

Original Article

Application of bronchoalveolar lavage combined with ambroxol in elderly patients with severe pneumonia and its effect on serum sTREM-1 level

Jianming Hu¹, Xue Bai¹, Long Li¹, Lihui Ding¹, Jiawei Hu²

¹Department of Respiratory and Critical Care Medicine, The First Hospital of Lanzhou University, Lanzhou 730030, Gansu Province, China; ²Department of Clinical Medicine in Medical College of Jinggangshan University, Ji'an 343000, Jiangxi Province, China

Received June 12, 2020; Accepted August 4, 2020; Epub November 15, 2020; Published November 30, 2020

Abstract: Objective: This study aimed to explore the treatment efficacy of bronchoalveolar lavage (BAL) combined with ambroxol for senile severe pneumonia (SP) and its effect on serum levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1). Methods: A total of 168 patients with SP treated in our hospital from May 2016 to December 2018 were randomized to receive routine treatment and BAL (76 cases, the control group, CG) or to receive routine treatment and BAL combined with ambroxol (92 cases, the research group, RG). The two groups were compared in terms of clinical efficacy, Acute Physiology and Chronic Health Evaluation II (APACHE II) score, recovery of clinical symptoms, markers for lung function, blood gas markers, and serum sTREM-1 level. Results: The improvement in clinical indicators was greater in the RG than in the CG. The improvement in blood gas markers and markers for lung function were greater in the RG than in the CG. The APACHE II score after treatment was markedly lower in the RG than in the CG. The concentration of sTREM-1 after treatment was markedly lower in the RG than in the CG. There was a positive correlation between the concentration of sTREM-1 and the APACHE II score after treatment in the RG. The overall response rate was markedly higher in the RG than in the CG. Conclusion: BAL combined with ambroxol is effective in treating senile SP and promotes the recovery and improvement of lung function. sTREM-1 is a potential reference index to evaluate the improvement of the patient's condition.

Keywords: Bronchoalveolar lavage, ambroxol, pneumonia, sTREM-1

Introduction

Pneumonia attacks 450 million individuals worldwide and causes nearly 4 million deaths each year. As a common reason for admission to the intensive care unit, severe pneumonia (SP), if not properly treated, may rapidly develop into septic shock, respiratory failure, or cause death within a few days [1-3]. SP is the main factor behind the morbidity and mortality in the elderly, what's worse, the number of elderly patients affected by SP is increasing according to epidemiological trends [4, 5]. Existing treatments for SP mainly rely on antibiotics [6, 7]. Development of the social economy in recent years leads to an increased incidence of common basic diseases and accompanying decreased sensitivity to antibiotics in patients [8]. Therefore, it is essential to find practical

and safe treatment therapies for SP with favorable efficacy.

Bronchoalveolar lavage (BAL), is minimally invasive and safe, and it can target the lesion under the view of the fiberoptic bronchoscope to clear the respiratory tract secretions, effectively relieve airway obstruction, and restore airway ventilation [9, 10]. BAL can also selectively drain out viscous secretions and sputum to ensure airway patency [11]. However, routine antibiotics used in patients receiving BAL can easily cause drug resistance, which impairs treatment efficacy. Here we used BAL combined with ambroxol to treat SP patients [12]. Ambroxol is a metabolite of bromhexine widely used clinically for airway diseases or phlegm-dispelling [13]. It can promote bronchial epithelial repair and accelerate the trans-

port of mucosal cilia [14]. Ambroxol has extensive pharmacological anti-inflammatory properties *in vivo* and *in vitro*, such as inhibiting or clearing oxidative stress or nitrosative stress and promoting local defense molecules associated with respiratory virus replication [15]. In the study by Yang Z et al., ambroxol effectively improved the lung function markers in newborns with pneumonia, reduced serum levels of inflammatory mediators, inhibited cell apoptosis, activated the NF- κ B pathway, and enhanced lung function [16]. sTREM-1 is a unique form of TREM-1 clinically recognized as a biomarker for infectious diseases, which can be tested directly in human body fluids to diagnose infectious diseases such as lung infections, pleural effusions, and viral infections and predict prognosis [17, 18].

