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Comparative analysis of MCP-1 and TF in elderly patients with acute exacerbations of COPD and its clinical significance

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Abstract. – OBJECTIVE: This study was conducted to investigate the variation of monocyte chemoattrant protein-1 (MCP-1) and tissue factor (TF) in elderly patients with acutely exacerbated chronic obstructive pulmonary disease (AECOPD) and their clinical significance.

PATIENTS AND METHODS: Serum specimens were obtained from 49 AECOPD. Patients and 30 health controls, with mean age of 76.1 ± 10.2 and 62.8 ± 6.5 years. Patients in AECOPD group were further grouped into two subgroups, with high or normal procaletonin (PCT) levels. Plasma TE, MCP-1 and PCT were qualified by enzyme-linked immunosorbent assay (ELISA).

RESULTS: TF and MCP-1 were found to be higher in AECOPD patients than in health people ($p < 0.01$), and TF was linearly and positively related to MCP-1. In the subgroups TF was significantly higher in patients with higher PCT than those with normal PCT ($p < 0.05$).

CONCLUSIONS: In AECOPD patients blood cells are activated to hypercoagulation state, particularly when their PCT level is high. Extrinsic pathway activated by TE plays important role in development of the hypercoagulation state. Our results indicate that plasma TF level was positively correlated to the level of MCP-1. This suggests that monitoring of plasma TF and MCP-1 levels in AECOPD patients could be a very useful way to prevent and cure blood hypercoagulability, cardiovascular and cerebrovascular thrombotic diseases.

Key Words:
Chronic obstructive pulmonary disease, Tissue factor, Monocyte chemoattractantprotein-1, Procalcitonin.

Introduction

Patients with chronic obstructive pulmonary disease (COPD) have been well demonstrated to accompany with hypercoagulability[1,2], and severe-pulmonary complications, such as venous thromboembolism and acute coronary syndrome, which causing high mortality in COPD patients. Monocytechemoattractant protein[1], MCP-1 is protein consisting of 76 amino acid, secreted specifically from monocytes and macrophages. The chemokine stimulates the secretion of a number of cell factors and inflammatory mediators, and is engaged in reperfusion-induced ischemia[3]. Tissue factor (TF) is the major regulator of blood coagulation and hemostasis. COPD is often accompanied by chronic or acute exacerbation of abnormal inflammatory reactions, leading to increased flow limitation.

Inflammatory reactions will damage lung endothelial cells, activate TE pathway to induce blood coagulation, resulting in microthrombus formation and acute venous thromboembolism events.

Due their close relationship with inflammatory reactions and blood coagulation[4,5], we measured the level of TF and MCP-1in plasma of elderly patients with acutely exacerbated chronic obstructive pulmonary disease (AECOPD), and investigate the correlations of these two factors in AECOPD and its potential clinical applications.

Patients and Methods

This was a single-center cross-sectional study. We sought to evaluate the relationships between MCP-1 and TF in elderly AECOPD patients and their clinical significance.

Patients were selected from AECOPD patients admitted in Emergency Department, Tongren Hospital, affiliated to Capital Medical University, Beijing, between May 2009 and December 2011, as well as healthy volunteers. AECOPD was confirmed according to the chronic obstructive pulmonary disease treatment guidelines issued by

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Chinese Society of Respiratory Disease (2007)[6]. We excluded patients with history of cancers, diabetes mellitus and blood clots. Patients selected had well functioned important vital organs and were free of thrombotic diseases in hearts, brains and lower limbs. Healthy volunteers were selected from patients attending the above mentioned hospital. They were examined to be free of heart, brain, liver, kidney, lung diseases and other diseases, nor cancer, diabetes mellitus, dyslipidemia, high blood or family history of blood hypercoagulation. All selected subjects had not administered any medicines that might affect platelet and clotting factors during the study.

