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EVALUATING THE PLASMA INTERLEUKIN-1 BETA AND INTERLEUKIN-8 LEVELS IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASES FOLLOWING TREATMENT WITH ALLOGENEIC MESENCHYMAL STEM CELL DERIVED FROM UMBILICAL CORD TISSUES AND PLATELET RICH PLASMA

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SUMMARY

Chronic obstructive pulmonary disease (COPD) is a common disease which negatively affects living quality and longevity of patients. Unfortunately, current COPD treatments are not radical. COPD is characterized by persistent neutrophil infiltrations mediated by inflammatory cytokines including interleukin (IL)-1β and IL-8 in the airway. Mesenchymal stem cells (MSCs) have been proven to suppress inflammation, modulate the immune system and can be regenerative. Allogeneic MSCs have been used to treat COPD patients across the world and produced positive clinical outcomes. In this study, we evaluated plasma IL-1β and IL-8 concentrations in 10 stage-D COPD patients before being transplanted with allogeneic MSCs derived from umbilical cord tissues (UC-MSCs) activated by platelet-rich plasma (PRP) and at 4 follow- ups (after 1, 3, 7, 12 months), as well as determined their associations with COPD clinical and sub-clinical parameters. Plasma IL-1β and IL-8 concentrations were measured using Multiplex Immunoassay. We found that plasma IL-8 levels were significantly reduced from 3.2 to 1.9 pg/ml after 12 months of treatment which is accompanied by the notable decrease in C-reactive protein (CRP). Exacerbation episodes were significantly decreased from 3 to 1. Plasma IL-1β levels were positively correlated with IL-8 levels and white blood cell (WBC) levels. Parallel measurements of plasma IL-1β and IL-8 may help assess the disease progression of COPD patients after the treatment with UC- MSCs and PRP. Blockage of IL-1β or IL-8 could be a valid strategy for the prevention and control of COPD.

Keywords: Chronic obstructive pulmonary disease, Umbilical cord, Mesenchymal stem cell, IL-1β, IL-8

INTRODUCTION

COPD is a lethal respiratory disorder with increasing incidence throughout the world. According to The Global Burden of Disease Study, the prevalence of COPD in 2016 was 251 million cases worldwide. Annually, approximately 3.2 million deaths are caused by COPD, which accounted for 5% of global deaths. Of which, 90% of the COPD deaths occur in low- and middle-income countries (World Health Organization 2017). In Vietnam, the incidence of COPD observed in the male population aged 40 and above is 7.1%, whereas this rate in the female population within the same age range is 1.9% (World Health Organization 2017).

COPD is mainly caused by chronic exposure to environmental pollution including noxious gases, particles or cigarette smoke giving rise to an imbalance between protease and antiprotease, dysregulation of oxidant-antioxidant actions and chronic airway inflammation, contributing to irreversible airway damage in terminal bronchioles or alveoli (Zou *et al.* 2017). Airway inflammation further leads to systemic inflammatory response and other COPD-related manifestations. Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) is defined as sudden worsening in airway function and respiratory symptoms in patients with COPD. AECOPD can range from self-limited diseases to severe respiratory failure that requires mechanical ventilation (MacIntyre and Huang 2008). Bacterial infections are the most common causes of AECOPD in highly susceptible patients. Other causes can be viral infection, air pollution and cardiac dysfunction (Sapey and Stockley 2006).

Chronic COPD inflammation is characterized by the release of a plethora of inflammatory cytokines, especially IL-8 and IL-1β. IL-8 is a neutrophil chemoattractant and activator which gives rise to increased intracellular calcium concentration, exocytosis with release of enzymes and proteins from intracellular storage organelles, and a respiratory burst. IL-8 also facilitates leukotriene B4 production in sputum from COPD patients which may contribute to the chemotactic activity. Effector cells such as activated macrophages and T-cells can also produce leukotriene B4, IL-8 and TNF-α capable of maintaining the neutrophilic inflammation. Leukotriene B4 can stimulate polymorphonuclear leukocytes (PMN) to produce more IL-8, creating a feedback mechanism to ensure consistent influx of PMN to inflammatory sites (McCain *et al.* 1994). In addition, IL-1β is upregulated in inflamed airways and induces many cell types to secret other cytokines such as IL-1, -2, -3, -4, -5, -6 and -8 and tumor necrosis factor (TNF). A recent study has shown that IL-1β stimulates the production of IL-8 in healthy human bronchial epithelial cells (Khan *et al.* 2014), thus, promotes neutrophil recruitment and activation. Together with TNF-α, IL-1β induces intercellular adhesion molecule (ICAM)-1 expression on endothelial cells, permitting trans endothelial migration of leukocytes into peripheral tissues (Chung 2001).

Chronic inflammation in COPD is also represented by an increment in C- reactive protein (CRP) level. CRP is an acute inflammatory protein that raises up to 1,000-fold at sites of infection or inflammation and is synthesized primarily in liver hepatocytes (Sproston and Ashworth 2018). Increased serum CRP in individuals with airway obstruction predicts future hospitalization and death from COPD (Dahl *et al.* 2007). Increased levels of IL-8, IL-1β and CRP have been found at COPD exacerbation compared with the stable state (Sapey and Stockley 2006, R.Hammad *et al.* 2015).

Current COPD therapies have been mainly focusing on relieving the symptoms instead of radical treatment. Thus, intensive research has been carried out to develop stem cell therapies which can repair or replace the damaged pulmonary structures which have been damaged by toxic substances from cigarette smoke (CS)

or polluted air, with a focus on mesenchymal stem cells (MSCs). Mesenchymal stem cells (MSCs) are a heterogeneous group of cells that can be harvested from bone marrow, adipose tissue, or the umbilical cord. MSCs can create a local immunosuppressive and tissue renewing microenvironment. Regarding the tissue repair capacity, MSCs have been shown to express Clara cell secretory protein (CCSP), cystic fibrosis transmembrane conductance regulator (CFTR), surfactant protein C, thyroid transcription factor-1 mRNA, which are crucial for lung epithelium architecture (Sueblinvong et al. 2008). Moreover, MSCs secret paracrine factors such as anti-inflammatory prostaglandin E2 (PGE2), transforming growth factor beta (TGF-β) to attenuate T cell-dependent inflammation (Gazdic *et al.*, 2015) while suppressing proinflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, INF-γ and IL-8 (Chung 2001). MSCs have been studied over the last two decades and showed promising clinical results on COPD patients.