This study investigated the application value of BAL combined with ambroxol in the treatment of senile SP, tested serum sTREM-1 concentrations before and after treatment, and assessed the efficacy and prognosis of all patients, aiming to provide a better reference for the treatment of SP in elderly patients.

Materials and methods

Basic information

A total of 168 patients with SP treated in our hospital from May 2016 to December 2018 were randomized to receive routine treatment and BAL (76 cases, the control group, CG) or to receive routine treatment and BAL combined with ambroxol (92 cases, the research group, RG). Inclusion criteria: Patients diagnosed with SP [19]; patients with stable vital signs; patients capable of independent thinking; patients with complete clinical data; patients with an expected survival of ≥ 1 year. This study was approved by the Ethics Committee of our hospital. All patients and their family members signed the written informed consent. Exclusion criteria: Patients lost to the follow-up; patients with liver, kidney, or hematopoietic dysfunction; patients with end-stage diseases, multiple organ dysfunction, or in shock; patients allergic to drugs used in this study; patients with severe pulmonary hypertension, impaired central nervous system, history of mental illness, or family history of SP; patients who withdrew from the study midway; patients who left the hospital without permission.

Treatment methods

Patients in the CG were given routine treatment first (conventional anti-infection treatment, nutritional support, postural drainage, and symptomatic treatment). Then patients were treated with BAL. We gave 5 mg of midazolam (Jiangsu Nhwa Pharmaceutical Co., Ltd., Jiangsu, China, China Food and Drug Administration Approval No. H20031037) for sedation, connected the fiberoptic bronchoscope to a negative pressure aspirator, and gave patients an inhalation of high-concentration oxygen for about 5 minutes under ECG monitoring. When the patient's blood oxygen saturation reached 95% or greater, we inserted the fiberoptic bronchoscope through the mouth, nose, or artificial airway, examined the patient's total trachea, trachea segments, and subsegmental bronchi quickly, and sucked out the secretions. Then we gave 15-20 mL of 0.9 sodium chloride solution (Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China, item number: R21092) to irrigate the lesion and sucked the solution 1 minute afterwards. The negative pressure was controlled ≤ 100 mmHg (1 mmHg = 0.133 kPa) during the wash. If the blood oxygen saturation was less than 85%, we stopped the operation immediately and took out the fiberoptic bronchoscope, followed by an oxygen supply. The wash continued when the patient's blood oxygen saturation reached 95% or greater again. Each lung segment of the lesion was washed repeatedly until the lavage fluid was clear (the number of lavages ≥ 3 times, the total lavage volume ≤ 100 ml/time).

Patients in the RG were given ambroxol based on the treatment regimen designed for patients in the CG. We mixed 250 mL of sterile saline with 120 mL of ambroxol (SHANGHAI ZZBIO Co., Ltd., Shanghai, China, item number: IR43676) to wash the lesion. The total amount of lavage fluid was 100-200 mL each time, 2-3 times per week. The treatment for both groups lasted for 1 week.

Outcome measures

Clinical symptoms: We recorded the mechanical ventilation time, time of recovery from atelectasis, time of recovery from abnormal temperature, time of recovery from respiratory failure, and hospitalization time after treatment intervention.

Treatment efficacy of BAL and ambroxol for senile severe pneumonia

Table 1. Basic information of patients [n (%)] (mean \pm SD)

