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Bacterial co-infection with H1N1 infection in patients admitted with community acquired pneumonia

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Summary *Background:* Bacterial co-infection is an important contributor to morbidity and mortality during influenza pandemics .We investigated the incidence, risk factors and outcome of patients with influenza A H1N1 pneumonia and bacterial co-infection.
Methods: Prospective observational study of consecutive hospitalized patients with influenza A H1N1 virus and community-acquired pneumonia (CAP). We compared cases with and without bacterial co-infection.
Results: The incidence of influenza A H1N1 infection in CAP during the pandemic period was 19% (*n*, 667). We studied 128 patients; 42(33%) had bacterial co-infection. The most frequently isolated bacterial pathogens were *Streptococcus pneumoniae* (26, 62%) and *Pseudomonas aeruginosa* (6, 14%). Predictors for bacterial co-infection were chronic obstructive pulmonary disease (COPD) and increase of platelets count. The hospital mortality was 9%. Factors associated with mortality were age ≥65 years, presence of septic shock and the need for mechanical ventilation. Although patients with bacterial co-infection presented with higher Pneumonia

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Severity Index risk class, hospital mortality was similar to patients without bacterial co-infection (7% *vs.* 11%, respectively, $p = 0.54$).
Conclusion: Bacterial co-infection was frequent in influenza A H1N1 pneumonia, with COPD and increased platelet count as the main predictors. Although associated with higher severe scales at admission, bacterial co-infection did not influence mortality of these patients.

Introduction

Influenza virus infection is an important cause of morbidity and mortality.[1] In April-2009, several patients were infected with a novel H1N1 swine-origin influenza virus A in North America[2] and the World Health Organization (WHO) declared an influenza pandemic, caused by novel S-OIV A (H1N1) in June 11, 2009.[3] In December 5, 2009, 208 countries had reported cases and over 10,000 deaths had been registered. In August 10, 2010 the WHO announced that the H1N1 pandemic had moved into the post-pandemic period, and reported a total of 18,500 confirmed deaths worldwide.[4]

Seasonal and pandemic influenza are frequently complicated by bacterial infections.[5] Bacterial co-infection has been found in around 30% of all cases with seasonal influenza, and the pathogens most often reported include *Haemophilus influenzae*, *Staphylococcus aureus* and *Streptococcus pneumoniae*.[6]

Bacterial co-infection is an important contributor to morbidity and mortality. Bacterial pneumonia complicating influenza infection was a major cause of death during the 1918 influenza pandemic,[7,8] and during periods of seasonal influenza activity in inter-pandemic periods.[9]

Bacterial co-infection was frequently reported in fatal cases during the 2009 influenza A H1N1 pandemic,[10,11] with *S. pneumoniae* as the most frequent pathogen identified. Reports on specific populations such as critically-ill patients found bacterial co-infection ranging from 18% to 33% patients with 2009 influenza A H1N1 virus pneumonia.[12–14] However, the incidence and the role of bacterial co-infection in the outcome of patients with influenza A H1N1 virus-associated pneumonia are not well described in the general population.

We therefore determined the incidence, risk factors and outcomes of patients with influenza A H1N1 virus-associated community-acquired pneumonia and bacterial co-infection.

Methods

Study design and patients

This was a prospective, observational study of 128 consecutive adult patients hospitalized with diagnosis of influenza A (H1N1) and community-acquired pneumonia (CAP). Patients were enrolled from 2 Spanish centers, Hospital Clinic of Barcelona and Hospital La Fe of Valencia, from May-2009 to February-2010. The following information was recorded: demographic data, co-morbidities, time of illness onset and hospital admission, previous antibiotic and corticosteroids therapy, influenza and pneumococcal vaccination, microbiological, chest radiologic, laboratory findings and complications. To determine the severity of illness, the Pneumonia Severity Index (PSI)[15] was calculated in all patients within 24 h from admission. We excluded patients with immunosuppression (e.g., patients with neutropenia after chemotherapy or bone marrow transplantation, patients with drug-induced immunosuppression as a result of solid-organ transplantation or corticosteroid or cytotoxic therapy, and patients with HIV-related disorders) and health care associated pneumonia (HCAP) patients.