The objectives of this study was to (1) evaluate plasma interleukin IL-1β and IL-8 levels and to (2) determine the correlations of these two inflammatory cytokines with the COPD sub-clinical parameters (blood count and biochemistry) and with each other in patients with COPD following UC-MSCs and platelet rich plasma transplantation.

MATERIALS AND METHODS

Patient selection

This was pilot clinical trial carried out at the Center of Internal Respiration, Miliraty Hospital 103 and Institute of Biomedicine and Pharmacy, Vietnam Military Medical University (VMMU). Ten male patients with clinical and laboratory diagnosis of COPD were enrolled in this study which belonged to the subject number KC.10.15/16-20. A control group was not provided because this study focused on evaluating the changes in plasma IL-1β and IL-8 levels of COPD patients before and after treatment.

Qualified patients, after consenting to participate in our study, received an intravenous infusion of standardized mesenchymal stem cells derived from umbilical cord tissues activated by platelet-rich plasma at a dose of 1.5 million cells per kilogram of body weight. After the infusion, patients were monitored at the hospital and periodically re-examined at 1 month, 3 months, 7 months and 12 months.

Patients were transplanted with allogeneic mesenchymal stem cell derived from umbilical cord tissues (UC-MSCs) and platelet rich plasma (PRR) via intravenous infusion according to the protocol approved by Independent Ethics Committee (4805/QD-HVQY). The detailed protocol were described by Phuong *et al.* 2020. All participants (COPD patients and umbilical cord donors) provided written informed consent.

Specimen collection

Blood samples were collected at five time points including (1) before and four scheduled follow-ups at (2) one, (3) three, (4) seven, (5) twelve months after MSCs transplantation. These samples were used to measure plasma IL-1β and IL-8 concentrations, Complete Blood Count (WBC, Neu%, Lymph%) and Blood biochemistry [C reactive protein (CRP)]. Blood was drawn in vacuum blood tube and centrifuged at 1,500 × g for 20 minutes in Eppendorf Centrifuge 5430 (Eppendorf, Germany) within 60 minutes after collection. The plasma supernatants were frozen at -80°C until analysis. COPD exacerbations were collected from medical records.

Measurements of plasma IL-1β and IL-8 levels

Plasma IL-1β and IL-8 concentrations were measured using the ProcartaPlex™ Multiplex Immunoassay in accordance with the manufacturer's protocols (Thermo Fisher Scientific, USA). The assay resembled

sandwich ELISA and can be used to simultaneously detect a large number of analytes (Figure 1). The sample is added to a mixture of color-coded beads which are pre-coated with analyte-specific capture antibodies. The antibodies bind to the target analytes. Biotinylated detection antibodies specifically recognizing the target analytes are added to form an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is added and binds to the biotinylated detection antibodies. Beads were read on a dual-laser flow-based detection instrument Luminex® 200™ (Thermo Fisher Scientific, USA).

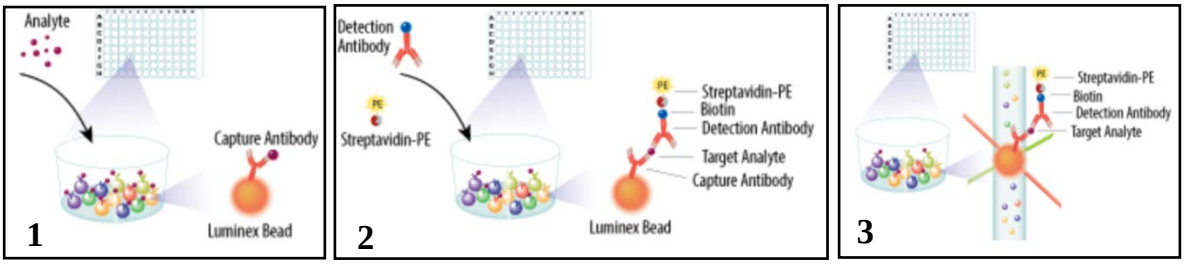


Figure 1. ProcartaPlex™ Multiplex Immunoassay Principle. (1) The sample is added to a mixture of color-coded beads, pre-coated with analyte-specific capture antibodies. The antibodies bind to the analytes of interest. (2) Biotinylated detection antibodies specific to the analytes of interest are added and form an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is added. It binds to the biotinylated detection antibodies. (3) Beads are read on a dual-laser flow-based detection instrument, such as the Luminex 200™ or FlexMap® analyzer. One laser classifies the bead and determines the analyte that is being detected. The second laser determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound. [Adapted from (R&DSystems)].

Statistical analysis

Data was collected and analysed by SPSS version 26.0 (IBM Corporation, Armonk, NY, USA). Data were shown as the mean ± standard deviation (SD). Paired- samples t-test was used to compared plasma IL-1β and IL-8 levels before and after treatment. Correlations between plasma IL-1β and IL-8 levels and blood count as well as blood biochemistry (CRP) were performed using Pearson methods. A two-tailed $p<0.05$ was considered significant.

RESULTS

Changes in plasma IL-1β, IL-8 levels, total blood counts and CRP

The sub- clinical outcomes before and after treatment of COPD patients (n=10) were compared in Figure 2 and Table 1.