Factors	RG (n = 92)	CG (n = 76)	t/ χ^2	P
Sex			0.872	0.383
Male	52 (56.52)	48 (63.16)		
Female	40 (43.48)	28 (36.84)		
Age (year)	65.79 \pm 11.32	66.83 \pm 11.38	0.591	0.555
BMI (kg/m ²)	24.67 \pm 4.79	25.09 \pm 4.82	0.564	0.573
Time of disease onset (d)	2.21 \pm 0.13	2.27 \pm 0.16	1.663	0.098
Place of residence			0.316	0.573
Urban area	56 (60.87)	43 (56.58)		
Rural area	36 (39.13)	33 (43.42)		
Ethnicity			1.791	0.181
Han nationality	59 (64.13)	41 (53.95)		
Minority nationality	33 (35.87)	35 (46.05)		
Educational degree			0.043	0.834
\geq high school	39 (42.39)	31 (40.79)		
< high school	53 (57.61)	45 (59.21)		
Smoking history			2.276	0.096
Yes	69 (75.00)	48 (63.16)		
No	23 (25.00)	28 (36.84)		
Drinking history			0.300	0.583
Yes	63 (68.48)	49 (64.47)		
No	29 (31.52)	27 (35.53)		
Hypertension history			0.499	0.479
Yes	57 (61.96)	43 (56.58)		
No	35 (38.04)	33 (43.42)		
Diabetes history			0.381	0.536
Yes	54 (58.70)	41 (53.95)		
No	38 (41.30)	35 (46.05)		
Systolic blood pressure (mmHg)	135.42 \pm 19.36	134.70 \pm 19.32	0.240	0.810
Diastolic blood pressure (mmHg)	74.36 \pm 8.87	73.98 \pm 8.85	0.276	0.782
Heart rate (beat/min)	90.43 \pm 11.32	91.36 \pm 11.28	0.530	0.596

Blood gas analysis: At 24 hours before and after treatment, we collected 5 mL arterial blood from each patient, injected it into the heparin cap, and stored the blood sample in a cooling package below 8°C. Then we measured the partial pressure of carbon dioxide (PaCO₂), partial pressure of oxygen artery (PaO₂), and pH value in the blood samples on a blood gas analyzer (Shanghai Yuyan Instruments Co., Ltd., Shanghai, China, item number: 57984).

Lung function markers: We measured the forced vital capacity (FVC), forced expiratory volume (FEV) in the first second (FEV₁), FEV₁/FVC, maximum mid-expiratory flow (MMF), and the peak expiratory flow (PEF) of patients before and after treatment on a lung function test

instrument (Beijing Zeao Medical Technology Co., Ltd., Beijing, China, item number: z00502).

Acute Physiology and Chronic Health Evaluation II (APACHE II) score [20]: The score was assessed based on three indicators, ranging from 0 to 71 points. A higher score indicates a more serious condition.

Concentration of soluble myeloid cell trigger receptor-1 (sTREM-1): We took 5 mL venous blood from the elbow of patients 24 hours before and after treatment and centrifugalized the blood sample at 1500 \times g for 10 minutes at 4°C. Then the sample was stored in a -70°C freezer. Then we detected the concentration of sTREM-1 using the enzyme-linked immunosorbent assay (ELISA) [21] following

Treatment efficacy of BAL and ambroxol for senile severe pneumonia

Table 2. Improvements in clinical symptoms in the two groups (mean \pm SD)

Group	Case number	Mechanical ventilation time (d)	Time of recovery from atelectasis (d)	Time of recovery from abnormal temperature (d)	Time of recovery from respiratory failure (d)	Hospitalization time (d)
RG	92	1.27 \pm 0.21	8.71 \pm 1.09	2.35 \pm 0.34	1.89 \pm 0.26	12.37 \pm 1.21
CG	76	3.19 \pm 0.25	13.67 \pm 1.13	6.79 \pm 0.67	4.62 \pm 0.37	17.86 \pm 1.34
t	-	54.100	28.870	55.520	56.000	27.880
P	-	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Table 3. Blood gas markers in the two groups (mean \pm SD)

Group	Case number	PaCO ₂ (mmHg)		PaO ₂ (mmHg)		PH level	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
RG	92	59.76 \pm 4.68	42.76 \pm 4.37	56.63 \pm 5.04	85.79 \pm 6.89	7.02 \pm 0.27	7.43 \pm 0.31
CG	76	60.03 \pm 4.79	46.98 \pm 4.42	57.12 \pm 5.07	56.03 \pm 6.91	7.04 \pm 0.28	7.31 \pm 0.29
t	-	0.368	6.198	0.625	27.830	0.469	2.571
P	-	0.713	< 0.001	0.532	< 0.001	0.639	0.011

the instructions of the sTREM-1 kit (Shanghai Hengdu Biotechnology Co., Ltd., Shanghai, China, item number: HD38923).