This study was approved by the Ethics Committees of both centers (Register: 2009/5251). Patients' identification remained anonymous and informed consent was waived due to the observational nature of the study and the fact that this activity is an emergency public health response.

Microbiological studies

Protocolized samples were performed in all patients with diagnosis of CAP at hospital admission in the two institutions. Samples considered valid for microbiological assessment included, sputum culture, two sets of blood cultures, and urine antigens of *S. pneumoniae* and *Legionella pneumophila* were applied for all patients. Detection of *S. pneumoniae* antigen in urine was performed by a rapid immunochromatographic assay (NowTM; Binax, Portland, ME, USA), detection of *L. pneumophila* serogroup I antigen in urine was performed by an immunoenzymatic comercial method (Legionella Urinary Antigen; Binax). Other additional diagnostic sampling techniques occasionally used were pleural puncture, tracheobronchial aspirates (predefined thresholds $\geq 10^5$ cfu/ml) and bronchoscopy with quantitative cultures of bronchoalveolar lavage (predefined thresholds $\geq 10^4$ cfu/ml).

Sputum and blood samples were obtained for bacterial culture before start of antibiotic therapy in the emergency department. Urine samples for *S. pneumoniae* and *L. pneumophila* antigen detection were obtained within 24 h after hospital admission. Valid sputum sample criteria were: purulent sample (polymorphonuclear leukocytes ≥ 25 per high power microscopic field and few squamous epithelial cells ≤ 10 per high power microscopic field). Blood samples for serology of atypical pathogens was performed at admission and within the third and sixth week thereafter when possible. This protocol of diagnosis was the same in the two institutions.

All patients admitted to the hospital in this period with a diagnosis of CAP were tested for influenza A (H1N1) in each institution. Nasopharyngeal-swab specimens were collected at admission, viral diagnosis was performed on RNA from nasopharyngeal-swab swabs in the Microbiology Services of the participant hospitals by reverse transcription-polymerase chain reaction (RT-PCR)-based methods using reagents provided free of charge by the Centers for Disease Control (CDC, Atlanta, GA, USA), the test was performed in accordance with published guidelines from the CDC.[16] In addition,

nasopharyngeal-swab specimens from all patients were tested with the use of multiplex PCR using the xTAG1 RVP FAST Assay (Luminex—Abbott Molecular, Wiesbaden, Germany) according to the manufacturer's instructions for qualitative detection of influenza virus A and B, respiratory syncytial virus, human coronavirus (strains 229E, OC43, NL63 and HKU1), parainfluenza types 1, 2, 3 and 4, human metapneumovirus, rhinovirus/enterovirus, adenovirus, and human bocavirus. The diagnosis of atypical pneumonia was based on the following tests: a fourfold increase in IgG levels for *Mycoplasma pneumoniae* $\geq$ 1:64; *Chlamidophila pneumoniae* $\geq$ 1:512; *L. pneumophila* $\geq$ 1:256; *Coxiella burnetii* $\geq$ 1:160 or a single increased IgM titer (*M. pneumoniae* $\geq$ 1:16; *C. pneumoniae* $\geq$ 1:16; *C. burnetii* $\geq$ 1:80). IgG was evaluated by complement fixation (Diesse) for *L. pneumophila*, *C. burnetti*, *C. pneumoniae* and *M. pneumoniae;* IgM for *C. pneumoniae* and *M. pneumoniae* were evaluated by enzyme immunoassay (ELISA) Vircell and Virotec respectively.

Definitions

Definition of CAP was based on current Infectious Disease Society of America (IDSA)/American Thoracic Society (ATS) guidelines.[17] Severe CAP was defined as the presence of either one of two major criteria, or at least three of nine minor criteria.[17] Fever was defined as two or more consecutive measurements $\geq$38 °C. We registered the presence of septic shock[18] and acute respiratory distress syndrome (ARDS) criteria.[19]

A confirmed case was defined as a patient with diagnosis of pneumonia with laboratory-confirmed pandemic influenza A H1N1 virus infection by RT-PCR. Only confirmed cases were included in the current study.

Bacterial co-infection was diagnosed in patients with one or more positive cultures obtained from blood, other normally sterile fluids, or valid sputum, bronchoscopic samples and/or positive urinary antigens (*S. pneumoniae* and *L. pneumophila*) at the time of hospital admission.