The increase in plasma IL-1β level after 12 months of treatment could be explained by the fact that some patients suffered exacerbations at this critical time point, thus, had higher plasma IL-1β levels than stable COPD patients. After 12 months, plasma IL-8 level was significantly reduced by nearly a half which was accompanied by the significant decrease in exacerbation episodes ($p< 0.05$), suggesting a therapeutic effect of MSCs transplantation. Neutrophil levels were significantly reduced after only 1 month of treatment ($p< 0.05$). In the contrary, the notable increase in lymphocyte levels suggested a state of chronic inflammation in COPD patients. We also observed a strong reduction in CRP level after 12 months, indicating that inflammation was notably suppressed ($p< 0.05$).

Changes in plasma IL-1β, IL-8 levels, total blood counts and CRP between three age groups

To primarily assess the beneficial effect of the MSCs transplantation on different age groups, we categorized patients into 3 subgroups: (1) 1 patient aged 50-59, (2) 5 patients aged 60-69 and (3) 4 patients aged 70-79. Figure 3 and Table 2 summarize the results. The plasma IL-1β level at the time point (5) of

the only patient belonged to the 50-59 age group was abnormally high (20.8 pg/ml), indicating a possible exacerbation. The most significantly improved parameter of this patient is the amount of exacerbations, which was reduced from 4 times before the treatment to only 1 time within 12 months post-treatment. However, this may be a case, thus the obtained results were not representative for all the COPD patients at ages of 50-59.

The plasma IL-1β levels in both age groups 60-69 and 70-79 did not change significantly throughout the period. In the other hand, the levels of plasma IL-8 were reduced by 34% (age group 60- 69) and 38.7% (age group 70- 79) compared with those before the treatment, but these reductions were not statistically significant. Patients aged 60- 69 presented significantly improved Neutrophil level (Neu) after 3 months of stem cell treatment ($p < 0.05$). The CRP value were decreased by approximately 70% after 12 months of treatment in both age groups 60-69 and 70-79, however, this reduction is only significant for the latter ($p < 0.05$). Regarding the exacerbations, we also observed a visible decrease in both age groups 60-69 (from 3 to 1.6) and 70-79 (from 3.3 to 1.3) but these results were not statistically significant.

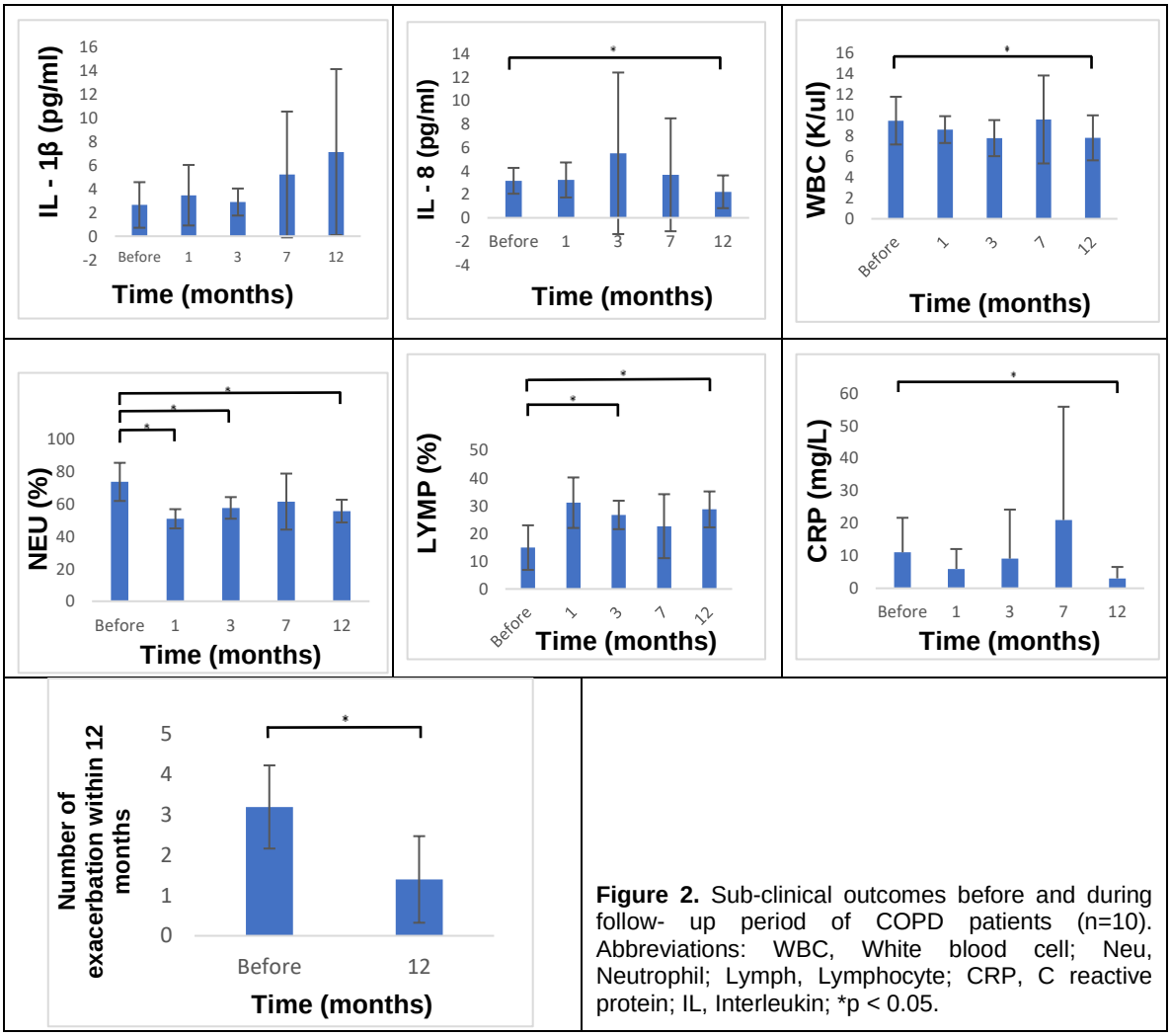
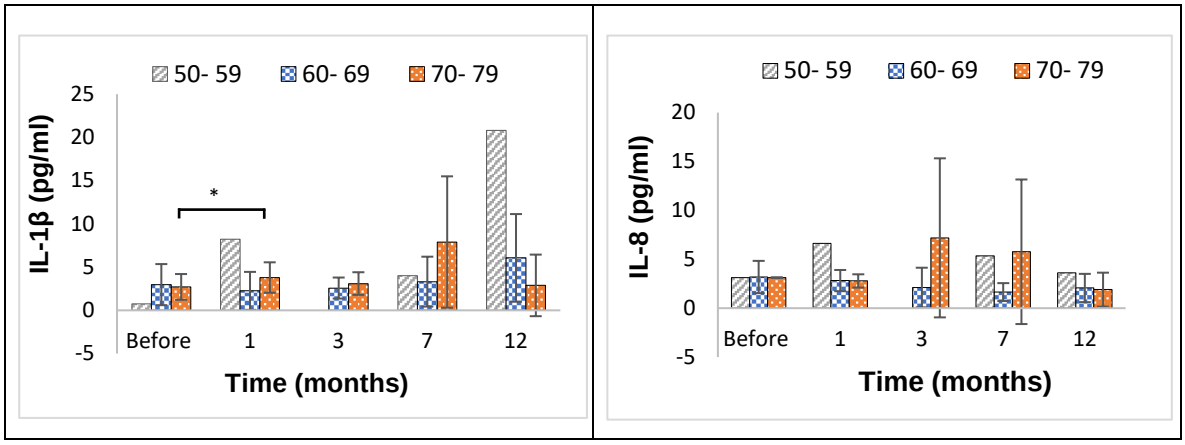


