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High Lymphocyte Percentage in Bronchoalveolar Lavage Fluid of Patients with H1N1-Associated Acute Respiratory Distress Syndrome

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Abstract

Distinguishing between bacterial and H1N1 infection in patients with acute respiratory distress syndrome (ARDS) is difficult based on clinical symptoms alone; moreover, rapid antigen test for influenza has poor sensitivity. The aim of this work was to determine whether the differential cell count in bronchoalveolar lavage (BAL) fluid could aid in early diagnosis of H1N1-ARDS. We retrospectively identified pneumonia-induced ARDS patients who underwent BAL in intensive care unit (ICU) of the Chang Gung Memorial Hospital, Keelung from January 1, 2014 to March 31, 2016. Patient characteristics, severity of illness scores, white blood count (WBC) and differential count, biochemical test, BAL fluid differential cell count, and semi-quantitative culture of lower respiratory tract sample were evaluated. Nine patients with H1N1-ARDS and 18 with non-H1N1-ARDS were identified. Patients with H1N1-ARDS had lower APACHE II scores. Lymphocyte percentage in BAL fluid was significantly higher in the H1N1-ARDS group (15.6 ± 7.5% vs. 7.6 ± 8.0%, p=0.009). The area under the ROC curve (AUC) was 0.829; with a sensitivity of 85.5%, specificity of 77.8%, positive predictive value of 79.4% and negative predictive value of 84.3% for H1N1-ARDS prediction at a cutoff value of 11%. Lymphocyte percentage in BAL fluid was higher in patients with H1N1-ARDS than in those without. This result has potential applicability for early detection of H1N1 influenza virus infection in patients with ARDS. (J Intern Med Taiwan 2018; 29: 46-53)

Key Words: Acute respiratory distress syndrome; Bronchoalveolar lavage; H1N1; Influenza; Respiratory failure

Introduction

In 2009, the novel swine-origin influenza A (H1N1) was identified as the cause of a global pandemic[1]. Thirty percent of patients hospitalized for H1N1 infection require admission to the intensive care unit (ICU)[2,3], and the most common cause of death is viral pneumonia with acute respiratory distress syndrome (ARDS)[2]. Early use of antiviral agents with neuraminidase inhibitor is recommended based on improved survival rates in critically ill patients with H1N1 infection[4,5].

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However, it may be difficult to distinguish between bacterial and H1N1 infection in patients with ARDS based on clinical symptoms alone[6]; moreover, rapid antigen test reportedly shows 34% false negative rate for H1N1 influenza detection[7]. The real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for influenza has high sensitivity and specificity, but is currently not readily available in general hospitals. Patients with ARDS usually present with leukocytosis of an average 14,000 white blood cells (WBC) per μL[8]. The differential cell counts of bronchoalveolar lavage (BAL) fluid from H1N1-ARDS patients are not well established, to our best knowledge.

We conducted a retrospective study to assess the sensitivity and specificity of differential cell count of BAL fluid for distinguishing between patients with H1N1-ARDS and non-H1N1-ARDS.

Materials and Methods

Subjects

We retrospectively identified patients who underwent BAL in the ICU of the Chang Gung Memorial Hospital, Keelung from January 1, 2014 to March 31,2016. Patients who were diagnosed with ARDS due to pneumonia were enrolled. The ICU is a medical and closed unit in our hospital. This study was approved by the Institutional Review Board at Chang Gung Memorial Hospital (201600787B0). The following patient data were recorded within the first 3 days of admission: age; gender; body mass index (BMI); medical history; WBC and differential count; blood urea nitrogen and creatinine; C-reactive protein; lactate levels; PaO2/FiO2 ratio; and Acute Physiology and Chronic Health Evaluation (APACHE) II score. Adverse events recorded within the first 5 days of admission included gastrointestinal bleeding, shock, acute kidney injury (AKI), jaundice, new arrhythmia, stroke, thrombocytopenia and bacteremia.

BAL

BAL was conducted within 3 days of admission. Three 50 ml syringes prefilled with room-temperature normal saline was instilled by bronchoscopy. Gentle suction was then performed. The BAL fluid was sent for differential cell count; semi-quantitative culture and rRT-PCR for influenza.