Efficacy: We assessed the efficacy for all patients after treatment, which was divided into three levels: marked response, moderate response, and no response. A marked response referred to normal clinical symptoms and arterial blood gas markers and regular chest radiograph results. A moderate response referred to mitigated clinical symptoms, improved arterial blood gas markers, and alleviated inflammation according to the chest radiograph. No response referred to the absence of notable changes in clinical symptoms and blood gas markers or a worsened condition, and severe inflammation according to the chest radiograph. Overall response rate = (number of cases with marked responses + moderate responses)/total case number \times 100%.

Statistical analysis

Statistical analysis was performed by SPSS 21.0 (EASYBIO, Beijing, China). The count data were represented by the number of cases/percentage [n (%)] and compared between the two groups using the chi-square test. The measurement data were represented by the mean \pm standard deviation (mean \pm SD), which were compared between the two groups using the independent sample t-test and compared within the group using the paired t-test. Pearson correlation coefficient was used for correlation analysis. Data visualization was performed on

GraphPad Prism 6 software. The difference was statistically significant when $P < 0.05$.

Results

Basic information

The comparison between the two groups showed no significant difference in sex, age, BMI, time of disease onset, place of residence, ethnicity, educational background, smoking history, drinking history, hypertension history, diabetes history, systolic blood pressure, and diastolic blood pressure ($P > 0.05$). More details are shown in **Table 1**.

Comparison of improvements in clinical symptoms

After treatment, the mechanical ventilation time, time of recovery from atelectasis, time of recovery from abnormal temperature, time of recovery from respiratory failure, and hospitalization time were markedly shorter in the RG than in the CG ($P < 0.05$). More details are shown in **Table 2**.

Comparison of blood gas markers

The differences between the two groups in PaCO₂, PaO₂, and pH levels before treatment were not significant ($P > 0.05$). After treatment, blood gas markers were improved in both groups ($P < 0.05$), with markedly lower PaCO₂ level and markedly higher PaO₂ and pH levels

Treatment efficacy of BAL and ambroxol for senile severe pneumonia

Table 4. Markers for lung function in the two groups (mean \pm SD)

Group	Case number	FVC (L)		FEV ₁ (L)		FEV ₁ /FVC (%)		MMF (L)		PEF (L/s)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
RG	92	2.03 \pm 0.14	2.57 \pm 0.41	1.31 \pm 0.21	1.84 \pm 0.26	59.86 \pm 4.61	71.04 \pm 5.92	1.36 \pm 0.27	2.94 \pm 0.45	2.67 \pm 0.33	3.17 \pm 0.51
CG	76	2.02 \pm 0.11	2.31 \pm 0.37	1.27 \pm 0.24	1.53 \pm 0.21	59.34 \pm 4.35	65.28 \pm 5.86	1.32 \pm 0.25	2.27 \pm 0.34	2.58 \pm 0.31	2.87 \pm 0.47
t	-	0.506	4.274	1.152	8.378	0.746	6.306	0.988	10.700	1.808	3.931
P	-	0.613	< 0.001	0.251	< 0.001	0.456	< 0.001	0.324	< 0.001	0.072	0.001

Treatment efficacy of BAL and ambroxol for senile severe pneumonia

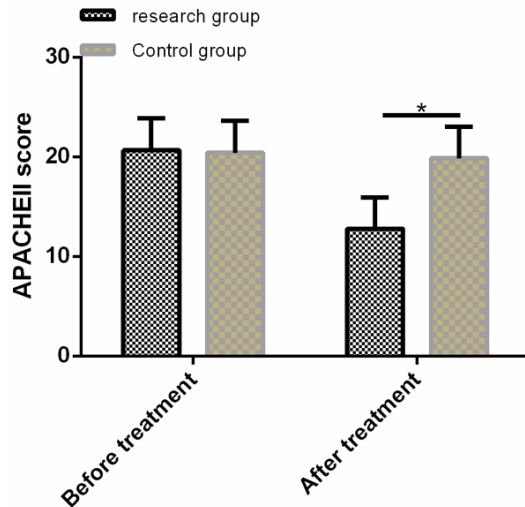


Figure 1. APACHE II scores in the two groups. The two groups were not notably different in the APACHE II score before treatment. After treatment, the APACHE II score was markedly lower in the RG than in the CG. Note: * indicates $P < 0.05$.