Figure 2. Sub-clinical outcomes before and during follow- up period of COPD patients (n=10). Abbreviations: WBC, White blood cell; Neu, Neutrophil; Lymph, Lymphocyte; CRP, C reactive protein; IL, Interleukin; *p < 0.05.

Table 1. Comparisons in sub-clinical outcomes of COPD patients (n=10) before and after treatment.

Outcome	T_1	T_2	$p_{2,1}$*	T_3	$p_{3,1}$*	T_4	$p_{4,1}$*	T_5	$p_{5,1}$*
Plasma IL-1β and IL-8 concentrations (pg/ml)									
IL- 1β (pg/ml)	2.6± 1.9 (0.7- 7.1)	3.5± 2.6 (0.4- 8.2)	p> 0.05	2.9± 1.1 (1.7- 4.6)	p> 0.05	5.2± 5.3 (0.4- 19.2)	p> 0.05	7.1± 7.0 (0.4- 20.8)	p> 0.05
IL – 8 (pg/ml)	3.2± 2.3 (1.8- 5.9)	3.4± 2.4 (1.3- 6.6)	p> 0.05	7.9± 10.1 (0.7- 19.4)	p> 0.05	3.9± 5.9 (0.2- 16.7)	p> 0.05	1.9± 1.5 (0.1- 3.8)	**p< 0.05**
Blood count and biochemistry									
WBC (10^9/L)	9.5± 2.3 (6.2- 12.6)	8.59± 1.3 (7.2- 10.6)	p> 0.05	7.77±1.7 (5.56-10.8)	p> 0.05	9.6± 4.2 (5- 18.02)	p> 0.05	7.8±2.2 (5.8- 11.4)	**p< 0.05**
Neu (%)	73.7± 11.8 (58,7-92,5)	50.9± 5.9 (41.8-59.7)	**p< 0.05**	57.7± 6.7 (48.8-69.6)	**p< 0.05**	61.6± 17.3 (20.3-83.4)	p> 0.05	55.7± 7.0 (43.8-69.2)	**p< 0.05**
Lymph (%)	14.9± 8.0 (6.1-29.3)	31.1± 9.1 (19.7-44.5)	p> 0.05	27.6± 5.1 (17.2-31.6)	**p< 0.05**	22.6± 11.5 (5.8- 48.4)	p> 0.05	28.7± 6.5 (17.7-40.7)	**p< 0.05**
CRP (mg/L)	11±10.7 (1.3-32.9)	5.9± 6.1 (1.06-15.7)	p> 0.05	9.1± 15.1 (0.6- 49.2)	p> 0.05	20.9±34.9 (0.8-114)	p> 0.05	3.04±3.5 (0.32-12.5)	**p< 0.05**
Exacerba -tions	3± 1 (2- 5)							1± 1.1 (0- 4)	**p< 0.05**

Statistics is median± standard deviation (interquartile range). *p value was calculated based on Paired-samples t test comparing outcomes before treatment with those at each follow-up (1, 3, 7 and 12 months). *Abbreviations:* WBC, White blood cell; Neu, Neutrophil; Lymph, Lymphocyte; CRP, C- reactive protein; IL, Interleukin; T_1, Before treatment; T_2, After 1 month; T_3, After 3 months; T_4, After 7 months; T_5, After 12 months.



