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## Can procalcitonin help identify associated bacterial infection in patients with severe influenza pneumonia? A multicentre study

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**Abstract Purpose:** To determine whether procalcitonin (PCT) levels could help discriminate isolated viral from mixed (bacterial and viral) pneumonia in patients admitted to the intensive care unit (ICU) during the A/H1N1v2009 influenza pandemic. **Methods:** A retrospective observational study was performed in 23 French ICUs during the 2009 H1N1 pandemic. Levels of PCT at admission were compared between patients with confirmed influenzae A

pneumonia associated or not associated with a bacterial co-infection. **Results:** Of 103 patients with confirmed A/H1N1 infection and not having received prior antibiotics, 48 (46.6%; 95% CI 37–56%) had a documented bacterial co-infection, mostly caused by *Streptococcus pneumoniae* (54%) or *Staphylococcus aureus* (31%). Fifty-two patients had PCT measured on admission, including 19 (37%) having bacterial co-infection. Median (range 25–75%) values of PCT were significantly higher in patients with bacterial co-infection: 29.5 (3.9–45.3) versus 0.5 (0.12–2) µg/l ( $P < 0.01$ ). For a cut-off of 0.8 µg/l or more, the sensitivity and specificity of PCT for distinguishing isolated viral from mixed pneumonia were 91 and 68%, respectively. Alveolar condensation combined with a PCT level of 0.8 µg/l or more was strongly associated with bacterial co-infection (OR 12.9, 95% CI 3.2–51.5;  $P < 0.001$ ). **Conclusions:** PCT may help discriminate viral from mixed pneumonia during the influenza season. Levels of PCT less than 0.8 µg/l combined with clinical judgment suggest that bacterial infection is unlikely.

**Keywords** Procalcitonin · Pneumonia · A/H1N1v influenza · Bacterial infection

## Introduction

The new pandemic A/H1N1 influenza virus emerged and spread globally in 2009, with a high rate of intensive care unit (ICU) admissions among hospitalised patients [1]. Causes of death included an overwhelming viral infection and primary or secondary bacterial infection. Because of the presumed high frequency of bacterial infection, most hospitalised patients with influenza pneumonia are administered antibiotics, even though bacterial co-infection is considered unlikely [2]. Indeed, bacterial pneumonia cannot be differentiated from viral pneumonia on the basis of the patients' characteristics, chest radiographic findings or routine laboratory results. Procalcitonin (PCT) is a recognised marker of bacterial infection and might be a prognostic marker in lower respiratory tract infections [3]. Few studies have assessed PCT levels in viral infections, except for paediatrics studies in which PCT was found to help distinguishing viral from bacterial meningitis [4] or pneumonia [5]. A small study in Singapore during the Coronavirus outbreak suggested that PCT remained at low levels in viral infections [6].

This study aimed to examine whether PCT levels may help discriminate between viral from mixed (bacterial and viral) pneumonia among patients presenting to the ICU with severe community-acquired pneumonia during the H1N1v2009 influenza pandemic.

## Methods

This was an observational multicentre study conducted in conjunction with the 'REVA-Grippe-SRLF' registry set up in France from November 2009 to April 2010 to record patients with severe A/H1N1v2009 influenza infection admitted to ICUs. Among the 103 participating centres, 23 volunteered to participate in this substudy and completed a specific case report form, whether or not a diagnosis of bacterial co-infection had been established. Some of these centres routinely performed measurements of PCT and/or C-reactive protein (CRP) levels, and the biomarker levels were recorded and analysed for the present study. Microbiological investigations and biomarker levels were obtained as part of the routine clinical management of patients, at the discretion of the treating physician. The study was approved by the ethics review board of the Société de Réanimation de Langue Française and informed consent was waived.

Patients with a confirmed diagnosis of H1N1 influenza infection (by PCR on nasopharyngeal secretions or bronchoalveolar lavage fluid), associated with a clinical pattern of community-acquired pneumonia as defined by the association of the acute onset of clinical symptoms (cough, fever, dyspnoea), and compatible infiltrates on the

chest radiograph, in the absence of an alternative diagnosis, were eligible for this study. To better distinguish patients with and without bacterial co-infection on admission, for this analysis we selected patients not having received antibiotics prior to ICU admission. We excluded patients with suspected hospital-acquired influenza infection, a documented non-pulmonary bacterial infection, and severely immunocompromised patients.