Disease definitions

ARDS was defined according to the Berlin definition[9]. Influenza was confirmed by rRT-PCR of BAL fluid. Disease severity was assessed with the APACHE II score[10]. Shock was identified by a vasopressor requirement of a mean arterial pressure of ≥ 65 mmHg and serum lactate level of > 2 mmol/L in the absence of hypovolemia[11]. AKI was defined as any of the following: (1) increase in serum creatinine level by ≥ 0.3 mg/dL within 48 hours; (2) increase in serum creatinine level to ≥ 1.5 times within 7 days; (3) urine volume < 0.5 mL/kg/hour for 6 hours[12]. Jaundice was defined as total serum bilirubin levels of > 2 mg/dL. Thrombocytopenia was defined as a platelet count of < 150 x 10^3/μL.

Statistical analysis

Results were expressed as means ± standard deviation (SD). Differences in continuous variables between the 2 groups were analyzed by Mann Whitney U test. Differences in categorical variables between the 2 groups were compared by the Fisher's exact test. To evaluate the lymphocyte percentage in BAL fluid as a method of early diagnosis of H1N1 infection in ARDS patients, we constructed receiver-operating-characteristic (ROC) curves from measurements within the first 3 days of admission. Cut-off values based on ROC curves were used to calculate sensitivities and specificities.

Statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS, Inc., USA). A p-value < 0.05 was considered statistically significant.

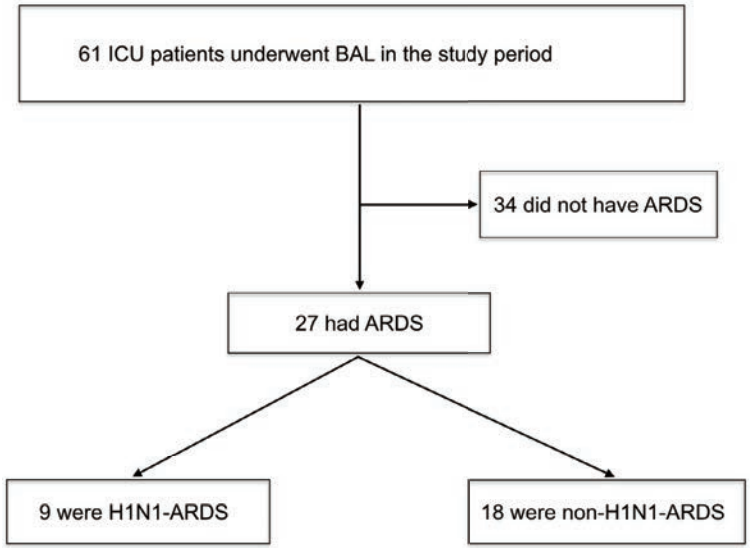
Results

From January 1, 2014, to March 31,2016, a total of 61 ICU patients underwent BAL (Figure 1). Thirty-four patients who failed to meet the Berlin definition of ARDS were excluded. Among the 27 cases with ARDS, 9 were due to H1N1 infection and 18 were due to bacterial infection. Clinical characteristics, interventions, and outcomes of pneumonia patients with ARDS were shown in Table 1. Patients with H1N1-ARDS had higher BMI and lower APACHE II score than patients with non-H1N1-ARDS. There were no differences in age and sex between groups. Percentages of cases with a history of diabetes mellitus, hypertension, chronic obstructive pulmonary disease (COPD), congestive heart failure (CHF), hemodialysis, and liver cirrhosis were similar between the 2 groups. Mean PaO2/FiO2 ratio was 61.1 mmHg in the H1N1-ARDS group and 87.9 mmHg in the non-H1N1-ARDS group, without statistically significant difference.

Percentage of cases with jaundice was higher in the H1N1-ARDS group. Other adverse events, including gastrointestinal bleeding, shock, AKI, new arrhythmia, stroke, thrombocytopenia, and bacteremia showed no group-wise differences. No differences were noted in the use of neuromuscular blockers, low tidal volume therapy, prone position, extracorporeal membrane oxygenation (ECMO) and 30-day mortality rates between patients with H1N1-ARDS and non-H1N1-ARDS.

The results of laboratory studies were shown in Table 2. WBC, differential count, and biochemical tests were similar between the 2 groups. BAL fluid showed significantly higher lymphocyte percentage in the H1N1-ARDS group, as compared to the non-HN1-ARDS group (15.6 ± 7.5% vs. 7.6 ± 8.0%, P=0.009). WBC count, RBC count, neutrophil percentage, and macrophage percentage in BAL fluid were similar between the 2 groups. Multivariate analysis revealed that lymphocyte percentage of BAL fluid is an independent factor of H1N1-ARDS after adjusting for gender, age, APACHE II and BMI (adjusted odds ratio, 1.15; 95% confidence interval, 1.02 to 1.32, P=0.03).