Molecular analysis was performed in the Department of Emergency. Freshly collected venous blood from fasting patients was used. TF and MCP-1were assayed by ELISA using human TF kit (American Diagnostica, Greenwich, CT, USA) and MCP-1 assay kit (Boster, Wuhan, China). The measurements were carried out with a microplate reader Elx800 (Bio-Tek, Winooski, VT, USA). For PCT (procalcitonin) determination, 3 ml of venous blood was collected and spin for 15 min at 2000 r/min to separate serum. The samples were kept at –20°C for subsequent analysis. PCT was assayed using a VIDAS Immunofluorescence analyzer (bioMerieux, Marcy I'Etoile, France) according to manufacturer's instruction. All measurements were conducted in three replicates.

Patients were interviewed and measured for the following variables: ages; COPD disease history (years, if any); gender; smoking history; systolic blood pressure (SBP); diastolic blood pressure (DBP); blood glucose (Glu) and creatinine (Scr) levels.

Statistical Analysis

In statistical analysis, the patients were grouped into AECOPD and control groups, and for AECOPD groups, patients were further divided into two subgroups high PCT and normal PCT with PCT of 0.5 ug L^{-1} as cutoff.

All statistical analyses were performed with the IBM SPSS Statistics software package, version 19.0 (IBM Corporation, Armonk, NY, USA). All tests were two-tailed and the significance was set at $p < 0.05$.

Descriptive analysis was performed, quantitative data being presented as mean ± SD or median (interqurtile range). Qualitative data were expressed as n (%).

Comparison between different groups was performed by ANOVA followed by the *t*-tests for AECOPD groups and control, and SNK-q tests for AECOPD subgroups. TF as dependent variable and MCP-1 as independent variable were analyzed for linear regression.

Results

Between May 2009 and December 2011, we evaluated 79 AECOPD patients and controls. All the patients completed the study protocols.

Table I shows the general characteristics of the patients. Mean ages in AECOPD group and control were 76.1 ± 10.2 and 62.8 ± 6.5 years, and they were 79.8 ± 4.8 and 72.9 ± 9.1 years in high PCT (PCT ≥ 0.5 ug L^{-1}) and normal PCT (PCT < 0.5 ug L^{-1}) groups, respectively, not significantly different each other. Among them 43.3% to 50% were smokers, with mean SBP of 123 mmHg (range 120-126 mmHg), DBP of 79 mmHg

Table I. General characteristics of the patients diagnosed to be AECOPD and control[1].

Variables	Groups				T or Chi square	*p* value
	Control (n=30)	AECOPD (n=49)	High PCT (n=19)	Normal PCT (n=30)		
Age, year	62.8 ± 6.5	76.1 ± 10.2	79.8 ± 4.8	72.9 ± 9.1	0.723	0.312
Male, %	50.0	71.4	52.6	53.3	0.459	0.058
Smoking, %	43.3	51.0	52.6	50.0	0.821	0.256
SBP (mmHg)[a]	120 ± 12	126 ± 13	124 ± 13	135 ± 15	0.967	0.362
DBP (mmHg)	76 ± 8	82 ± 9	80 ± 9	88 ± 10	0.915	0.402
Glu (mmol .L^{-1})	5.82 ± 0.61	6.40 ± 1.02	6.67 ± 1.09	7.58 ± 1.27	0.936	0.137
SCr (μmol .L^{-1})	64.4 ± 10.5	62.1 ± 9.3	69.3 ± 2.5	70.6 ± 3.6	0.634	0.359

[a]1 mmHg = 0.133 kPa; Smoking: Smoking history; SBP: systolic blood pressure; DBP: diastolic blood pressure; Glu: blood glucose; SCr: serum creatinine,Values expressed as mean ± SD.

(range, 76-82 mmHg), Glu of 6.21 mmol.L^{-1} (range, 5.82-6.40 mmol.L^{-1}) and Scr of 63.25 μmol.L^{-1} (range, 62.1-64.4 μmol.L^{-1}) in control and AECOPD patients. These parameters between and the groups and between subgroups were not significantly different.

