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Bacterial coinfection is associated with severity of avian influenza A (H7N9), and procalcitonin is a useful marker for early diagnosis

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ABSTRACT

Patients contracting avian influenza A (H7N9) often develop severe disease. However, information on the contribution of bacterial coinfection to the severity of H7N9 is limited. We retrospectively studied 83 patients with confirmed H7N9 infection from April 2013 to February 2014. The severity of patients with bacterial coinfection and markers for early diagnosis of bacterial coinfection in H7N9 were analyzed. We found *Staphylococcus aureus* was the most prevalent pathogen. Higher Acute Physiology and Chronic Health Evaluation II score, shock, renal replacement treatment, mechanical ventilation, and extracorporeal membrane oxygenation treatment were more frequently observed in patients with bacterial coinfection. Procalcitonin is more sensitive than C-reactive protein in determining bacterial coinfection in H7N9 patients. In conclusion, H7N9 infection patients with bacterial coinfection had a more severe condition. Elevated procalcitonin is an accurate marker for diagnosing bacterial coinfection in H7N9 patients, thus enabling earlier antibiotic therapy.

1. Introduction

A novel avian-origin influenza A (H7N9) virus had emerged and spread among humans in China and led to 435 cases by June 27, 2014, with a mortality rate as high as 36.7% (WHO, nd). Early reports have shown that H7N9 infection could cause progressive pneumonia and multiorgan dysfunction, with 63–76.6% of patients being admitted to an intensive care unit (ICU) (Gao et al., 2013a; Li et al., 2014). Much effort has been devoted to investigate the risk factors for the progression and the fatal outcome of the disease. Our early findings have indicated the presence of a coexisting medical condition as the only independent risk factor for acute respiratory distress syndrome (Gao et al., 2013a). Elevated angiotension II levels are associated with the severity of H7N9-induced disease and may potentially predict patient mortality (Huang et al., 2014). However, initial studies of H7N9 influenza describe few details of patients with bacterial coinfection and the reasonable selection of antibiotic therapy.

We conducted a retrospective study that focused on the presence of bacterial coinfection in confirmed cases of H7N9 infection. We aimed to investigate the incidence of bacterial coinfection, the most common pathogen, and an accurate diagnosis marker before obtaining positive cultures for this potential fatal disease and to determine which patients should receive antibiotic therapy at the time of admission.

2. Methods

2.1. Study design and patients

We performed a retrospective review of medical records of all patients with laboratory-confirmed avian H7N9 influenza infection (by polymerase chain reaction of nasopharyngeal secretions or bronchoalveolar lavage fluid) in a large tertiary hospital, the First Affiliated Hospital, School of Medicine, Zhejiang University, China, from April 2013 to February 2014. The following data were recorded retrospectively: demographic details, microbiologic findings, comorbidities, severity of illness score, the presence of shock, clinical laboratory findings within 24 h of admission, and outcome on hospital day 90. The need for mechanical ventilation, renal replacement therapies, and extracorporeal membrane oxygenation (ECMO) treatment was also recorded. The study was approved by the Ethical Board of the First Affiliated Hospital, School of Medicine, Zhejiang University. Written informed consent was obtained from all patients.

2.2. Definitions

A confirmed case was defined as a positive test result for avian H7N9 using reverse transcriptase polymerase chain reaction, as described previously (Gao et al., 2013b). Bacterial coinfection was defined as any bacterial infection presumed by the expert group according to clinical manifestations, with one or more positive cultures or with positive

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urine *Streptococcus pneumoniae* or *Legionella pneumophila* antigen detection. The cultures should have been obtained from blood, valid sputum, lower respiratory tract (endotracheal or bronchoalveolar lavage) samples, or other normally sterile fluids within the first 72 h of hospitalization. Meanwhile, the urine samples subjected to urine antigen detection should have been positive within 24 h after admission.

To determine the severity of illness, Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al., 1985) was calculated for all patients within 24 h of admission. Shock was defined as that being treated with vasopressors. Mortality was defined as death occurring anytime after admission and up to day 90.