Table 3 showed the pathogens isolated from BAL specimens in ARDS patients. One patient had co-infection with H1N1 and *Stenotrophomonas*



*Abbreviations: BAL= bronchoalveolar lavage; ARDS= acute respiratory distress syndrome

Figure 1. Patients selection process. H1N1 infection is confirmed by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) of BAL fluid.

Table 1. Comparison of demography, clinical parameters, interventions, and outcomes between H1N1 and non-H1N1-ARDS patients

	H1N1-ARDS (N=9)	Non-H1N1-ARDS (N=18)	P value
Age, years (mean ± SD)	60.7 ± 7.1	69.1 ± 17.3	0.145
Sex, No. (%)			
Male	6 (66.7)	13 (72.2)	1.000
Female	3 (33.3)	5 (27.8)	
BMI (Kg/m^2) (mean ± SD)	27.4 ± 3.9	22.9 ± 4.4	0.006
APACHE II score (mean ± SD)	19.7 ± 7.2	26.9 ± 8.3	0.031
History, No. (%)			
Diabetes mellitus	3 (33.3)	3 (16.7)	0.367
Hypertension	6 (66.7)	8 (44.4)	0.420
Chronic obstructive pulmonary disease	1 (11.1)	3 (16.7)	1.000
Congestive heart failure	1 (11.1)	4 (22.2)	0.636
Hemodialysis	1 (11.1)	3 (16.7)	1.000
Liver cirrhosis	0 (0.0)	0 (0.0)	
PaO2/FIO2 ratio (mean ± SD)	61.1 ± 13.0	87.9 ± 41.5	0.095
Adverse events, No. (%)			
Gastrointestinal bleeding	4 (44.4)	6(33.3)	0.683
Shock	7 (77.8)	14 (77.8)	1.000
Acute kidney injury	6 (66.7)	13 (72.2)	1.000
Jaundice	4 (44.4)	1 (5.6)	0.030
New arrhythmia	1 (11.1)	4 (22.2)	0.636
Stroke	1 (11.1)	0 (0.0)	0.333
Thrombocytopenia	9 (100.0)	14 (77.8)	0.268
Bacteremia	0 (0.0)	1 (5.6)	1.000
Intervention, No. (%)			
Low tidal volume ventilation	9 (100%)	11 (61.1)	0.059
Neuromuscular blockade	8 (88.9)	11 (61.1)	0.201
Prone position	4 (44.4)	4 (22.2)	0.375
ECMO	4 (44.4)	2 (11.1)	0.136
30-day mortality, No. (%)	4 (44.4)	10 (55.6)	0.695

*Abbreviations: ARDS= acute respiratory distress syndrome; SD= standard deviation; BMI= body mass index; APACHE= Acute Physiology and Chronic Health Evaluation; ECMO= Extracorporeal Membrane Oxygenation.

maltophilia, and one with H1N1 and *Candida tropicalis.* In non-H1N1-ARDS group, 3 patients had co-infection with 2 pathogens (*Pseudomonas aeruginosa* and *Aspergillus fumigatus*; *Acinetobacter baumannii* and *Elizabethkingia meningoseptica*; *Klebsiella pneumonia* and *Candida albicans*). Seven patients with H1N1-ARDS and 6 patients with non-H1N1-ARDS showed no detectable pathogens in BAL culture. *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter bauman-*

Table 2. Comparison of white blood cell count, biochemical test and bronchoalveolar lavage (BAL) (mean ± standard deviation) among the two groups

	H1N1-ARDS(N=9)	Non-H1N1-ARDS(N=18)	P value
WBC (μl)	7188.9 ± 2830.4	11258.9 ± 6619.1	0.194
Neutrophil (%)	81.2 ± 6.1	81.6 ± 11.3	0.463
Lymphocyte (%)	14.5 ± 6.4	10.2 ± 8.4	0.059
BUN (mg/dL)	22.6 ± 13.9	43.5 ± 35.2	0.085
Cr (mg/dL)	1.94 ± 1.96	3.45 ± 3.29	0.232
CRP (mg/dL)	21.63 ± 10.59	17.98 ± 9.88	0.379
Lactate (mg/dL)	20.8 ± 11.2	20.8 ± 13.6	1.000
Bronchoalveolar lavage			
WBC (μL)	856 ± 1181	1091 ± 1597	0.883
RBC (μL)	11821 ± 15897	43900 ± 163347	0.495
Neutrophil (%)	59.9 ± 14.5	72.4 ± 25.0	0.085
Lymphocyte (%)	15.6 ± 7.5	7.6 ± 8.0	0.009
Macrophage (%)	21.3 ± 14.7	19.8 ± 19.5	0.495