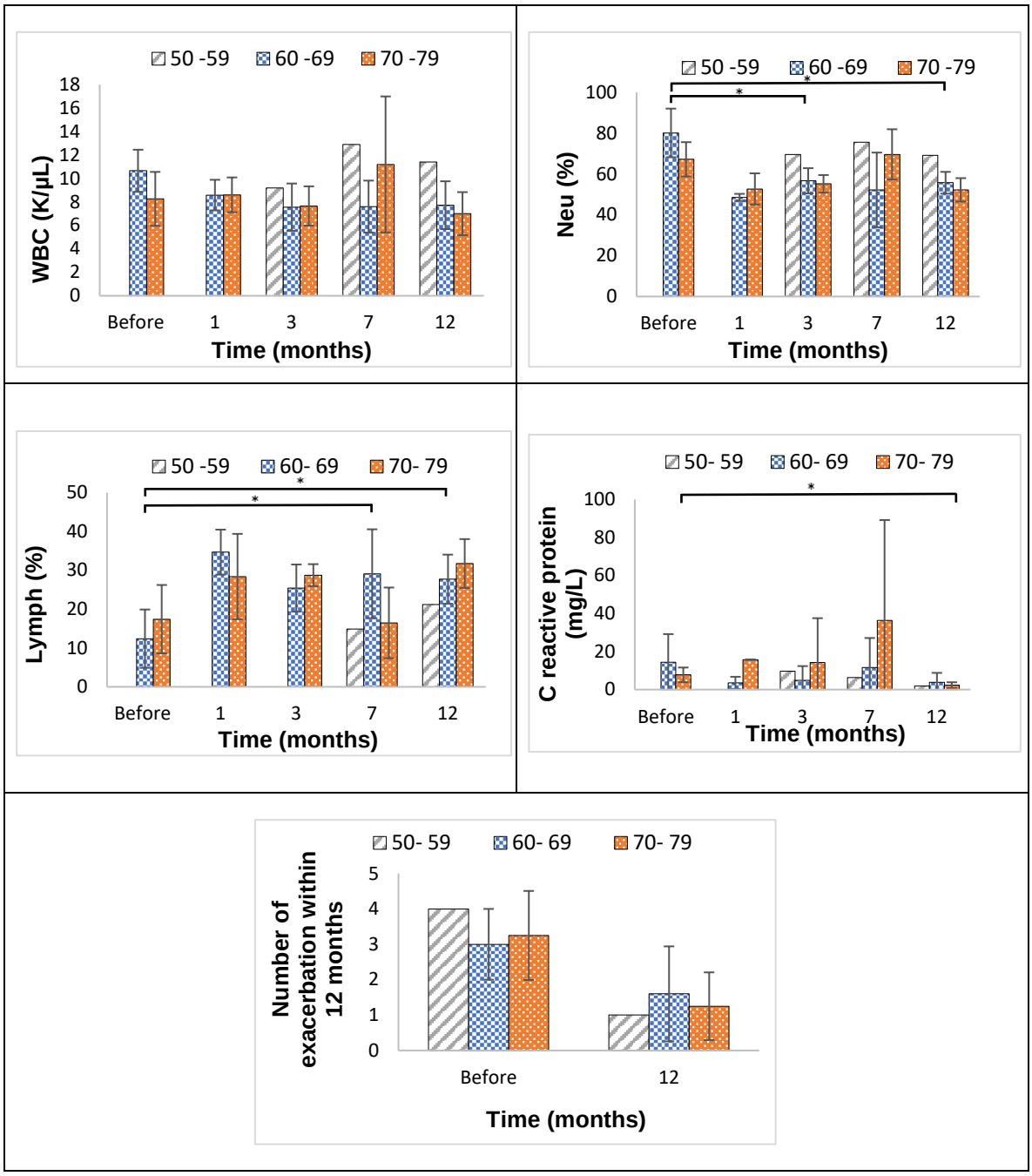


Figure 3. Sub-clinical outcomes before and during follow-up period by age groups; (1) 50-59 years old, (2) 60-69 years old and (3) 70-79 years old. Abbreviations: WBC, White blood cell; Neu, Neutrophil; Lymph, Lymphocyte; CRP, C reactive protein; IL, Interleukin; *p < 0.05.

Table 2. Comparison of sub-clinical outcomes before and after treatment between three age groups: (1) 50-59 years old, (2) 60-69 years old and (3) 70-79 years old.