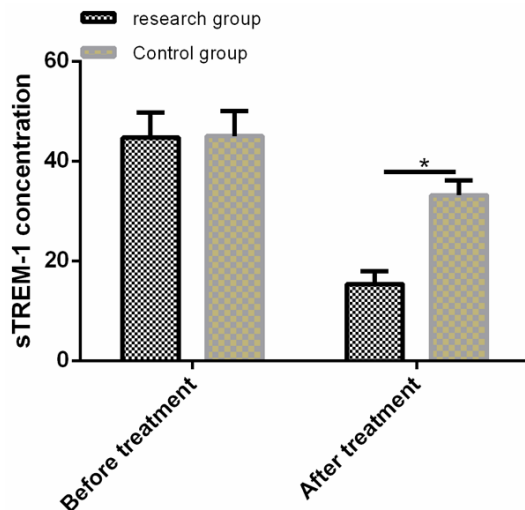


Figure 2. sTREM-1 concentrations in the two groups. The two groups were not different in the sTREM-1 concentration before treatment. After treatment, the sTREM-1 concentration was lower in the RG than in the CG. Note: * indicates $P < 0.05$.

in the RG than in the CG ($P < 0.05$). More details are shown in **Table 3**.

Comparison of markers for lung function

The two groups were not notably different in markers for lung function before treatment ($P > 0.05$). After treatment, markers for lung function were markedly improved in both groups ($P < 0.05$), with markedly higher FVC,

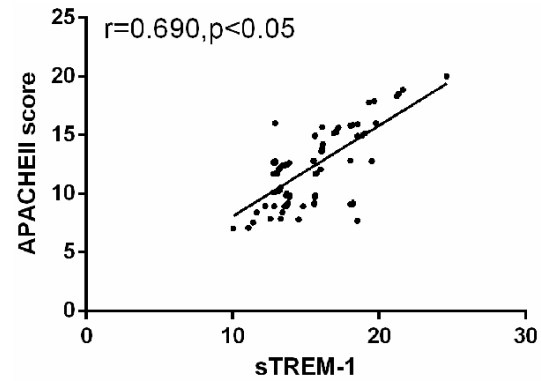


Figure 3. Correlation between serum sTREM-1 concentration and APACHE II score after treatment in the RG. There was a positive correlation between the serum sTREM-1 concentration and the APACHE II score after treatment in RG. Note: * indicates $P < 0.05$.

FEV₁, FEV₁/FVC, MMF, and PEF levels in the RG than in the CG ($P < 0.05$). More details are shown in **Table 4**.

Comparison of APACHE II score

The two groups were not notably different in the APACHE II score before treatment ($P > 0.05$). After treatment, the APACHE II score was markedly lower in the RG than in the CG ($P < 0.05$). More details are shown in **Figure 1**.

Comparison of sTREM-1 concentration

The two groups were not notably different in the sTREM-1 concentration before treatment ($P > 0.05$). After treatment, the sTREM-1 concentration was markedly lower in the RG than in the CG ($P < 0.05$). More details are shown in **Figure 2**.

Correlation between serum sTREM-1 concentration and APACHE II score after treatment in the RG

The results of the Pearson correlation coefficient revealed a positive correlation between the serum sTREM-1 concentration and the APACHE II score after treatment in the RG ($r = 0.690$, $P < 0.05$). More details are shown in **Figure 3**.

Comparison of treatment efficacy

The overall response rate was markedly higher in the RG than in the CG (91.3% vs. 80.26%, $P < 0.05$). More details are shown in **Table 5**.