2.3. Method for procalcitonin (PCT) and C-reactive protein (CRP) measurement

PCT was tested using the electrochemical luminescence method (Elecsys Brahms PCT, Mannheim, Germany). CRP was measured using immunoturbidimetric assays (Beckman, Carlsbad, CA 92010, USA).

2.4. Statistical analyses

Categorical variables are expressed in percentages and frequencies. Continuous variables are expressed means and SD or median. Categorical variables were compared using the chi-square test. Continuous variables were compared using independent-samples *t*-test or Mann–Whitney *U* test. Univariate and multivariate logistic regression analyses were performed to predict 90-day mortality. The variables analyzed were age; gender; comorbidities; presence of shock; APACHE II score; serum creatinine; serum creatinine kinase (CK); serum lactate dehydrogenase (LDH); CRP, leukocyte count, platelet count; D-dimer; PCT; bacterial coinfection; and need for mechanical ventilation, renal replacement, and ECMO. The diagnostic accuracy of biomarkers (CRP and PCT) for predicting bacterial coinfection was examined by their receiver operating characteristic curve (ROC), and the area under the curve (AUC), sensitivity, and specificity were also calculated. All tests were 2 tailed, and the significance was set at 5%. Data analysis was performed using SPSS for Windows, version 18.0.

3. Results

During the study period, 83 consecutive patients with laboratory-confirmed avian H7N9 influenza infection who were admitted to the infectious disease department in our hospital were analyzed. The mean age was 57.71 years (57.71 ± 14.60, range, 21–86 years); 41 (49.4%) patients were older than 60 years, and 55 (66.3%) were males. Forty-eight patients (57.8%) had comorbidities, of which hypertension and diabetes were the most common. Two (2.4%) patients were pregnant. The mean APACHE II score at admission was 19.93 ± 8.25. The main demographic and clinical characteristics of patients with and without bacterial coinfection are detailed in Table 1.

In our study, 16 (19.3%) patients were diagnosed with bacterial coinfection in the first 72 h of admission based on clinical manifestations, cultures, and urinary antigen detection. However, urine samples of all patients were negative for *S. pneumoniae* and *L. pneumophila* antigens. The pathogens that caused bacterial coinfections were *Staphylococcus aureus* in 4 cases (25%), 3 of which involve methicillin-resistant *S. aureus* (MRSA); *Staphylococcus hominis* in 3 cases; *Staphylococcus epidermidis* in 2 cases; *Ralstonia mannitolilytica* in 2 cases; *Acinetobacter baumannii* in 2 cases; *Pseudomonas aeruginosa* in 1 case; *Escherichia coli* in 1 case; and *Burkholderia cepacia* in 1 case. Eight patients with bacterial coinfection had bacteremia (*S. hominis* in 3 cases, *S. epidermidis* in 2 cases, MRSA in 2 cases, and *A. baumannii* in 1 case). Three patients (3.6%) had both positive respiratory tract cultures and bacteremia (MRSA in 2 cases and *A. baumannii* in 1 case).

Patients with bacterial coinfection had a more severe condition than those without coinfection and had higher APACHE II score (25.63 ± 5.30 versus 18.57 ± 8.27, $P = 0.000$) and were more likely to present with shock (62.5% versus 28.4%, $P = 0.010$); were more likely to need renal replacement treatment (81.2% versus 23.9%, $P = 0.000$), mechanical ventilation (93.8% versus 43.3%, $P = 0.000$), and ECMO treatment (50.0% versus 20.9%, $P = 0.018$); had higher serum levels of LDH, CRP, PCT, D-dimer, and leukocyte counts; and showed higher 90-day hospital mortality (62.5% versus 23.9%, $P = 0.003$). However, there was no significant trend for longer hospital stay. No differences in age, gender, comorbidities, serum creatinine, CK, and platelets counts were observed (Table 1).

Table 1
Demographic and clinical characteristics of 83 avian H7N9 patients with and without bacterial coinfection.