Statistical analysis

Categorical variables were described by frequencies and percentages. Continuous variables were described by means and standard deviations (SD) or the median and interquartile range (IQR) for data not normally distributed (Kolmogorov—Smirnov test). Categorical variables were compared with the chi-square test or Fisher's exact test where appropriate. Continuous variables were compared using the Student's *t*-test once normality was demonstrated; otherwise the non-parametric Mann—Whitney *U* test was performed. Univariate and multivariate logistic regression analyses were performed to identify variables predictive of patients with bacterial co-infection (dependent variable). The variables analyzed were: age, gender, body mass index (BMI), smoking, alcohol consumption, previous antibiotic, influenza vaccination, pneumococcal vaccination, chronic obstructive pulmonary disease (COPD), chronic cardiovascular disease, diabetes mellitus, neurological disease, chronic renal disease, chronic liver disease, Pneumonia Severity Index (PSI) risk class, serum creatinine, serum creatinine kinase, serum lactate dehydrogenase, C-reactive protein, leukocyte, platelets, mechanical ventilation, septic shock, and multilobar infiltration. Univariate and multivariate logistic regression analyses were performed to predict 30-day mortality (dependent variable). The independent variables analyzed were those mentioned above plus, $Pa0_2/Fio_2$, mechanical ventilation, bacterial co-infection, bacteremia and ARDS criteria. Variables that showed a significant result univariately ($p < 0.1$) were included in the multivariate logistic regression backward stepwise model to determine which of them were independently related to prognosis. The Hosmer—Lemeshow goodness-of-fit test was performed to assess the overall fit of the model.[20] The predictive capacity for bacterial co-infection of continuous variables was assessed with receiver operating characteristic (ROC) curves; the area under the curve (AUC), optimal cut-off value, sensitivity, specificity, predictive positive value, predictive negative value, positive likelihood ratio, and negative likelihood ratio were calculated. All tests were two-tailed and significance was set at 5%. All analyses were performed with SPSS version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Study population

During the study period 667 consecutive patients admitted with CAP in both hospitals (302 in Barcelona, 365 in Valencia) were registered. Among them, 128 (19%) patients had influenza A H1N1 pneumonia (57, 19% in Barcelona, and 71, and 19% in Valencia).

The mean age was 44 $\pm$ 17 years (range 18—90); only 15 (12%) patients were older than 65 years. Fifty-one (40%) patients had co-morbidities, 14 (12%) patients were obese (BMI $\geq$ 30 and $<$40) and 1 (1%) patient was morbidly obese (BMI $\geq$ 40). Five (4%) patients were pregnant women. The main demographic and clinical characteristics of patients are detailed in Table 1.

The median (IQR) time from the onset of symptoms to hospitalization was 5 (3—9) days. The most frequent symptoms and signs on hospital admission were cough (88%), fever (88%), dyspnea (57%), arthromyalgia (46%), chills (46%), gastrointestinal manifestations (32%), pleural pain (24%), rhinorrhea (10%). Thirty-three (26%) patients had received previous antibiotic treatment before admission. The vast majority of patients were classified as low risk, according to a PSI risk class $\leq$3 (112, 88%).

Bacterial co-infection

Overall, 42 (33%) patients had bacterial co-infection. The bacterial pathogens identified are summarized in Table 2. Four (10%) patients with bacterial co-infection had bacteremia (*S. pneumoniae* in 3 cases and *Fusobacterium* sp. in 1).

Patients with bacterial co-infection had more frequently COPD, higher PSI risk class and leukocyte and platelets counts, and longer length of hospital stay. There was a non-significant trend for higher serum levels of C-reactive protein, and more frequent need for mechanical ventilation. However, the need for ICU admission, and the rates of septic shock and 30-day hospital mortality were similar among patients with and without bacterial co-infection (Table 1).

Table 1 Demographic and clinical characteristics of patients with influenza A H1N1 pneumonia.