*Abbreviations: BUN= blood urea nitrogen; Cr= Creatinine; WBC= white blood cell; CRP= C-reactive protein; RBC=red blood cell.

Table 3. Identification of pathogens from BAL culture

Pathogens (No. [%])	H1N1-ARDS (N=9)	Non-H1N1-ARDS (N=18)
Bacteria	1 (11.1)	13 (72.2)
Pseudomonas aeruginosa	0	4 (22.2)
Stenotrophomonas maltophilia	1 (11.1)	3 (16.7)
Acinetobacter baumannii	0	2 (11.1)
Klebsiella pneumoniae	0	1 (5.6)
Haemophilus parainfluenzae	0	1 (5.6)
Staphylococcus aureus	0	1 (5.6)
Elizabethkingia meningoseptica	0	1 (5.6)
Aspergillus species	0	1 (5.6)
Candida species	1 (11.1)	1 (5.6)

nii were the most frequently isolated pathogens from non-H1N1-ARDS patients, in descending order.

For distinguishing between H1N1-ARDS and non-H1N1-ARDS patients, the ROC curve of the lymphocyte percentage in BAL fluid had an area under the curve (AUC) of 0.829 (95% CI, 0.654 to 1.000; p=0.012) (Figure 2); and a cutoff value of 11% had sensitivity of 85.5%, specificity of 77.8%, positive predictive value of 79.4% and negative predictive value of 84.3% for H1N1-ARDS detection.

Discussion

The results of our study suggested that the lymphocyte percentage in BAL fluid could be used in the diagnosis of H1N1-ARDS. BAL fluid from healthy volunteers reportedly shows macro-

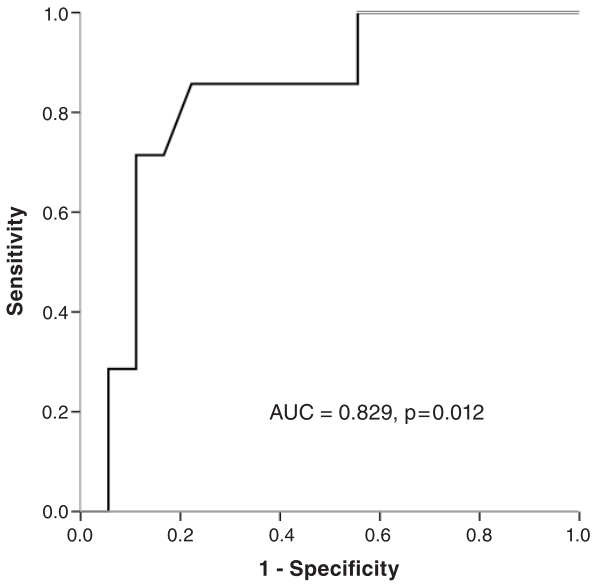


Figure 2. Receiver-operating-characteristic (ROC) curves of lymphocyte percentage from bronchoalveolar lavage fluid shows good ability to distinguish between H1N1-ARDS and non-H1N1-ARDS patients. The area under the curve (AUC) is 0.829 with p=0.012.

phages, 85.2%; neutrophils, 1.6%; and lymphocytes, 11.8%[13]. Increased neutrophil counts in BAL fluid are usually seen in patients with pneumonia, including bacterial infection and virus infection (H1N1). Our study showed that the lymphocyte percentage in BAL fluid was significantly higher in patients with H1N1-ARDS. Lymphocytes, especially T cells, play an important role in host defense mechanisms against H1N1 influenza virus[14]. On the other hand, T cells accumulate around the lung area[14], which might explain the higher lymphocyte percentage in BAL fluid from H1N1-ARDS patients.