Characteristics	Bacterial coinfection (n = 16)	H7N9 only (n = 67)	*P* value
Age (years), mean	58.25 ± 17.14	57.58 ± 14.07	0.871
Sex (male), n (%)	8 (50.0%)	47 (70.1%)	0.126
Comorbidities	9 (56.2%)	39 (58.2%)	0.973
Laboratory finding at admission			
Serum creatinine (μmol/L)	110.44 ± 91.11	78.99 ± 67.35	0.122
Serum CK (U/L)	463.81 ± 513.88	403.06 ± 775.13	0.768
Serum LDH (U/L)	783.19 ± 584.47	498.13 ± 300.27	0.007
CRP (mg/dL)	144.82 ± 84.66	85.10 ± 70.01	0.004
PCT (ng/mL)	18.80 ± 28.97	0.58 ± 1.18	0.000
D-Dimer (μg/L)	11914.44 ± 9789.27	5030.78 ± 5527.12	0.000
Leukocyte count (10^9/L)	6.96 ± 4.43	4.77 ± 3.52	0.037
Platelets counts (10^9/L)	127.5 ± 82.55	136.07 ± 69.23	0.669
APACHE II score at admission	25.63 ± 5.30	18.57 ± 8.27	0.002
Renal replacement	13 (81.2%)	16 (23.9%)	0.000
Mechanical ventilation	15 (93.8%)	29 (43.3%)	0.000
Shock	10 (62.5%)	19 (28.4%)	0.010
ECMO treatment	8 (50.0%)	14 (20.9%)	0.018
Hospital stay (days), mean	29.88 ± 23.13	24.21 ± 19.77	0.322
30-day mortality, n (%)	7 (43.8%)	11 (16.4%)	0.017
90-day mortality, n (%)	10 (62.5%)	16 (23.9%)	0.003

PCT and CRP levels were obtained, respectively, in 53 and 83 of the total 83 patients, and 53 patients had both biomarkers measured simultaneously within 24 h after admission. Fig. 1 demonstrates the scatter plot of PCT and CRP values in the 2 groups on admission. Using ROC analysis, AUC to diagnose bacterial coinfection was 0.96 (95% confidence interval [CI]: 0.91–1.00) for PCT compared with 0.68 (95% CI: 0.50–0.87) for CRP ($P = 0.005$) (Fig. 2). For PCT at a cutoff value of 0.81 μg/L for diagnosis of bacterial coinfection, the sensitivity was 91.7%, and the specificity was 90.2%.

All patients received antiviral therapy in our study. The median time from the onset of illness to the initiation of antiviral therapy was 7 days (range, 1–20); 9.6% (8/83) of the patients received antiviral therapy within 48 h after the onset of symptoms. The antiviral regimes include oseltamivir at a daily dose of 150–300 mg in 43 patients, oseltamivir combined with peramivir at a daily dose of 600 mg in 39 patients, and peramivir alone in 1 patient.

The mean time from onset to admission was 6.67 (range, 1–15) days. About 91.2% of our patients had received antibiotics before admission, and empiric antibiotic therapy was administered to 58 (69.9%) patients after admission. The antibiotic regimens were beta-lactam monotherapy (in 41 patients, 69.5%), beta-lactam plus vancomycin (in 6 patients, 10.1%), fluoroquinolone monotherapy (in 4 patients, 6.8%), carbapenem monotherapy (in 2 patients, 3.4%), vancomycin monotherapy (in 1 patient, 1.8%), fluoroquinolones plus beta-lactam (in 2 patients, 3.4%), and fosfomycin monotherapy and other combinations (in 2 patients, 3.4%). Empiric antibiotic treatment was inappropriate in 4 (25%) of 16 cases with bacterial coinfection, and 3 patients with inappropriate treatment died. The pathogens associated with inappropriate treatment were *A. baumannii* in 2 cases, 1 case each of MRSA and *E. coli*.

The all-cause hospital mortality was 28.9% (24/83), and the 90-day mortality was 31.3% (26/83). In the univariate analysis, age; bacterial coinfection; comorbidities; APACHE II score; the presence of shock;

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