Characteristics	No bacterial co-infection ($N = 86$)	Bacterial co-infection ($N = 42$)	p-value
Age (years), mean ± SD	44 ± 16	44 ± 19	0.98
Sex (male), *n* (%)	45 (53)	23 (55)	0.79
Current smoking, *n* (%)	16 (19)	7 (17)	0.90
Current alcohol abuse, *n* (%)	8 (9)	3 (7)	0.71
Previous antibiotic, *n* (%)	24 (29)	9 (22)	0.43
Influenza vaccine, *n* (%)	12 (15)	9 (22)	0.29
Pneumococcal vaccine, *n* (%)	5 (6)	2 (5)	>0.99
BMI (kg/m^2), mean ± SD	25.7 ± 4.2	25.8 ± 5.7	0.89
Co-morbidities, *n* (%)			
Chronic respiratory disease	20 (23)	14 (33)	0.22
COPD	2 (2)	10 (24)	<0.001
Asthma	15 (17)	4 (10)	0.23
Chronic cardiovascular disease	7 (8)	3 (7)	0.84
Diabetes mellitus	7 (8)	2 (5)	0.48
Neurological disease	6 (7)	2 (5)	0.62
Chronic liver disease	2 (2)	1 (2)	0.98
Chronic renal disease	1 (1)	1 (2)	0.60
Laboratory finding, median (IQR)			
Serum creatinine (mg/dL)	0.8 (0.7–1.0)	0.9 (0.7–1.2)	0.064
Serum CK (U/L)	90 (57–240)	91 (54–152)	0.67
Serum LDH (U/L)	501 (373–912)	548 (403–900)	0.81
C-reactive protein (mg/dL)	9.3 (5.1–19.2)	14.9 (10.8–21.3)	0.052
Leukocyte count (10^9/L)	7.3 (4.7–11.4)	9.9 (6.1–14.7)	0.037
Platelets count (per mm^3)	180 (147–256)	222 (169–292)	0.014
PSI risk class IV–V, *n* (%)	6 (7)	10 (24)	0.007
Severe CAP, *n* (%)	26 (39)	18 (44)	0.60
ICU admission, *n* (%)	24 (28)	14 (33)	0.48
PaO_2/FIO_2, median (IQR)	288 (232–310)	260 (162–311)	0.40
Mechanical ventilation, *n* (%)	9 (11)	9 (22)	0.10
Septic shock, *n* (%)	17 (22)	11 (28)	0.46
Multilobar infiltration, *n* (%)	35 (43)	17 (42)	0.91
Pleural effusion	5 (6)	2 (5)	0.71
ARDS criteria, *n* (%)	6 (8)	4 (10)	0.59
Hospital stay (days), median (IQR)	5 (3–9)	7 (4–9)	0.036
30-day mortality, *n* (%)	9 (11)	3 (7)	0.54

Abbreviations: COPD = chronic obstructive pulmonary disease; PSI = pneumonia severity index; IQR = interquartile range; LDH = lactate dehydrogenase; CAP = community-acquired pneumonia; ICU = intensive care unit; PaO_2/FIO_2 = arterial oxygen tension to inspired oxygen fraction ratio; ARDS = acute respiratory distress syndrome; BMI = body-mass index. Percentages were based on the number of patients with non-missing information.

Statistically significant variables in the univariate analysis are reported in Table 3. In multivariate analysis the independent predictors of bacterial co-infection were underlying COPD and increased platelets count at admission. The model was well calibrated with p-value in Hosmer–Lemeshow test 0.41. Using ROC analysis, the optimal cut-point for bacterial co-infection was 181,000 per mm^3, with AUC 0.63 (0.53–0.73) (74% sensitivity, 51% specificity, 43% predictive positive value, 80% predictive negative value, 1.51 positive likelihood ratio, and 0.51 negative likelihood ratio).

Antimicrobial treatment

The antibiotic regimens were fluoroquinolone monotherapy (63, 49%), beta-lactam plus macrolide (30, 23%), fluoroquinolones plus beta-lactam (19, 15%), beta-lactam monotherapy (6, 5%), and other combinations (10, 8%). All patients received oseltamivir at doses of 75 mg bid or 150 mg bid, for 5–10 days.[21] Twenty-seven (24%) patients received prior steroids at admission.

The empirical antibiotic treatment was inappropriate in 7 (17%) out of 42 cases with bacterial co-infection and only one patient with inappropriate treatment died. The pathogens most frequently associated to inadequate treatment were *P. aeruginosa* in 5 cases, *M. pneumoniae* and *Fusobacterium* in 1 case each.