Outcome	T_1	T_2	$p_{2,1}$*	T_3	$p_{3,1}$*	T_4	$p_{4,1}$*	T_5	$p_{5,1}$*
IL-1β (pg/ml)									
50- 59 (n=1)	0.7	8.2		No data		4		20.8	
60- 69 (n=5)	3.0± 2.4 (1.2- 7.1)	2.3± 2.2 (0.4-5.6)	p> 0.05	2.6± 1.2 (1.7- 3.4)	p> 0.05	3.3± 2.9 (0.4- 7.6)	p> 0.05	6.1± 5.1 (0.5- 14.0)	p> 0.05
70- 79 (n=4)	2.7± 1.5 (1.2- 4.7)	3.8± 1.8 (2.0- 6.2)	**p< 0.05**	3.1± 1.3 (2.2- 4.6)	p> 0.05	7.9± 7.6 (2.8- 19.2)	p> 0.05	2.9± 3.6 (0.4- 5.4)	p> 0.05
IL-8 (pg/ml)									
50- 59 (n=1)	3.1	6.6		No data		5.4		3.6	
60- 69 (n=5)	3.2± 1.7 (1.8- 5.9)	2.8± 1.1 (1.3- 3.8)	p> 0.05	2.1± 2 (0.7- 3.5)	p> 0.05	1.6± 0.9 (0.9- 3.1)	p> 0.05	2.1±1.4 (0.1- 3.8)	p> 0.05
70- 79 (n=4)	3.1± 0 (3.1- 3.1)	2.8± 0.7 (1.8- 3.1)	p> 0.05	7.2± 8.1 (3.1- 19.4)	p> 0.05	5.8± 7.4 (0.2- 16.7)	p> 0.05	1.9± 1.7 (0.7- 3.1)	p> 0.05
WBC (K/µL)									
50- 59 (n=1)	No data	No data		9.2		12.9		11.4	
60- 69 (n=5)	10.7± 1.8 (8.6-12.6)	8.6± 1.3 (7.2- 9.8)	p> 0.05	7.6± 2 (5.6- 10.8)	p> 0.05	7.6± 2.2 (5.0- 11.0)	p> 0.05	7.7± 2 (6.1- 10.0)	p> 0.05
70- 79 (n=4)	8.3± 2.3 (6.2-11.5)	8.6± 1.5(7.3-10.6)	p> 0.05	7.7± 1.7 (6.1- 9.4)	p> 0.05	11.2± 5.8 (6.3- 18.0)	p> 0.05	7.0± 1.8 (5.8- 9.7)	p> 0.05
Neu (%)									
50- 59 (n=1)	No data	No data		69.6		75.7		69.2	
60- 69 (n=5)	80.2±11.9 (67.1-92.5)	48.6± 1.8 (47.3-50.6)	p> 0.05	56.7± 6.2 (48.8-65.2)	**p< 0.05**	52.2± 18.3 (20.3-65.1)	p> 0.05	55.7± 5.3 (51.1-64.7)	**p< 0.05**
70- 79 (n=4)	67.3± 8.4 (58.7-78.6)	52.7± 7.7(41.8-59.7)	p> 0.05	55.2± 4.3(50.3-58.5)	p> 0.05	69.7± 12.3 (55.2-83.4)	p> 0.05	52.3±5.7 (43.7-55.4)	p> 0.05
Lymph (%)									
50- 59 (n=1)	No data	No data		No data		14.9		21.2	
60- 69 (n=5)	12.4± 7.5 (6.1-21.4)	34.7± 5.8 (28.5-40.0)	p> 0.05	25.4± 6.1 (17.2-31.6)	p> 0.05	29.1± 11.4 (17.8-48.4)	**p< 0.05**	27.7± 6.3 (17.7-34.9)	**p< 0.05**
70- 79 (n=4)	17.4± 8.8 (8.0-29.3)	28.4± 11 (19.7-44.5)	p> 0.05	28.8± 2.8 (25.5-30.6)	p> 0.05	16.5± 9.1 (5.8- 27.7)	p> 0.05	31.8± 6.3 (26.8-40.7)	p> 0.05
CRP (mg/L)									
50- 59 (n=1)	No data	No data		9.6		6.3		1.9	
60- 69 (n=5)	14.3± 14.9 (1.3- 32.9)	3.5± 3.2 (1.1- 8.0)	p> 0.05	4.9± 7.4 (0.6- 18.0)	p> 0.05	11.5± 15.6 (0.8- 38.5)	p> 0.05	3.9± 4.9 (0.3- 12.5)	p> 0.05
70- 79 (n=4)	7.7± 3.9 (2.7-10.9)	15.7 (15.7-15.7)	p> 0.05	14.2± 23.3 (1.9- 49.2)	p> 0.05	36.4± 52.7 (2.5-114.1)	p> 0.05	2.3± 1.5 (1.1- 4.6)	**p< 0.05**
Exacerbation episodes									
50- 59 (n=1)	4							1	
60- 69 (n=5)	3.0±1.0 (2-4)							1.6± 1.3 (1-4)	p> 0.05
70- 79 (n=4)	3.3± 1.3 (2-5)							1.3± 1.0 (0-2)	p> 0.05

Statistics is median± standard deviation (interquartile range). *p value was calculated based on Paired-samples t test comparing outcomes before treatment with those at each follow-up (1, 3, 7 and 12 months). *Abbreviations:* WBC, White blood cell; Neu, Neutrophil; Lymph, Lymphocyte; CRP, C- reactive protein; IL, Interleukin; T_1, Before treatment; T_2, After 1 month; T_3, After 3 months; T_4, After 7 months; T_5, After 12 months.

Association of plasma IL-1β or IL-8 levels with blood count and blood biochemistry (CRP levels) in COPD patients

Table 3 showed all possible correlations between blood count, CRP and the plasma levels of IL-1β or IL-8 but only significant correlations ($p< 0.05$ and $p<0.01$) were illustrated in Figure 4. The WBC levels had significant positive correlations with both plasma IL-1β levels ($r=0.833$, $p< 0.01$; Figure 4A and plasma IL-8 levels ($r=0.665$, $p< 0.05$; Figure 4B).

Table 3. Association of plasma IL-1β or IL-8 levels with blood count and CRP.

	IL-1β		IL-8	
WBC (10^9/L)	r= 0.833	**p < 0.01**	r= 0.665	**p < 0.05**
Neu (%)	r= 0.560	p > 0.05	r= 0.441	p > 0.05
Lymph (%)	r= - 0.539	p > 0.05	r= - 0.343	p > 0.05
CRP (mg/L)	r= - 0.343	p > 0.05	r= 0.214	p > 0.05

WBC, White blood cell; Neu, Neutrophil; Lymph, Lymphocyte; CRP, C reactive protein. Data was analysed using Pearson method.

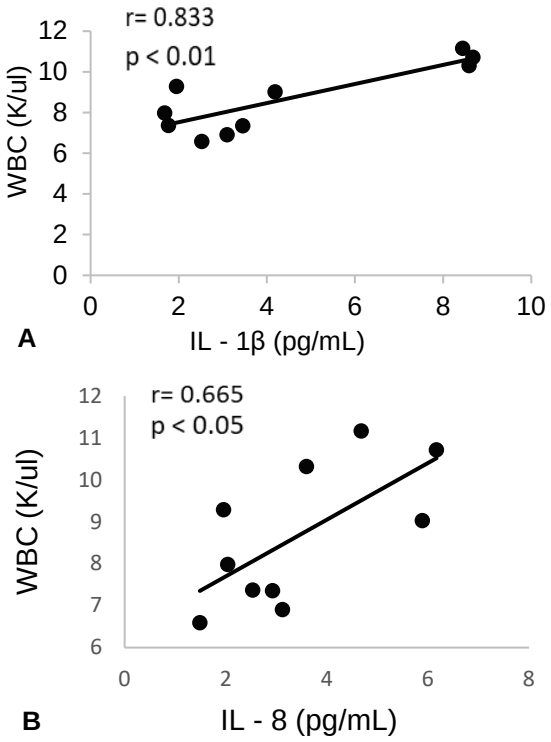


Figure 4. Association of WBC with plasma IL-1β levels (A) and IL-8 levels (B). *Abbreviations*: WBC, White blood cell; IL, Interleukin. Data was analysed using Pearson method.