Demographics, clinical and microbiological data obtained within the first 48 h of ICU admission were collected retrospectively. Confirmation of bacterial pulmonary infection was obtained through blood cultures, urinary antigen (pneumococcal and Legionella) tests and/or culture of a respiratory tract secretions sample. Patients were thus categorised as having or not having associated bacterial co-infection and levels of biomarkers were compared between these two subgroups.

Procalcitonin was assayed using time-resolved amplified cryptate emission technology on a Kryptor analyser (Brahms Diagnostica, Berlin, Germany) and functional assay (detection concentration 0.06 µg/l). Total PCT assay imprecision was reported by the manufacturer to be 10% at 0.20 µg/l and less than 6% at more than 0.30 µg/l.

## Statistics

Fisher's exact test was used to compare proportions for categorical variables. For continuous variables, Student's *t* test and Mann-Whitney *U* test were used for comparing parametric and non-parametric data, respectively. The diagnostic accuracy of biomarkers was examined by their receiver-operating curve (ROC). Statistical analyses were performed with PASW Statistic 18.0 (SPSS Inc, Chicago, USA).

## Results

This substudy from the French REVA-SRLF influenza registry was conducted in 24 centres, where data on 188 patients with or without a diagnosis of bacterial co-infection were recorded; 32 of these patients were excluded from analysis based on our predefined exclusion criteria, and a further 53 were excluded because of administration of antibiotics prior to ICU admission. Thus, 103 patients formed the main cohort, of whom 48 (46.6%) had a documented bacterial co-infection associated with influenza A/H1N1v2009 infection (Table 1). Microorganisms identified included *Streptococcus pneumoniae* (*n* = 26, 54%), *Staphylococcus aureus* (*n* = 13, 17%), group A *Streptococcus* (*n* = 4, 8%) and other microorganisms (*n* = 5, 11%). They were recovered from blood cultures (*n* = 19, 40%) and/or a respiratory tract

**Table 1** Demographic, clinical and laboratory features of 103 patients having influenza pneumonia, with or without associated bacterial co-infection

	Bacterial co-infection (n = 48)	Influenza only (n = 55)	p	OR (95% CI)
Male, n (%)	26 (54.2)	27 (49.1)	0.74	0.61 (0.56–2.7)
Age, years, median (IQR)	43 (27–56)	43 (25–55)	0.87	0.99 (0.97–1.02)
No comorbidity, n (%)	21 (43.7)	12 (21.8)	0.02	2.8 (1.15–6.8)
SAPS 3 score, median (IQR)	54 (42–70)	44 (34–54)	0.006	1.03 (1.007–1.05)
CURB-65 $\geq 3$ , n (%)	21 (43.8)	14 (25.5)	0.051	2.3 (0.97–5.3)
Peak temperature, °C (mean $\pm$ SD)	39 (38.8–39.6)	39 (38.5–40)	0.76	–
Alveolar infiltrates on chest X-ray, n (%)	40 (83.3)	29 (52.7)	0.001	4.5 (1.7–12.4)
PaO <sub>2</sub> /FiO <sub>2</sub> ratio, median (IQR)	116 (80–214)	178 (137–252)	0.04	–
PaO <sub>2</sub> /FiO <sub>2</sub> <200	27 (56.2)	20 (36.4)	0.04	2.3 (1.0–5.1)
Admission WBC ( $\times 10^9/l$ ), median (IQR)	8.7 (3.3–12.0)	8.4 (5.0–13.3)	0.38	–
Admission CRP (mg/l) (IQR) <sup>a</sup>	260 (110–347)	95 (57–161)	0.002	–
Admission PCT ( $\mu g/l$ ) (IQR) <sup>b</sup>	29.5 (4.0–45.4)	0.5 (0.1–1.8)	<0.001	–
Mechanical ventilation, n (%)	36 (75.0)	26 (47.3)	0.01	3.3 (1.4–8.1)
Length of ICU stay (mean $\pm$ SD)	12.5 (4.5–29)	5 (3–16)	0.009	–
Death n (%)	10 (20.8)	8 (14.6)	0.4	1.5 (0.5–4.3)