Table 5. Treatment responses in the two groups [n (%)]

Group	Case number	Marked response	Moderate response	No response	Overall response rate
RG	92	58 (63.04)	26 (28.26)	8 (8.70)	84 (91.30)
CG	76	22 (28.95)	39 (51.32)	15 (19.74)	61 (80.26)
t	-	-	-	-	4.294
P	-	-	-	-	0.038

Discussion

Pneumonia is a major public concern especially for the elderly, with high morbidity and mortality among senior people [22]. Pneumonia in the elderly, has quick onset and a poor prognosis, it can easily develop into SP, which causes a mortality rate of 24% mainly due to impaired respiratory function [23]. The airway secretions increase and get sticky in patients with pneumonia, eventually causing a vicious circle [24]. Damaged airway ventilation and aggravated local airway inflammation resulting from airway obstruction often impairs the effect of antibiotics [25]. Therefore, it is quite crucial to effectively clean up the respiratory secretions in patients and relieve airway obstruction.

Here we used BAL combined with ambroxol to treat elderly patients with SP and found a better prognosis in patients. The study by Wang LP and his team [26] suggests that early alveolar lavage with flexible bronchoscopy can relieve clinical symptoms of children with respiratory diseases and improve the prognosis. The study by Chen F et al. [27] revealed that ambroxol hydrochloride alleviated alveolitis and pulmonary fibrosis in rat models of pulmonary fibrosis, as well as supported anti-infection treatment in the early stages of fibrosis. In this study, the improvements in clinical indicators of patients were markedly greater in the RG than in the CG, indicating that BAL combined with ambroxol can effectively alleviate clinical symptoms in elderly patients with SP, control disease progression, and facilitate recovery. The results of arterial blood gas analysis can intuitively reflect the patient's body acid-base balance, the degree of hypoxia, and the severity of SP [28]. Here we detected markedly lower PaCO₂ level and markedly higher PaO₂ and pH levels in the RG than in the CG after treatment, indicating that BAL combined with ambroxol can improve the alveolar oxygenation of patients. Moreover, the improvements in markers for lung function were markedly

greater in the RG than in the CG, which shows that BAL combined with ambroxol can effectively dissolve the viscous substances in the airway, decrease airway obstruction, increase inhaled oxygen flow, and improve the patient's lung function.

The APACHE II score is a prognostic indicator for patients in the intensive care unit [29]. A previous study revealed that the increase in the APACHE II score leads to increased incidence of delirium in elderly patients with SP receiving invasive mechanical ventilation, suggesting that the APACHE II score is important in evaluating the incidence of delirium in elderly patients with SP [30]. In the present study, the APACHE II score after treatment was markedly lower in the RG than in the CG, indicating that BAL combined with ambroxol can effectively decrease the severity of the patient's condition. sTREM-1 is a pro-inflammation mediator that is involved in the inflammatory process of SP and works as a marker for the diagnosis and severity assessment of patients with SP [31, 32]. In the present study, the sTREM-1 concentration after treatment was markedly lower in the RG than in the CG, indicating that BAL combined with ambroxol can reduce inflammation and improve the patient's condition and prognosis. Here we detected a decrease in both the APACHE II score and the sTREM-1 concentration during the treatment and the decrease in the two groups was significantly and positively correlated, indicating that patients undergoing BAL combined with ambroxol have a good prognosis. After treatment, the overall response rate was markedly higher in the RG than in the CG, indicating that BAL combined with ambroxol is highly effective in treating senile SP and relieving conditions.

This study confirmed that BAL combined with ambroxol is a feasible treatment plan for elderly patients with SP, but it is subject to some deficiencies. For example, we did not evaluate the quality of life of patients and analyze the diagnostic value of sTREM-1 for SP, nor did we

explore risk factors that affect the efficacy of treatment for elderly patients with SP. We will address these deficiencies in future research. In summary, BAL combined with ambroxol is markedly effective in the treatment of SP in elderly patients and promotes the recovery and improvement of lung function. sTREM-1 is a potential reference index to evaluate the improvement of patients conditions.

Disclosure of conflict of interest

None.