The correlation between plasma IL-1β and IL-8 levels in COPD patients

There was a significant positive correlation between the plasma IL-1β levels and the levels of IL-8 in COPD patients ($r= 0.716$, $p< 0.05$, Figure 5A). Additional regression analysis demonstrated that IL-1β might be a critical factor giving rise to the elevation of IL-8 in patients with COPD ($R^2= 0.512$, regression coefficient= 0.397, Figure 5B).

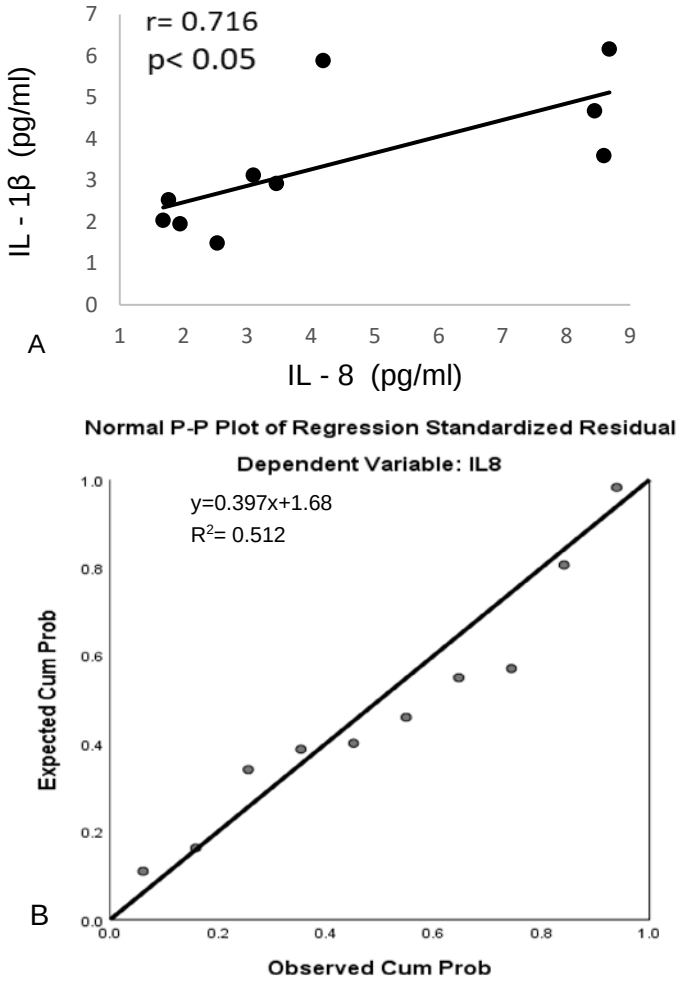


Figure 5. (A) The correlation between plasma IL-1β and IL-8 levels in COPD patients. (B) Regression analysis with IL- 8 as dependent variable. *Abbreviations*: IL, Interleukin.

DISCUSSION

We have obtained some important findings from the study, which are (1) plasma IL-8 levels were significantly reduced in COPD patients after 12 months of MSCs transplantation which is accompanied by the notable decrease in C-reactive protein (CRP), WBC and Neu% ($p< 0.05$) and (2) more prominently, plasma IL-1β levels were positively correlated with IL-8 levels and white blood cell (WBC) levels.

Circulating C-reactive protein (CRP) levels are a biomarker of systemic inflammation and a significant predictor of future chronic obstructive pulmonary disease (COPD) outcome (Deng *et al.* 2014). Thus, the decrease of CRP can be considered as the positive control for the suppression of inflammation in COPD patients after MSCs transplantation. After 12 months, the levels of CRP were significantly reduced which were reflected by the notably decrease in plasma IL-8 levels and the amount of exacerbations, suggesting that MSCs can suppress the activities of inflammatory modulators, thus, repress the disease progression.

Neutrophils can produce and release neutrophil extracellular traps (NETs) in patients with COPD (Wright *et al.* 2016). NETs are web-like structures containing DNA, histones, NE and myeloperoxidase and they can prime macrophages to produce a precursor form of IL-1β (pro-IL-1β). NETs collaborate with another activation signal, such as cholesterol crystals or heat shock protein, to promote the release of IL-1β (Warnatsch *et al.* 2015). In reply, IL-1β initiated and maintained neutrophil airway inflammation in COPD via two main mechanisms: first, IL-1β stimulated normal human bronchial epithelial cells to produce many inflammatory cytokines, such as IL-6 and IL-8 which can facilitate neutrophil recruitment and activation (Khan *et al.* 2014); second, IL-1β could promote the expression of IL-17 in the lung by increasing the number of IL-17–producing T lymphocytes (αβ T cells and γδ T cells) (Warnatsch *et al.* 2015). Therefore, neutrophils enhance the production of IL-1β, consequently facilitates neutrophil recruitment into airways, creating a feedback mechanism that contributes to the continuing growth of COPD. This finding supports our data that both cytokines are positively correlated with WBC levels which include neutrophils in COPD patients.

Interestingly, we observed a significant positive correlation between the plasma IL-1β levels and the levels of IL-8 in COPD patients. IL-1β might be a critical factor giving rise to the elevation of IL-8 in patients with COPD. Therefore, plasma IL-1β or IL-8 maybe an important biomarker to assess the disease progression of COPD patients after the treatment with UC- MSCs and PRP.

The significantly increased level of lymphocytes accompanied by significant reduction of neutrophil level after 3 months of treatment indicated possible viral infections and low probabilities for bacterial infection in some patients as commonly observed (Naess *et al.* 2017). Consistently high lymphocyte level should not be considered as worsening progression of COPD patients after MSCs transplantation since it represented a state of chronic infection which is the main characteristic of COPD (Fleit 2014). Of note, significant decrease in neutrophils levels mirrored by the decrease in exacerbation events notably confirmed the therapeutic effect of MSCs transplantation.