Table II compares the TF and MCP-1 levels between AECOPD and control. The results show that mean TE level in AECOPD group (203.6 ± 92.93 ng.L^{-1}) was significantly higher than that in control (136.9 ± 24.3 ng.L^{-1}). Mean MCP-1 level was significantly higher in AECOPD (152.8 ± 99.9 ng.L^{-1}) than in control group (87.5 ± 41.5 ng.L^{-1}).

Compares TF and MCP-1 levels between high PCT and normal PCT patients in AECOPD group. Mean TF levels in high and normal PCT groups were 215.3 ± 71.2 and 192.4 ± 79.3 ng.L^{-1}, respectively, and were significantly different each other. Mean MCP-1 levels in high PCT group were significantly higher than in normal PCT groups (181.1 ± 61.6 vs. 137.3 ± 74.4 ng.L^{-1}) (Table III).

In Table IV we further analyzed the linear regression between TF and MCP-1 separately in the three groups, using TF as dependent variable and MCP-1 as independent variable. Statistically significant positive linear correlations were found between the two variables in all the three groups.

Table II. Comparison of TF and MCP-1 in AECOPD and control groups[1].

Variables	Groups		p
	AECOPD (n=49)	Control (n=30)	
TF (ngL^{-1})	203.6 ± 92.9*	136.9 ± 24.3	0.0021
MCP-1 (ngL^{-1})	152.8 ± 99.9*	87.5 ± 41.5	0.0045

[1]Values expressed as mean ± SD; *Donates figures different from control at $p < 0.01$ level.

Discussion

Recent studies have shown that inflammation mediates the development of thrombosis through a number of steps, in which monocytes, endothelial cells, platelets as well as a variety of other inflammatory factors are involved. Of them, the interactions between inflammation and the TF have been studied extensively. TF exists in vascular endothelial cells, myoblast cells, myofibroblasts, monocytes and tumor cells. Under normal physiological conditions, endothelial cells and monocytes do not secrete TF. However, once activated by bacterial toxins or immune complexes, TF could be expressed and secreted TF into blood. It has been shown that when complexed with FVII, TF could convert inactive FVII to active FVIIa, and FX to FXa, to change fibrinogen into fiber protein cascading extrinsic route of blood coagulation. When binding to FVIIa, TF will change FIX to FIXa to function as co-factor for FX activation, which is involved in intrinsic route of blood coagulation[7]. Therefore, increased TF level has been regarded as an indicator of vascular endothelial injury and blood hypercoagulability[8].

AECOPD is acute event occurring in the normal courses of COPD. It is characterized by difficulty in breathing, coughing and/or sputum aggravated beyond the normal daily variations. When it happens, changes in the conventional medication are often recommended[9]. Bacterial infection is believed to be the major causes of COPD exacerbation. Studies have shown that 80% of AECOPD are resulted from respiratory infections in low respiratory tract and 20% from non-infectious factors such as environmental factors and poor medication compliance. PCT is produced as a result of stimulation by bacterial toxins and inflammatory cytokines. Serum PCT

Table III. Comparison of TF and MCP41 in high and normal PCT groups.

	Groups		p value
	High PCT (N = 19)	Normal PCT (N = 30)	
TF (ng.L^{-1})	215.3 ± 71.2	192.4 ± 79.3	0.035
MCP-1 (ng.L^{-1})	181.1 ± 61.7	137.3 ± 74.4	0.024

Note: High PCT: patient with PCT ≥ 0.5 Mg.L^{-1}; Normal PCT: patient with PCT < 0.5 Mg.$^{-L}$.

Table IV. Correlation analysis of TF and MCP-1 in AECOPD groups.