Analysis of mortality

Twelve (9%) patients died in the hospital, in all cases in the ICU. The characteristics of survivors and non-survivors are detailed in Table 4.

Bacterial co-infection was similarly frequent in non-survivors and survivors.

Several variables were significantly associated with death in univariate analysis (Table 5). In multivariate

Table 2 Bacterial co-infection in study populations.[a]

Pathogen	Number of patients[b] ($n = 42$)	Blood culture ($n = 38$)	Sputum culture ($n = 16$)	Urinary antigen ($n = 39$)	BAL/BAS ($n = 9$)	Pleural effusion culture ($n = 3$)	Serology ($n = 30$)
S. pneumoniae	26 (62)	3 (7.8)	7 (43.7)	24 (61.5)	3 (33.3)	—	—
S. pyogenes	1 (2)	—	1 (6.3)	—	—	1 (33.3)	—
S. aureus	2 (5)	—	2 (12.5)	—	—	—	—
M. pneumoniae	3 (7)	—	—	—	—	—	3 (10)
M. catarrhalis	1 (2)	—	1 (6.3)	—	—	—	—
C. burnetti	1 (2)	—	—	—	—	—	1(3.3)
E. coli	1 (2)	—	—	—	1 (11.1)	—	—
P. aeruginosa	6 (14)	—	4 (25.0)	—	5 (55.5)	—	—
Fusobacterium sp.	1 (2)	1(2.6)	—	—	—	—	—

[a] Data are presented as number (percentage).
[b] Total number of patients for each etiologic agent.

logistic regression analysis, independent predictors of 30-day hospital mortality were age ≥65 years, the need for mechanical ventilation and the presence of septic shock. Bacterial co-infection was not associated to an increased mortality. The model was well calibrated with *p*-value in Hosmer–Lemeshow test 0.27.

Discussion

Bacterial co-infection was frequent (33%) in patients hospitalized with influenza A H1N1 pneumonia. The most relevant predictors of bacterial co-infection were underlying COPD and higher platelet count at admission. Although associated with higher PSI risk class, bacterial co-infection was not related with increased mortality in these patients.

This is the first investigation that reports all consecutive patients admitted with influenza A H1N1 pneumonia during the whole 2009–2010 pandemic period at two Spanish hospitals with experience in the study of respiratory infections. Unlike previous trials,[12–14] we included both critically and non-critically ill patients. All patients admitted to the hospital with CAP during this period underwent a systematic microbial investigation that included detection tests for Influenza A H1N1 virus and bacterial pathogens. Interestingly, both hospitals found that 19% cases of hospitalized pneumonia during this pandemic period presented with influenza A H1N1 infection.

The rate of bacterial co-infection in our series, 33%, was slightly higher than 21% reported for patients with seasonal influenza-associated CAP[22] and for critically-ill ICU patients during the 2009 influenza A H1N1 pandemic (18%–33%).[12–14]

Reports in fatal cases of influenza A H1N1 shown a great variability in the rate of bacterial pathogens detected at autopsy, ranging from 25% to 55%.[10,11,23,24] The rate of bacterial co-infection in our series could possible be underestimated since 26% of our patients had received previous antibiotics, thus limiting the chance to detect bacterial co-infection. Thus, the true bacterial co-infection rate might be even higher. Indeed, a study using molecular techniques such as MassTag PCR testing for 33 microbial agents in nasopharyngeal swabs found 76% rate of bacterial pathogens in a sample of patients with influenza A H1N1.[25]

S. pneumoniae was the most frequent bacterial pathogen in our series. This is in accordance with recent studies evaluating seasonal[22] and novel influenza A H1N1-associated pneumonia.[11,13,21] Unexpectedly, *P. aeruginosa* was the second most frequent bacterial pathogen, whereas *S. aureus* was rarely found, and we did not identify *H. influenzae*. The presence of *P. aeruginosa* may be related to the high proportion patients with severe CAP[26,27] and COPD[17] in the bacterial co-infection group. The absence of *H. influenzae* in our patients is also unusual. This pathogen was the

Table 3 Significant univariate and multivariate logistic regression analyses of bacterial co-infection.