Besides, there are some limitations existing in this study. First, its moderately small sample size was insufficient for the detection of the associations between cytokine markers and most sub-clinical parameters. Second, drugs commonly used for COPD patients such as inhaled corticosteroid and bronchodilators were not examined, thus we could not compare the effect of these drugs on the systemic levels of IL-1β and IL-8 with that of UC- MSCs. If time and condition permits, we will carry on the study with the addition of a control group while recruiting more COPD patients and expanding the examinations on COPD-related inflammatory cytokines other than just IL-1β and IL-8.

CONCLUSION

In this study, we found that plasma IL-8 levels were significantly reduced in COPD patients after 12 months of UC-MSCs transplantation. This decrease was accompanied by the significant reduction in the levels of three pre-clinical parameters (CRP, WBC, Neu%) and exacerbation episodes. Furthermore, plasma IL-1β levels were positively correlated with IL-8 levels and white blood cell (WBC) levels. Parallel measurements of plasma IL-1β and IL-8 may help assess the disease progression of COPD patients after the treatment with UC-MSCs and PRP. Blockade of IL-1β or IL-8 could be a valid strategy for the prevention and control of COPD.

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REFERENCES

Chung KF (2001) Cytokines in chronic obstructive pulmonary disease. *Eur Respir J Suppl* 34: 50s-59s.

Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG (2007) C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 175: 250-255.

Deng ZC, Zhao P, Cao C, Sun SF, Zhao F, Lu CY, Ma HY (2014) C-reactive protein as a prognostic marker in chronic obstructive pulmonary disease. *Exp Ther Med* 7: 443-446.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315-317.

Fleit HB (2014) Chronic Inflammation. Pathobiology of Human Disease. *Elsevier BV***:** 300-314.

Gao F, Chiu SM, Motan DAL, Zhang Z, Chen L, Ji HL, Tse HF, Fu QL, Lian Q (2016) Mesenchymal stem cells and immunomodulation: current status and future prospects. *Cell Death Dis* 7: e2062.

Gazdic, M, Volarevic V, Arsenijevic N, Stojkovic M (2015) Mesenchymal stem cells: a friend or foe in immune-mediated diseases. *Stem Cell Rev Rep* 11: 280-287.

Khan YM, Kirkham P, Barnes PJ, Adcock IM (2014) Brd4 is essential for IL-1β-induced inflammation in human airway epithelial cells. *PLoS One* 9: e95051.

Le Thi Bich P, Nguyen Thi H, Dang Ngo Chau H, Phan Van T, Do Q, Dong Khac H, Le Van D, Nguyen Huy L, Mai Cong K, Ta Ba T, Do Minh T, Vu Bich N, Truong Chau N, Van Pham P. (2020 Allogeneic umbilical cord-derived mesenchymal stem cell transplantation for treating chronic obstructive pulmonary disease: a pilot clinical study. *Stem Cell Res Ther* 11(1):60. doi: 10.1186/s13287-020-1583-4. PMID: 32054512; PMCID: PMC7020576.

MacIntyre N, Huang YC (2008) Acute exacerbations and respiratory failure in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 5: 530-535.

McCain RW, Holden EP, Blackwell TR, Christman JW (1994) Leukotriene B4 stimulates human polymorphonuclear leukocytes to synthesize and release interleukin-8 in vitro. *Am J Respir Cell Mol Biol* 10: 651-657.

Naess A, Nilssen SS, Mo R, Eide GE, Sjursen H (2017) Role of neutrophil to lymphocyte and monocyte to lymphocyte ratios in the diagnosis of bacterial infection in patients with fever. *Infection* 45: 299-307.

R&DSystems Luminex Assay Principle.

Hammad RD, Elgazzar AG, Essawy TS, Sameie SAAE (2015) Evaluation of serum interleukin-1 beta as an inflammatory marker in COPD patients. *Egypt J Chest Dis Tubercs* 64 347-352.

Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, Roberts AI, Le AD, Shi S, Shao C, Shi Y (2010) Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. *J Immunol* 184: 2321-2328.

Sapey E, Stockley RA (2006) COPD exacerbations . 2: aetiology. *Thorax* 61: 250-258.

Sheng H, Wang Y, Jin Y, Zhang Q, Zhang Y, Wang L, Shen B, Yin S, Liu W, Cui L, Li N (2008) A critical role of IFNgamma in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1. *Cell Res* 18: 846-857.

Sproston NR, Ashworth JJ (2018) Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front Immunol* 9: 754.

Sueblinvong V, Loi R, Eisenhauer PL, Bernstein IM, Suratt BT, Spees JL, Weiss DJ (2008) Derivation of lung epithelium from human cord blood-derived mesenchymal stem cells. *Am J Respir Crit Care Med* 177: 701-711.

Sun Z, Li F, Zhou X, Chung KF, Wang W, Wang J (2018) Stem cell therapies for chronic obstructive pulmonary disease: current status of pre-clinical studies and clinical trials. *J Thorac Dis* 10: 1084-1098.

Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V (2015) Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science* 349: 316-320.

World Health Organization (2017) "Chronic obstructive pulmonary disease (COPD)." Retrieved 9 September, 2020, from https://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd).

World Health Organization (2017) "Chronic obstructive pulmonary disease (COPD) in Viet Nam." Retrieved 9 September, 2020, from https://www.who.int/vietnam/health-topics/chronic-obstructive-pulmonary-disease-copd#.

Wright T K, Gibson PG, Simpson JL, McDonald VM, Wood LG, Baines KJ (2016) Neutrophil extracellular traps are associated with inflammation in chronic airway disease. *Respirology* 21: 467-475.

Zou Y, Chen X, Liu J, Zhou DB, Kuang X, Xiao J, Yu Q, Lu X, Li W, Xie B, Chen Q (2017) Serum IL-1β and IL-17 levels in patients with COPD: associations with clinical parameters. *Int J Chron Obstruct Pulmon Dis* 12: 1247-1254. doi: 10.2147/COPD.S131877. PMID: 28490868; PMCID: PMC5413485.