)	22/14	20/16	0.229	0.663
Cases of deep vein thrombosis (n)	17	15	0.225	0.635
Heart rate (times/min)	113.4±6.9	115.2±7.4	1.625	0.114
Systolic pressure (mmHg)	101.59±7.54	99.83±8.67	0.788	0.432
Pulmonary arterial pressure (mmHg)	68.37±4.26	67.02±5.11	1.543	0.146
PaO_2(mmHg)	54.03±6.20	53.79±5.89	0.268	0.795
$PaCO_2$(mmHg)	25.32±3.41	24.96±3.56	1.055	0.312

Table 3: The improvement of clinical symptoms before and after the treatment

	Thoracodynia	Dyspnea	Syncope	Hemoptysis	Tachycardia
Reteplase group					
Pre-	32	30	19	28	34
Post-	2	0	0	0	1
Improvement rate (%)	93.75	100	100	100	97.06
Urokinase group					
Pre-	35	31	21	31	34
Post-	9	7	7	5	8
Improvement rate (%)	74.29	77.42	66.67	83.87	76.47

STATISTICS ANALYSIS

All the data were subjected to statistical analysis using SPSS 16.0 software. Measurement data was conducted for normality test, the averages were showed as mean ±SD. The comparisons between groups were adopted *t* test and χ^2 test. The paired *t* test was adopted for changes before and after treatment. $p<0.05$ was considered as significant difference; $p<0.01$ was considered as extremely significant difference.

RESULTS

Table 2 showed that there was no significant differences between two groups before treatment in age, sex, smoking history, drinking history, deep venous thrombosis in lower limbs, HR, SBP, PAP, PaO_2 and $PaCO_2$ ($p>0.05$).

Outcomes and bleeding complications

After treatment, the clinical symptoms of patients in both groups (chest pain, dyspnea, syncope, hemoptysis and tachycardia, etc.) were significantly alleviated, and reteplase group showed a more obvious improvement than urokinase group (table 3). table 4 indicated that PaO_2, $PaCO_2$, SBP, PAP and HR of both groups after treatment were obviously improved than those before treatment ($p<0.001$) and the reteplase group showed a more obvious improvement than urokinase group ($p<0.001$). The effective rate of reteplase group was also higher than

Table 4: General conditions before and after thrombolytic therapy

		Reteplase group	Urokinase group	*p* value
PaO_2(mmHg)	Baseline	54.03±6.20	53.79±5.89	0.795
	Post-treatment	97.68±5.37	88.76±3.92	<0.001
	p value	<0.001	<0.001	
$PaCO_2$(mmHg)	Baseline	25.32±3.41	24.96±3.56	0.312
	Post-treatment	45.93±3.86	37.25±4.07	<0.001
	p value	<0.001	<0.001	
SBP(mmHg)	Baseline	101.59±7.54	99.83±8.67	0.432
	Post-treatment	127.84±8.14	111.22±7.56	<0.001
	p value	<0.001	<0.001	
PAP(mmHg)	Baseline	68.37±4.26	67.02±5.11	0.146
	Post-treatment	30.37±4.17	40.58±5.64	<0.001
	p value	<0.001	<0.001	
HR(times/min)	Baseline	113.4±6.9	115.2±7.4	0.114
	Post-treatment	74.1±5.2	86.5±6.0	<0.001
	p value	<0.001	<0.001	

Table 5: Outcomes of reteplase group and urokinase group

Groups	Recovery	Excellence	Improvement	Ineffectiveness	Exacerbation	Effective rate
Reteplase group	19	9	6	2	0	94.44%
Urokinase group	18	4	3	6	5	69.44%
χ^2 value	9.950					
p value	0.041					

Table 6: The incidence of bleeding complications after thrombolytic therapy

Groups	Gingiva bleeding	Gross or microscopic hematuria	Bloody sputum	Nasal mucosa bleeding	Hematemesis
Reteplase group	2	2	1	1	0
Urokinase group	3	2	1	1	1
χ^2 value	0.933				
p value	0.920				

urokinase group (p<0.05, table 5), but there was no difference between the two groups in bleeding complication incidence (p>0.05, table 6).