Variable	Univariate			Multivariate		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
COPD	11.79	2.42–57.29	0.002	9.66	1.93–48.31	0.002
C-reactive protein (+1 mg/dL)	1.04	1.00–1.07	0.070	—	—	—
Platelets count (per mm^3) (+10 units)	1.06	1.02–1.11	0.009	1.05	1.00–1.11	0.041
PSI risk class IV – V	4.17	1.40–12.42	0.010	—	—	—

Abbreviations: OR, odds ratio; CI, confidence interval; NA, not available; "+1 mg/dL" indicates the increase by one mg/dL; "+10 units" indicates the increase by ten units.

Table 4 Comparison of the clinical characteristics and laboratory between influenza A (H1N1) pneumonia patients who died and those who survived.

Characteristics	Survivors ($N = 116$)	Non-survivors ($N = 12$)	p-value
Age (years), mean ± SD	43 ± 16	51 ± 25	0.34
Age > 65 years, *n* (%)	10 (9)	5 (42)	<0.001
Sex (male), *n* (%)	59 (51)	9 (75)	0.11
Current smoking, *n* (%)	22 (19)	1 (8)	0.089
Current alcohol abuse, *n* (%)	8 (7)	3 (25)	0.10
Previous antibiotic, *n* (%)	30 (27)	3 (25)	0.90
Influenza vaccine, *n* (%)	17 (15)	4 (33)	0.022
Pneumococcal vaccine, *n* (%)	7 (6)	0 (0)	0.44
Obesity (BMI ≥ 30), *n* (%)	14 (13)	1 (13)	0.75
Co-morbidities, *n* (%)			
Chronic respiratory disease	30 (26)	4 (33)	0.57
COPD	11 (10)	1 (8)	0.89
Asthma	16 (14)	3 (25)	0.29
Chronic cardiovascular disease	5 (4)	5 (42)	<0.001
Diabetes mellitus	7 (6)	2 (17)	0.17
Neurological disease	7 (6)	1 (8)	0.75
Chronic liver disease	2 (2)	1 (8)	0.26
Chronic renal disease	1 (1)	1 (8)	0.25
PSI IV–V, *n* (%)	11 (10)	5 (42)	0.001
Laboratory finding, median (IQR)			
Serum creatinine (mg/ldL)	0.8 (0.7–1.0)	1.0 (0.8–1.6)	0.098
Serum LDH (U/L)	484 (374–902)	758 (456–1138)	0.069
C-reactive protein (mg/dL)	11.3 (5.2–19.4)	18.8 (9.0–23.6)	0.20
Leukocyte count (10^9/L)	7.7 (4.8–13.5)	7.8 (7.1–12.5)	0.35
Platelets count (per mm^3)	198 (153–261)	201 (135–249)	0.78
$Pa0_2/Fio_2$, median (IQR)	279 (224–328)	261 (186–300)	0.18
ICU admission, *n* (%)	26 (22)	12 (100)	<0.001
Severe CAP criteria, *n* (%)	32 (33)	12 (100)	<0.001
Mechanical ventilation, *n* (%)	10 (9)	8 (67)	<0.001
Septic shock, *n* (%)	19 (18)	9 (82)	<0.001
Multilobar infiltration, *n* (%)	43 (39)	9 (75)	0.016
ARDS criteria, *n* (%)	7 (7)	4 (36)	0.002
Bacterial co-infection, *n* (%)	39 (34)	3 (25)	0.54
Bacteremia, *n* (%)	3 (3)	1 (8)	0.27
Hospital stay (days), median (IQR)	5 (3–8)	7 (3–14)	0.38

Abbreviations: COPD = chronic obstructive pulmonary disease; PSI = pneumonia severity index; IQR = interquartile range; LDH = lactate dehydrogenase; CAP = community-acquired pneumonia; ICU = intensive care unit; PaO_2/FIO_2 = arterial oxygen tension to inspired oxygen fraction ratio; ARDS = acute respiratory distress syndrome; BMI = body-mass index. Percentages were based on the number of patients with non-missing information.

most frequent bacterial pathogen identified in patients with Influenza A H1H1 in Argentina.[25] However, the molecular techniques such as PCR used in this study are well known to improve diagnosis of the etiology of CAP.[28] Despite a recent report on three cases of H1N1-associated pneumonia and c-MRSA,[29] we did not find any case of MRSA in our series. As regards to *S. aureus*, this was the most frequent bacterial pathogen identified in a much selected population of severely immunosuppressed patients with solid organ transplant,[30] which is substantially different that that from the present study.

