

The prognosis value of C-reactive protein and endothelin-1 in chronic obstructive pulmonary disease patients with pulmonary artery pressure

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Abstract: Chronic Obstructive Pulmonary Disease (COPD), which is caused by various proinflammatory cytokines, will present some kinds of complication with progression such as Pulmonary Artery Hypertension (PAH). CRP (C-Reactive Protein) and ET-1(Endotheline-1) play pivotal function in inflammatory responses. The aim of this study is to investigate the prognosis value of CRP and ET-1 in COPD patients with pulmonary artery hypertension. CRP and ET-1 were measured in the plasma, sputum and exhaled breath condensate (EBC) of 55 COPD patients, 75 COPD patients with PAH and 57 healthy controls. Then, we analyzed the influence of these two proteins on the lung function, pulmonary artery pressure, exercise capacity and other clinical indices. The amount of CRP and ET-1 were the highest in COPD patients with PAH, followed by the COPD patients and the healthy controls. And there were statistically significant differences ($p < 0.05$). The amount of CRP and ET-1 were negatively correlated with the lung function and exercise capacity, positively correlated with the pulmonary artery pressure. CRP and ET-1 are two valuable indicators in the diagnosis of COPD patients with PAH in clinic.

Keywords: Chronic obstructive pulmonary disease; proinflammatory cytokines; c-reactive protein; endotheline-1; pulmonary artery hypertension

INTRODUCTION

Smoking and infection are the two major causes of COPD which usually accompanies some complications such as pulmonary artery hypertension (PAH), leading to the fifth mortality and low life quality all over the world (Wu *et al.*, 2011). The COPD patients with PAH always have a worse survival and accelerated risk of hospitalization (Pauk *et al.*, 2013). So it is necessary to pursue valuable prognosis markers to diagnose COPD with PAH effectively and simply. COPD is highly associated with the imbalance of oxidant and antioxidant (Ekstrom, 2014), the imbalance of protease and anti-protease (Pant *et al.*, 2009), and it is also correlated with apoptosis and inflammation throughout the whole body. In the progress of COPD, PAH caused by the right ventricular dysfunction is a pretty common complication all over the world (Pauk *et al.*, 2003; Wu *et al.*, 2011).

In COPD patients, the acute phase protein are highly synthesized such as C-Reactive Protein (CRP), fibrinogen and IL-6 and other inflammatory cytokines are dramatically increased throughout the whole body such as TNF- α , LPS binding protein and so on (Broekhuizen *et al.*, 2006; Brightling *et al.*, 2015; Li *et al.*, 2014; Tashkin, 2015). A great number of amino acids are needed to synthesize CRP in COPD with PAH patients, there is abundant CRP in smooth muscle cells leading to the accelerated degradation of the muscle fibrous proteins and

then decreased motor ability (Salvi, 2015; The Lancet Respiratory, 2015).

In the late severe COPD patients accompanied by PAH complication, they have a poor outcome and all of the symptoms are mainly caused by the pulmonary vascular constriction responding to the hypoxia (Matthay and Shub, 1990; Palasiewicz *et al.*, 1996). When the poison particles in the smoke travel into the pulmonary vascular, the abnormal changes are happened to the vascular systolic, diastolic and vascular cells proliferation, and finally resulting into the pulmonary vascular remodeling. There is abundant Endotheline-1 (ET-1) in the plasma, sputum and urine of the COPD patients, otherwise the concentration and distribution of ET-1 in COPD patients with PAH needs further investigation (Kaparianos *et al.*, 2011).

Cardiovascular disease is one major cause of the high mortality of COPD, CRP and ET-1 are the two principle indicators in the COPD exacerbation (Colice, 2010; Nillawar *et al.*, 2013; Virchow, 2005), so it is meaningful in clinic to measure and analyze CRP and ET-1 using a systemized and high sensitivity way. The aim of this study is to investigate the prognosis value of CRP and ET-1 in COPD with PAH and to further demonstrate the pathogenic roles of these two factors.

MATERIALS AND METHODS

In this study, we recruited 55 COPD patients, 75 COPD with PAH and 57 healthy controls. All of the diagnosis are

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according to the guidelines in the diagnosis handbook of COPD published in 2007. The content of CRP and ET-1 in plasma, sputum and exhaled breath condensate was measured by immune non equilibrium method; the pulmonary function was tested by the AS-407 apparatus made in Japan; The pulmonary artery pressure was determined by the doppler sonography; The pulmonary volume was detect by N₂ and the FEV1 and FVC were recorded accordingly. And then evaluate the correlation of CRP and ET-1 and the severity of COPD with PAH as well as motor ability. All of the dataset were conducted by the SPSS19.0 software, t-test was used to compare the difference between two groups, and p<0.05 or p<0.01 was indicated as significance. This study is approved by the First Affiliated Hospital of Xinjiang Medical University Ethics Committee.