Address correspondence to: Jianming Hu, Department of Respiratory, The First Hospital of Lanzhou University, No. 1 Donggangxi Road, Chengguan District, Lanzhou 730030, Gansu Province, China. Tel: +86-0931-8356210; E-mail: Hujianming19@outlook.com

References

- [1] Jiang J, Hu C, Li Y, Pan P, Su X, Deng P, Wang J and Wu X. Transmission electron microscopy improves the diagnostic sensitivity in nonbacterial etiology of severe pneumonia: a retrospective study. *Am J Med Sci* 2019; 357: 289-295.
- [2] Falcone M, Russo A, Cangemi R, Farcomeni A, Calvieri C, Barilla F, Scarpellini MG, Bertazzoni G, Palange P, Taliani G, Venditti M and Violi F. Lower mortality rate in elderly patients with community-onset pneumonia on treatment with aspirin. *J Am Heart Assoc* 2015; 4: e001595.
- [3] Memish ZA, Almasri M, Turkestani A, Al-Shangiti AM and Yezli S. Etiology of severe community-acquired pneumonia during the 2013 Hajj-part of the MERS-CoV surveillance program. *Int J Infect Dis* 2014; 25: 186-190.
- [4] Faverio P, Aliberti S, Bellelli G, Suigo G, Lonni S, Pesci A and Restrepo MI. The management of community-acquired pneumonia in the elderly. *Eur J Intern Med* 2014; 25: 312-319.
- [5] Aronen M, Viikari L, Kohonen I, Vuorinen T, Hameenaho M, Wuorela M, Sadeghi M, Soderlund-Venermo M, Viitanen M and Jartti T. Respiratory tract virus infections in the elderly with pneumonia. *BMC Geriatr* 2019; 19: 111.
- [6] Wongsurakiat P and Chitwarakorn N. Severe community-acquired pneumonia in general medical wards: outcomes and impact of initial antibiotic selection. *BMC Pulm Med* 2019; 19: 179.
- [7] Lassi ZS, Imdad A and Bhutta ZA. Short-course versus long-course intravenous therapy with the same antibiotic for severe community-acquired pneumonia in children aged two months to 59 months. *Cochrane Database Syst Rev* 2017; 10: CD008032.
- [8] Kawczyk-Krupka A, Pucelik B, Miedzybrodzka A, Sieron AR and Dabrowski JM. Photodynamic therapy as an alternative to antibiotic therapy for the treatment of infected leg ulcers. *Photodiagnosis Photodyn Ther* 2018; 23: 132-143.
- [9] Connett GJ. Bronchoalveolar lavage. *Paediatr Respir Rev* 2000; 1: 52-56.
- [10] Zhang Y, Liu Z, Li S, Wang M, Dai D, Jing H and Liu L. Upregulation of E-cadherin in bronchoalveolar lavage fluid-derived exosomes in patients with lung cancer. *Thorac Cancer* 2020; 11: 41-47.
- [11] Wang Z, Yu HB, Luo Q and Liu YY. Treatment of bronchopleural fistula with carbolic acid instilled through bronchofiberscope in post-pulmonectomy patients. *J Cardiothorac Surg* 2015; 10: 120.
- [12] Cohen ME, Salmasian H, Li J, Liu J, Zachariah P, Wright JD and Freedberg DE. Surgical antibiotic prophylaxis and risk for postoperative antibiotic-resistant infections. *J Am Coll Surg* 2017; 225: 631-638, e633.
- [13] Takeda K, Miyahara N, Matsubara S, Taube C, Kitamura K, Hirano A, Tanimoto M and Gelfand EW. Immunomodulatory effects of ambroxol on airway hyperresponsiveness and inflammation. *Immune Netw* 2016; 16: 165-175.
- [14] Yang YG, Song LX, Jiang N, Xu XT, Di XH and Zhang M. Pharmacokinetics of ambroxol and clenbuterol tablets in healthy Chinese volunteers. *Int J Clin Exp Med* 2015; 8: 18744-18750.
- [15] Beeh KM, Beier J, Esperester A and Paul LD. Antiinflammatory properties of ambroxol. *Eur J Med Res* 2008; 13: 557-562.
- [16] Yang Z, Xiao X, Huang Y, He X, Lu Q, Chen S and Lin Z. Effects and mechanisms of ambroxol inhalation (Mucosolvan((R))) in the treatment of neonatal pneumonia. *Pharmazie* 2017; 72: 604-607.
- [17] Cao C, Gu J and Zhang J. Soluble triggering receptor expressed on myeloid cell-1 (sTREM-1): a potential biomarker for the diagnosis of infectious diseases. *Front Med* 2017; 11: 169-177.
- [18] Wang HX and Chen B. Diagnostic role of soluble triggering receptor expressed on myeloid cell-1 in patients with sepsis. *World J Emerg Med* 2011; 2: 190-194.
- [19] Russell FM, Reyburn R, Chan J, Tuivaga E, Lim R, Lai J, Van HMT, Choumanivong M, Sychareun V, Khanh DKT, de Campo M, Enarson P, Graham S, La Vincente S, Mungan T, von Mollendorf C, Mackenzie G and Mulholland K. Impact of the change in WHO's severe pneumonia case definition on hospitalized pneumonia