Content of inflammatory factors

Before thrombolytic therapy, the inflammatory factors content of reteplase group was significantly higher than the control group (p<0.01, fig. 1); since 6h after thrombolytic therapy, the content of hs-CRP, IL-1β and IL-6 of reteplase group declined significantly (p<0.05 or 0.01, fig. 2). But until 24h after thrombolytic therapy, the content of hs-CRP, IL-1β and IL-6 of reteplase group was still higher than control group. These results implied that these inflammatory factors may be used to monitor APE occurrence and thrombolytic effect.

DISCUSSION

PE has a variety of clinical manifestations. The most common symptoms include dyspnea or short of breath, chest pain, syncope, hemoptysis and tachycardia, etc. There is also patient without any symptoms (Gulsen *et al*., 2015, Rugolotto and Favretto, 2015). In the study, the clinical symptoms and clinical indexes of two patient groups after treatment were obviously alleviated (p<0.001), and reteplase group showed a more obvious improvement than urokinase group (p<0.001). The effective rate of reteplase group was also higher than urokinase group (p<0.05), however, there was no difference between the groups after treatment in bleeding complication incidence (p>0.05).

As the third generation fibrinolytic drug, reteplase is modified form of alteplase with longer half-time and has only been applied in trials yet (Frendl and Csiba, 2011). Recently, there have been reported that reteplase was used to treat ischemic stroke. In patients with acute ischemic stroke (AIS), Misra *et al* found concurrent mechanical thrombolysis (MT) was attempted in 59.7% of urokinase-treated patients and 72.0% of reteplase-treated patients, and supposed that high doses of reteplase may be safe when given with or without MT in patients with