RESULTS

The CRP and ET-1 are much more highly distributed in the plasma, sputum and EBC in COPD with PAH who are more prone to hypertension

We compared the baseline characteristics of 55 COPD, 75 COPD with PAH and 57 healthy controls and found that smoking is highly associated with COPD and COPD accompanied by PAH, the lower BMI index indicated that short stature and the weight loss are the main characters of COPD with PAH. But surprisingly, there is no difference in pulmonary function demonstrated by the FEV1 and FVC. The concentration of CRP and ET-1 is much higher in the plasma, sputum and EBC of COPD with PAH than that of COPD patients (table 1). These results demonstrated that CRP and ET-1 have prognosis value in COPD with PAH patients and can be used as ideal indicators in clinic.

Raised CRP and ET-1 levels are highly correlated with weaker pulmonary function and lower BMI index in older male smokers

We compared the clinical parameters between patients with normal and raised CRP and ET-1 levels and found that the raised CRP and ET-1 levels are highly correlated with weaker pulmonary function and lower BMI (body mass index) index in older male smokers, and the difference between normal and raised CRP and ET-1 levels is significant (table 2). So we postulated that raised CRP and ET-1 are two major causes of COPD with PAH patients, and these two factors will be considered as the ideal therapeutic targets.

The COPD with PAH patients presented with raised CRP and ET-1 levels have a much weaker exercise activities than those with normal CRP and ET-1 levels

We compared the exercise parameters in COPD, COPD with PAH and normal controls with normal and raised CRP and ET-1 levels and found that the COPD accompanied by PAH patients with raised CRP and ET-1

levels have a much weaker exercise activities when compared with those with normal CRP and ET-1 levels, and there is a significant difference (p<0.05) (table 3). The workload and load in patients with raised CRP level are 52.7 (23.9) and 1.09 (0.49), otherwise 55.3 (20.5) and 1.17 (0.39) are presented in those with raised ET-1 level respectively and the difference is significant (p<0.05). The inhaled O₂ volume and exhaled CO₂ volume remain almost the same in patients with normal and raised CRP and ET-1 levels, and the detailed explanation needs further investigation. There is no difference in RER (respiratory exchange ratio). The duration time is much shorter in the patients with raised CRP (7min) and ET-1 (9 min) than those with normal CRP and ET-1 (15min and 19 min). But efficiency is slightly changed in the normal and raised groups. The 6-min walking distance is much shorter in patients with raised CRP (369m) and ET-1 (352m) levels than those with normal levels (457 m and 521m). These results further demonstrated that CRP and ET-1 have high prognosis value in COPD with PAH, and shed some lights on the improved strategies in therapeutic targets.

DISCUSSION

ET-1 regulates vasoconstriction and remodeling, both are manifested in PAH and COPD with PAH (Tuder, 2014). Although there were some investigations about the pivotal roles of CRP and ET-1 in COPD or PAH as mentioned above, the prognosis value of CRP and ET-1 in COPD with PAH remains elusive and needs further illustrated.

COPD is highly associated with PAH in the pathogenesis. NO, synthesized by the epithelial cells, is the major stimulator of the pulmonary artery systolic and diastolic. The synthesis of NO will consume O₂ and the process is interrupted in the COPD patients just due to the hypoxia, and eventually resulting into the PAH caused by the chronic hypoxia (Pauk *et al.*, 2013; Wu *et al.*, 2011). ET-1 is mainly synthesized by the endothelial cells but only a small part is released by the epithelial cells of the respiratory tract inducing the bronchitis contraction (Kaparianos *et al.*, 2011).

The typical characteristics of PAH in COPD patients includes right ventricular insufficiency caused by the pulmonary vascular resistance increase, pulmonary vascular remodeling and the changes of thoracic cavities. PAH is grouped into iPAH and hPAH due to the BMPR2 exon deletion or Smad8 mutation and so on, the his to-morphology of PAH includes the intimal fibrosis and intima thickening, the increased proliferation of vascular endothelial cells and smooth muscle cells and finally the thickening of the blood vessel wall or even the intimal lesions as well as vascular constriction. The pulmonary artery remodeling increases the blood vessel resistance and decreases vascular dilation and leads to the right ventricular insufficiency (Johnson *et al.*, 2012).

Table 1: Baseline characteristics of COPD, CoPD with PAH and healthy controls

Characteristics	COPD (n=55)	COPD with PAH (n=75)	Healthy controls (n=57)
Sex (F/M)	55/5	65/10	50/7
Age (years)	65.5(7.1)	70(10.5)	60(9.5)
Smoking, pack years	25(9)	35(10)*	0
BMI, kg/m ²	25.5(4.1)	23.8(5.2)*	27.7(8.5)
FEVI (% predicted)	58.45(1595)	48.37(1032)	89.87(1245)
FVC (% predicted)	70.23(1957)	66.733(13.85)	90.48(1297)
CRP (mg/L) plasma	27	53*	15
sputum	3.1	6.8*	18
EBC	23	49*	14
ET-1(ug/L) plasma	843	1836*	695
sputum	539	1035*	395
EBC	958	2059*	749
Hypertension. N (%)	89(23%)	95(33%)*	35(15%)

*Significant difference compared to COPD group. $p < 0.01$. Definition of abbreviation: COPD, Chronic Obstructive pulmonary Disease; PAH Pulmonary Artery Hypertension; BMI, Body Mass Index; FEVI, Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; CRP.C-reaction protein; ET-1, Endothelin-1; EBC, Exhaled Breath Condensate.