Groups	N	*R* value	*p* value	95% CI
AECOPD	49	0.67295	0.029	0.19879-0.91287
High PCT	19	0.73856	0.009	0.58349-1.08013
Normal PCT	30	0.65386	0.038	0.39472-0.89352

Note: High PCT: patient with PCT ≥ 0.5 ug.L^{-1}; Normal PCT: patient with PCT < 0.5 ug.L^{-1}.

is generally not high when there is no bacterium infection[10], and it has been shown to be directly correlated to the severity of the infection[11]. Luyt[12] reported that at 0.5 μg L^{-1} cutoff value, PCT's sensitivity and specificity are 72% and 24% for the diagnosis of ventilator associated pneumia. Daubin et al[13] proposed that antibiotics be administered for low respiratory tract infection when PCT is higher than 0.1 μg L^{-1}. Currently, it is generally agreed that no antibiotics treatment is necessary if serum PCT is 0.25 μg L^{-1} or less, even when sputum culture is positive. These bacteria can be considered as colonized bacteria[14]. Taking consideration of these studies as well as the PCT values used in our hospital, we used PCT value of 0.5 μg L^{-1} as the cutoff to group the patients in this study.

Our study shows that serum TF levels in AECOPD patients were significantly higher than those in healthy controls, indicating that there are great risks for endothelial cell injury and blood hypercoagulation in COPD patients. These may be resulted from long term and chronic pathogenic damage along with acute disease exacerbation. During the acute exacerbation, the patient's airflow obstruction gets aggravated. This is mainly caused by inflammation in small airways and lung systemic inflammatory syndrome. It is well known that bacterial infections often result in systemic inflammatory response syndrome that activates inflammatory cells and structural cells, leading to the secretion of a variety of cytokines and inflammatory mediators. In addition, lung hypoxia will directly or indirectly generate vascular endothelial injury, resulting in secretion of TF into blood, thereby, remarkably increasing plasma TF in COPD patients.

It has been demonstrated that blood cell TF is crucial for the activation of the TF pathway. Monocytes are commonly recognized cells that can synthesis TF, while weither other blood cells can synthesize TF is still controversial[15]. Aggregation and functionality of monocytes are regulated by MCP-1 and other factors. MCP-1 is an important member in chemotactic factor CC subfamily, which is primarily produced by monocytes, epithelial cells, fibroblasts and other cells. It can chemotactically elicit and activate T cells, monocytes, and basophils cells to engage in inflammatory process. In this study, we found that TF and MCP-1 in AECOPD patients were positively correlated, and were significantly higher than in control. This implies that the increased TF levels were associated with MCP-1 levels in AECOPD patients, probably due to the increased chemotactic activity. Increased chemotactic activity of MCP-1has been shown to promote the aggregation of monocytes *in vivo* inducing monocytes to secrete TF into the blood[16]. Our study shows that MCP-1levels were higher in AECOPD patients with high PCT than with normal PCT. This is in consistent with previous report[17]. Elevation of serum MCP-1 may be due to their increased secretions from lung epithelial cells or monocytes. This implies that MCP-1 may modulate inflammatory processes to induce and aggravate the formation of blood clots.

Intra-alveolar thrombin formation and fibrin deposition in AECOPD patients are pathological hallmarks of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). The activation of TF is a critical event that results in thrombin formation. Thrombin is a key intermediate molecule with several biological functions including augmentation of vascular permeability and enhancement of inflammation. Thrombin generation leads to fibrin polymerization and deposition with resultant formation of hyaline membranes, a pathological hallmark of ARDS. In the systemic vascular space, TF procoagulant activity is balanced by its natural inhibitor, tissue factor pathway inhibitor (TFPI). TFPI is a Kunitz-type serine protease inhibitor and is the only endogenous specific inhibitor of TF that has been described. In the absence of TFPI, the increased TF as seen in AECOPD patients may coagulate blood cells through the TF pathway. It can also

induce the expression of MCP-1 to enhance inflammation, resulting in infernal circle between inflammation and hypercoagulation, which is further exacerbated by platelet aggregation, resulting in local thrombosis[18].

Conclusions

TF and MCP-1 in AECOPD patients have been associated with high blood coagulation and thromboembolism, as well as high mortality. Clinically, improving hypercoagulation should be given special attention, especially in patients with elevated PCT. Our study demonstrates that monitoring of blood TF and MCP-1 levels, along with reducing the hypercoagulable state in COPD patients, are particularly useful to the prevention and treatment of thrombotic cardiovascular and cerebrovascular diseases in these patients in a long run with better outcomes.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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