This is the first study to assess the predictors of bacterial co-infection in influenza A H1N1 pneumonia in multivariate analysis. Underlying COPD and increasing platelet counts were independently predictive of the presence of bacterial co-infection in our series. Previous tracheobronchial colonization is frequent in COPD patients.[31] Bacterial pathogens such as *S. pneumoniae* and *P. aeruginosa* are frequently reported in COPD exacerbations of bacterial etiology,[32–34] and this may explain the presence of these bacteria as the most frequent bacterial isolates in our patients. Thrombocytosis, as well as thrombocytopenia, was recently described as independent predictor of death from CAP.[35] Platelets are inflammatory cells with an important role in antimicrobial host defenses and hence appear to be a marker of bacterial infection in patients with CAP.

Table 5 Significant univariate and multivariate logistic regression analyses of mortality.

Variable	Univariate			Multivariate		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Age $\geq$ 65 years	7.57	2.03–28.29	0.003	10.06	1.48–68.21	0.018
Influenza vaccine	4.61	1.12–18.93	0.034	–	–	–
Chronic cardiovascular disease	15.86	3.70–68.01	<0.001	–	–	–
Serum creatinine (+1 mg/dL)	1.84	1.04–3.28	0.038	–	–	–
Serum LDH (+100 U/L)	1.11	1.00–1.25	0.060	–	–	–
PSI IV – V	6.82	1.85–25.14	0.004	–	–	–
Mechanical ventilation	20.20	5.16–79.08	<0.001	12.27	2.02–74.40	0.006
Septic shock	21.08	4.21–105.48	<0.001	8.80	1.45–53.60	0.018
Multilobar infiltration	4.74	1.22–18.51	0.025	–	–	–
ARDS criteria	7.35	1.72–31.3	0.007	–	–	–

Abbreviations: OR = odds ratio; CI = confidence Interval; LDH = lactate dehydrogenase; PSI = pneumonia severity index; ARDS = acute respiratory distress syndrome. "+1 mg/dL" indicates the increase by one mg/dL; "+100 U/L" indicates the increase by one hundred U/L.

In our study higher levels of C-reactive protein at admission were nearly significantly higher in patients with bacterial co-infection. Although we did not measure Procalcitonin, a recent study showed that Procalcitonin and C-reactive protein may potentially assist in the discrimination between severe lower respiratory tract infections of bacterial and 2009 influenza A H1N1 origin.[36] Due to the increasing evidence on the usefulness of biomarkers in the diagnosis and management of CAP.[37] the role of biomarkers to discriminate between patients with influenza A H1N1 with and without bacterial co-infection needs further prospective investigation.

The overall hospital mortality from influenza A H1N1 pneumonia in our population, 9%, was slightly higher than that expected in patients with CAP in general, 5%[38] and seasonal influenza-associated pneumonia, 4.4%.[22] Although our patients were relatively young in average, age >65 years, together with major severity criteria such as septic shock and the need for invasive ventilation were independent predictors of mortality, as observed in previous studies.[39]

Our data do not support a specific impact of bacterial co-infection in the outcome of influenza A H1N1 pneumonia, despite the fact that bacterial co-infection was associated with higher PSI risk class at admission and a trend for worse renal function and more need for mechanical ventilation. However, current severity scores such as PSI have limited value in influenza A H1N1 pneumonia, since they underestimate mortality rates likely due to the your average age of these patients, as recently reported.[40]

Two important strengths of our study were that patients were included consecutively avoiding in that way potential bias and that we used a systematic microbiological diagnostic protocol in two centers.

Several limitations need to be addressed. First, bacterial co-infections might have been underestimated because 26% cases had received previously antibiotics. Second, the relatively low number of deaths, possibly due to the favorable influence of the administration of oseltamivir to all patients,[21,41] may limit the identification of other factors potentially related with death.

In conclusion, our data indicate that bacterial co-infection was frequent in influenza A H1N1 pneumonia, with COPD and increased platelet count as the main predictors. Although associated with higher severe scales at admission, bacterial co-infection did not influence mortality of these patients.

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