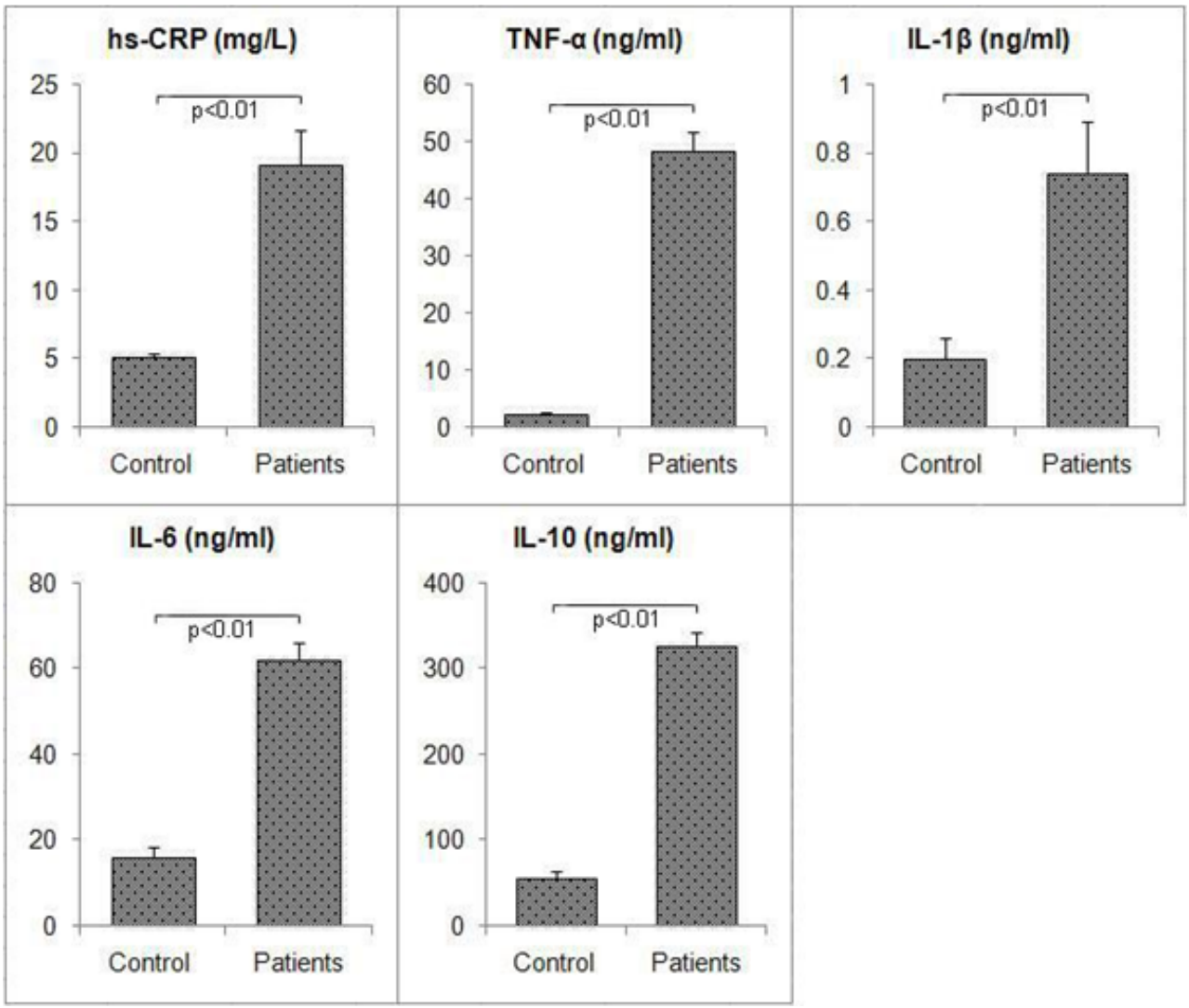

Fig. 1: Comparisons of the inflammatory factors in control group and reteplase group before thrombolytic therapy

AIS(Misra *et al*., 2011). Study of Hire math pointed out that in patients with acute myocardial infarction, the future of intravenous thrombolysis will remarkably be based on whether Indian physicians switch to direct Fibrin Inhibitors like retelplase and angiography (Hiremath, 2011). In this study, the effective rate of reteplase group was higher than urokinase group, which indicates that reteplase has a higher fibrinolytic efficiency than urokinase; concerning the routine item detection, both groups improved obviously after treatment and the reteplase group had a more obvious improvement than urokinase group. These results indicated that using reteplase in clinic can reduce thrombolytic time, improve thrombolytic effect, quickly return hemoperfusion of pulmonary artery, timely reduce pulmonary hypertension and alleviate anoxic condition (Aghaabdollahian *et al*., 2014).

Bleeding after thrombolysis is closely related to prognosis, and affects the selection of thrombolytic therapy on massive APE. In this paper, the patients of reteplase group had the complications of gingiva bleeding, hematuresis, bloody sputum and nasal mucosa bleeding. The total bleeding rate was equal to that of urokinase therapy, which confirmed the safety of reteplase.

In addition, many studies showed that APE is closely related to inflammation(Kundi *et al*., 2015, Piazza, 2015). In fact, a reciprocal relationship exists: patients with thromboembolism have an increased risk of myocardial infarction and PE, and vice versa. In epidemiologic studies, systemic inflammation has been closely associated with an increased risk of venous thromboembolism (VTE).And increased levels of the inflammatory biomarker hs-CRP are associated with an increased risk of VTE(Folsom *et al*., 2009). Activation of the innate immunity, marked by increased levels of TNF-α, IL-1β, IL-6 and IL-8 increases the risk of VTE even after adjustment for hs-CRP(Reitsma and Rosendaal, 2004). This study showed that since 6h after thrombolytic