Table 2: Comparison of clinical parameters between patients with normal and raised CRP and ET-1 levels

Characteristics	CRP<3mg/L (n=30)	CRP>3mg/L (n=100)	P value	ET-1<5ug/L (n=15)	ET-1>5ug/L (n=115)	P value
Years of age	60.9(7.8)	63.5(9.1)	<0.001	621(6.8)	62.9(8.6)	<0.001
Male, N (%)	75(55%)	139(65%)	0.005	642(35%)	65.9(73%)	0.008
Current smoker, N (%)	40(27%)	50(35%)	0.0389	50(31%)	62(42%)	0.0452
FEVI, % Pred	41.2(12.9)	412(12.8)	00093	519(118)	51.9(117)	0.0072
FVC, % Pred	89.6(20.9)	853(19.8)	00356	793(213)	78.6(19.7)	0.0328
FEVI/FVC (%)	41.9(10.5)	408(11.8)	00126	508(10.8)	49.9(1.8)	0.0139
BMI, kg/m ¹	25.9(4.1)	235(3.8)	0.0168	293(2.9)	30.8(1.8)	0.0219

Table 3: Exercise Parameters in COPD, COPD with PAH and normal controls with normal and raised CRP and ET-1 Levels

Characteristics	Normal CRP	Raised CRP	P Value	Normal ET-1	Raised ET-1	P Value
Worldoad (W)	65.0(20.8)	52.7(23.9)	<0.001	69.2(22.3)	55.3(20.5)	<0.001
Load/FFM(W/kg)	149(035)	1.09(049)	0.015	1.58(042)	117(039)	0029
Vo, (ml/min)	15.1	16.2	0.053	14.6	15.9	0058
Vco, (ml/min)	14.7	15.9	0.058	15.3	16.2	0.063
RER	0.96	0.92	0.358	0.91	0.97	0.295
Duration time (min)	15	7	0.004	19	9	0.006
Efficiency (%)	15.6	14.9	0.0139	16.2	15.8	00248
6-min walkingdistance	457	369	0.0058	521	352	0.0047

CRP is a well-known marker of acute systemic inflammation and a risk predictor of cardiovascular diseases (Brunner *et al.*, 2008). COPD patients with an increased concentration of CRP in plasma have an impaired metabolic system and exercise capacity (Broekhuizen *et al.*, 2006). It is also active in pulmonary artery hypertension, and PAH patients have a higher CRP levels which is a biological marker of PAH (Maciocia, 2010).

ET-1 is an effective pulmonary angiotensin and suppresses the apoptosis of vascular endothelial cells, fibroblasts and smooth muscle cells. Pulmonary vasoconstriction is regulated by lots of signals includes ET-1 signal, exerting their functions through changing the electrical activity. ET-1 binds to the two GPCR receptors ET_A and ET_B, promotes the pulmonary artery vasoconstriction. ET_A receptors are specifically expressed on human pulmonary artery smooth muscle cells, but ET_B

receptors are universally distributed in capillaries, respiratory smooth muscle cells and pulmonary endothelial cells (Roberts, 2013). In the blood vessels of iPAH patients, there is a high expression of ET-1. The ET_A and ET_B receptors antagonists are the ideal therapeutic medicine for PAH patients, such as bosentan, sitaxentan, ambrisentan and so forth (Guha, 2013; Lim, 2013; Pope, 2013). ET_A signals through RhoA, and ET_B signals through ERK and Akt and ET-1 signal is cross talked with MAPK signal indirectly.

In PAH patients with BMPR2 or Smad8 mutation, the ET_B receptors decrease a lot and the ET_A signal through ERK and Akt increase compensatory. Generally speaking, in PAH patients, both the ET_A and ET_B receptors decreased dramatically, especially the ET_B receptors and so do the ET_B signal mediated by protein kinases in PAH patients with BMPR2 mutation, but there are no significant changes in iPAH patients. Because the receptor expression and signal transduction are varied in different PAH individuals, so special caution will be paid to the PAH patients when choosing the appropriate ET-1 receptor antagonists (Burton, 2011).

In this study, we demonstrated that the raised CRP and ET-1 are two major causes of COPD with PAH patients, CRP and ET-1 have prognosis value which can be used as ideal indicators in clinic and will be considered as the ideal therapeutic targets. Overall, we indicated that CRP and ET-1 are highly distributed in the plasma, sputum and EBC in COPD with PAH patients who are prone to hypertension. Raised CRP and ET-1 levels are highly correlated with weaker pulmonary function and lower BMI index in older male smokers. The COPD with PAH patients presented with raised CRP and ET-1 levels have a much weaker exercise activities than those with normal CRP and ET-1 levels. This study highlights the prognosis value of CRP and ET-1 and paves the way to the therapeutic intervention of COPD with PAH.

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