- epidemiology: case studies from six countries. Bull World Health Organ 2019; 97: 386-393.
- [20] Cheng JY. Mortality prediction in status epilepticus with the APACHE II score. J Intensive Care Soc 2017; 18: 310-317.
- [21] Rao M, Kapila K and Gupta RM. Enzyme linked immunosorbent assays revisited. Med J Armed Forces India 1997; 53: 45-51.
- [22] Barnett JB, Hamer DH and Meydani SN. Low zinc status: a new risk factor for pneumonia in the elderly? Nutr Rev 2010; 68: 30-37.
- [23] Li W, Ding C and Yin S. Severe pneumonia in the elderly: a multivariate analysis of risk factors. Int J Clin Exp Med 2015; 8: 12463-12475.
- [24] Alishlash AS, Atkinson TP, Schlappi C, Leal SM Jr, Waites KB and Xiao L. Mycoplasma pneumoniae carriage with de novo macrolide-resistance and breakthrough pneumonia. Pediatrics 2019; 144: e20191642.
- [25] Xiao B, Wang M, Hu X, Li J, Wang F and Sun J. Antibiotic de-escalation principle in elderly patients with chronic obstructive pulmonary disease complicated with severe pneumonia. Exp Ther Med 2017; 13: 1485-1489.
- [26] Wang LP, Chen SP, Huang YY, Qin FF, Ou W, Liu HD, Deng XF, Lou L, Tao J and Cui YX. Application of flexible bronchoscopy in children with respiratory diseases. Zhongguo Dang Dai Er Ke Za Zhi 2017; 19: 1174-1179.
- [27] Chen F, Zhang YX and Zhang CQ. Effect of ambroxol on the concentration of cefotaxime in the bronchoalveolar lavage fluid of rats with pulmonary fibrosis. Exp Ther Med 2015; 9: 539-542.
- [28] Mizutani T and Hojo M. Severe hypoxaemia due to methaemoglobinemia and aspiration pneumonia. BMJ Case Rep 2011; 2011: bcr0320113937.
- [29] Naeini AE, Abbasi S, Haghighipour S and Shirani K. Comparing the APACHE II score and IBM-10 score for predicting mortality in patients with ventilator-associated pneumonia. Adv Biomed Res 2015; 4: 47.
- [30] Pei X, Yu H, Wu Y and Zhou X. Correlation between APACHE II scores and delirium probability of senile severe pneumonia patients undergoing invasive mechanical ventilation. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2017; 29: 821-824.
- [31] Liu CY, Jin P, Yang YX, Liu HX, Liu YQ, Xu JJ, Huang WD and Wang SJ. Relationship between soluble intercellular adhesion molecule-1 and severe pneumonia. Zhongguo Dang Dai Er Ke Za Zhi 2007; 9: 537-539.
- [32] Zhong MF and Zhao JM. Diagnostic values of plasma CD64 and sTREM-1 for pediatric pneumonia. Zhongguo Dang Dai Er Ke Za Zhi 2016; 18: 599-602.