A study in California on 1950 cases showed that neuraminidase inhibitor treatment within 5 days of symptom onset is associated with increased survival, as compared with no treatment in ICU patients with H1N1 infection (survival rate: 75% vs. 58%, $p<0.001$)[4]. In addition, there was a significant trend toward improved survival among those treated earliest[4]. Rapid influenza antigen tests yield results in approximately 15 minutes but with low sensitivity of 62.3%[15]. Currently, rRT-PCR is the most sensitive and specific test for influenza, with results available within 6 hours of specimen submission[16]. However, since rRT-PCR is not readily available in general hospitals, it usually takes 2 to 7 days to obtain results because of specimen transportation. BAL fluid differential cell count can be analyzed in general hospitals with results available within 30-60 minutes. In this study, lymphocyte percentage in BAL fluid showed approximately 80% positive and negative predictive values. Approximately 20% ARDS patients were misdiagnosed as non-H1N1 ARDS. Thus, physicians could use lymphocyte percentage in BAL fluid for early detection and treatment of suspicious influenza pneumonia; however, more studies are needed to confirm this method.

The non-H1N1-ARDS group showed a tendency of older age and more severe renal insufficiency, which could explain the significantly higher APACHE II score in this group. However, the 30-day mortality rates showed no significant group-wise difference, likely due to the same frequency of shock between the 2 groups (77.8%). Moreover, shock is associated with a greater risk of mortality due to profound circulatory, cellular and metabolic abnormalities[11].

In our study, 44.4% of patients with H1N1-ARDS developed jaundice within the first 5 days of admission, as compared to 5.6% of patients with non-H1N1-ARDS. The perihilar extrahepatic and the intrahepatic biliary tree receive blood exclusively from the hepatic artery[17], which could account for the cholestasis from decreased hepatic perfusion in critically ill patients. The reason for the higher percentage of cases with jaundice in the H1N1-ARDS group is unclear to our best knowledge.

In the current study, we showed that 16.7% patients had co-infection in the non-H1N1-ARDS group and 22.2% in the H1N1-ARDS group, similar to the previous report of 37.3% patients with bacterial co-infection among ICU patients with influenza

pneumonia[18]. Thus, empiric antibiotic treatment is still needed because of the high risk of bacterial co-infection in influenza pneumonia.

Our study has several limitations. First, this is a retrospective and small-size study although our finding has fair AUC for early diagnosis of H1N1-ARDS. Second, two H1N1-ARDS patients had co-infection with other pathogens. Third, virus culture is not routinely performed in these patients although virus other than influenza induced ARDS is not common. Accordingly, further prospective and large study is needed to confirm our result.

Our study showed a higher lymphocyte percentage in BAL fluid from H1N1-ARDS patients, as compared to non-H1N1-ARDS patients. This result has potential applicability for early detection of H1N1 infection in patients with ARDS during the highly prevalent period, with consequent early antiviral treatment, and increased survival rates.

Conflicts of Interest

None of the authors have any financial or personal relationships with other organizations that could inappropriately influence this work.

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支氣管肺泡沖洗液高淋巴球比例於H1N1流感急性呼吸窘迫症候群

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摘 要

早期使用抗病毒藥物治療H1N1流感重症患者可以改善病人的存活率。然而臨床上要區分H1N1或細菌造成的急性呼吸窘迫症候群是困難的，而且流感快篩檢驗的敏感度不夠高。一般的醫院無法以即時聚合酶鏈鎖反應偵測流感病毒。我們的研究目的是以支氣管肺泡沖洗液中的淋巴球比例來早期診斷H1N1流感引起的急性呼吸窘迫症候群。我們回朔性分析2014年到2016年3月基隆長庚醫院內科加護病房因為肺炎引起急性呼吸窘迫症候群並且有接受支氣管肺泡沖洗的病人，有9位是H1N1流感而18位是細菌引起的。H1N1-急性呼吸窘迫症候群的病人的病人有較低的APACHE II分數，支氣管肺泡沖洗液中的淋巴球比例比起細菌-急性呼吸窘迫症候群的病人還要高 (15.6 ± 7.5% vs. 7.6 ± 8.0%, p=0.009)。以支氣管肺泡沖洗液中的淋巴球比例11%來區分是H1N1流感或是細菌造成的急性呼吸窘迫症候群，ROC曲線下面積為0.829，敏感度85.5%，特異度77.8%，陽性預測值79.4%，陰性預測值84.3%。這個結果可以幫助我們對於急性呼吸窘迫症候群的病人早期區分是H1N1流感所致，早期給予抗病毒藥以改善病人的存活率。