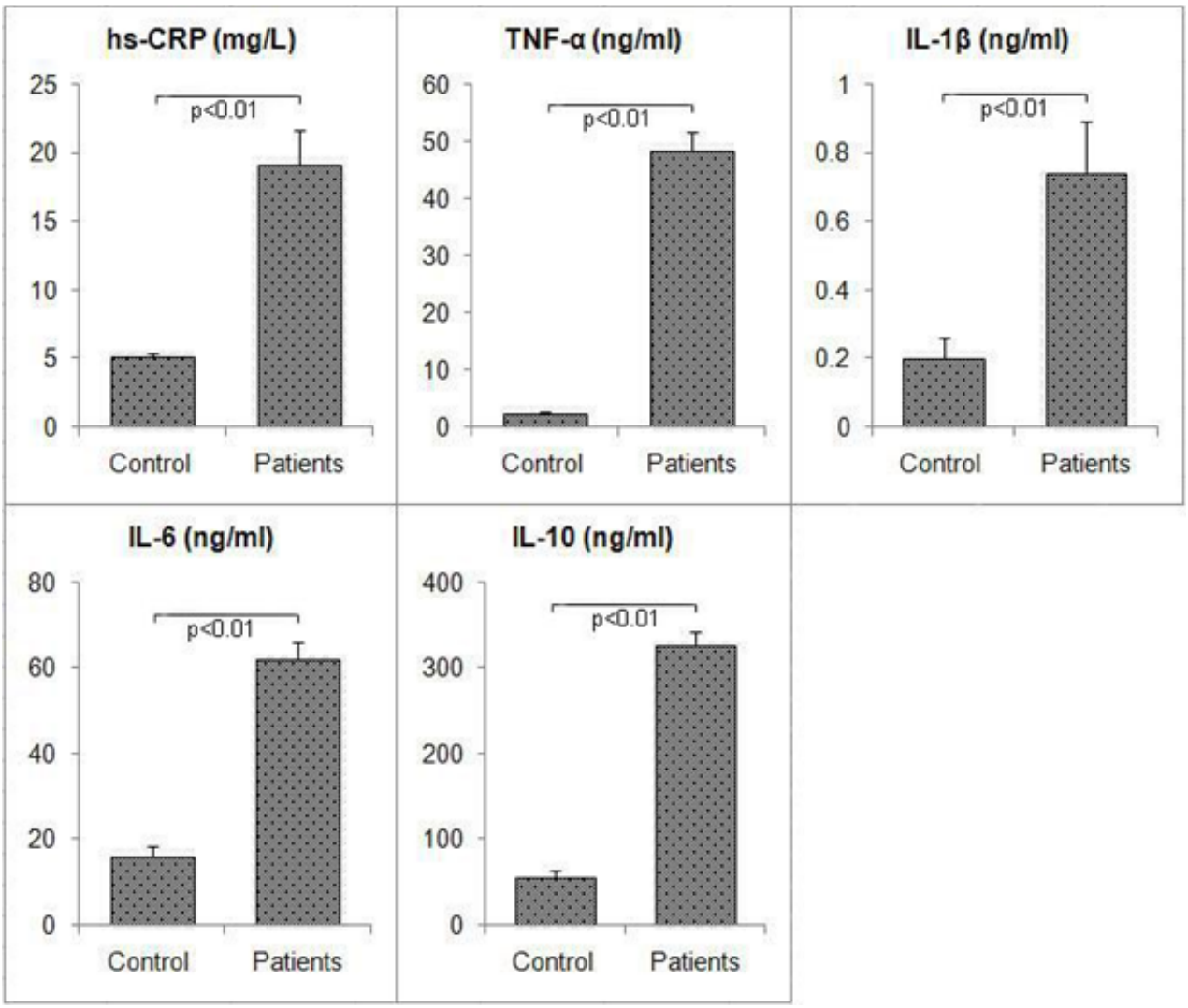

Fig. 2: Trends of inflammatory factors after thrombolytic therapy Note: *vs pre-treatment, $p<0.05$; **vs pre-treatment, $p<0.01$

therapy, the contents of hs-CRP, IL-1β and IL-6 of reteplase group decreased dramatically, which indicated that thrombolytic therapy can significantly reduce the inflammatory reaction. As one of monitoring indexes, hs-CRP has been widely applied to APE progression, though there was a decreasing trend about the content of hs-CRP, IL-1β and IL-6 in plasma after thrombolytic therapy, the content of above-mentioned factors were still higher than the normal content until 24 h after thrombolytic therapy, which reflected the continuance of inflammatory condition after thrombolytic therapy. Therefore, according to the reciprocal relationship between thrombus and the level of inflammation, the clinicians can conjecture the thrombus condition of patient through the level of hs-CRP, IL-1β and IL-6.

CONCLUSION

In summary, reteplase can be adopted to the rescue of high-risk PE patients and based on the hypothesis of supporting inflammation as a key component to the pathogenesis of thromboembolism, levels of hs-CRP, IL-1β and IL-6 in blood may reflect the prognosis of APE.

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