CURB-65 confusion, urea  $\geq 7$  mmol/l, respiratory rate  $\geq 30$ , systolic blood pressure  $< 90$  mmHg or diastolic  $< 60$  mmHg, and age  $\geq 65$  years; WBC white blood cells count, IQR interquartile (25th–75th percentile) range, OR odds ratio, SAPS 3 simplified acute physiology score 3

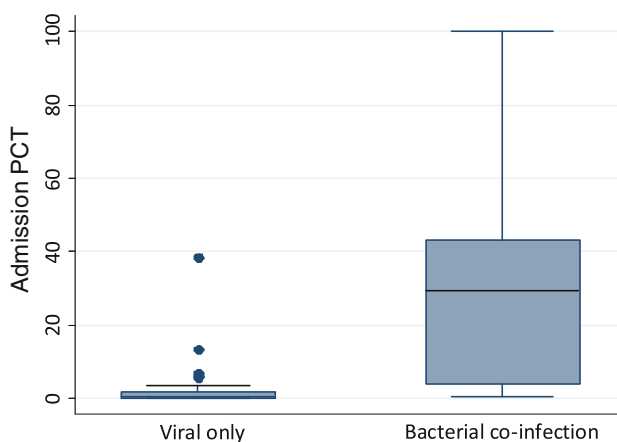
<sup>a</sup> CRP levels were available for 54 patients (24 with and 30 without bacterial co-infection)

<sup>b</sup> PCT levels were available for 52 patients (19 with and 33 without bacterial co-infection)

secretion specimen (n = 34, 71%), including bronchoalveolar lavage (n = 20, 42%) or protected distal sampling (n = 14, 29%) and/or urinary antigen tests (n = 19, 40%).

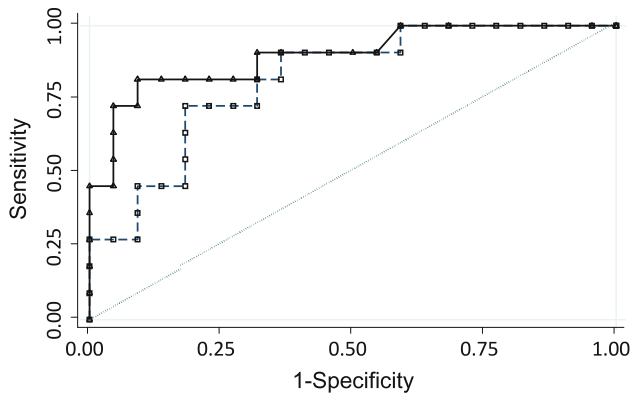
Table 1 compares the admission characteristics of the two subgroups of patients, with and without bacterial co-infection. The former subgroup less often had comorbidities (56.3 vs. 78.2%;  $P = 0.02$ ), had a higher SAPS 3 score (54 vs. 44;  $P = 0.006$ ), and more often had alveolar condensation on chest X-ray (83.3 vs. 52.7%,  $P = 0.001$ ). The overall mortality in the ICU was 17.5% (18/103 patients), and did not differ between those with and without bacterial co-infection (20.8 vs. 14.6%, respectively). Patients with bacterial co-infection more often required invasive mechanical ventilation (34 vs. 26, OR 2.7, 95% CI 1.2–6.1;  $P = 0.016$ ), and had a longer length of stay in the ICU (median 12.5 vs. 5 days;  $P = 0.009$ ).

PCT and CRP levels were obtained respectively in 52 and 54 of the 103 patients, and 32 had both biomarkers measured simultaneously. Of the 52 patients having PCT levels measured on admission, 19 (36.5%) had a documented bacterial co-infection associated with influenza A/H1N1v2009 infection, mostly caused by *Streptococcus pneumoniae* (52%) or *Staphylococcus aureus* (35%). Median (IQR) PCT levels on ICU admission were significantly higher in patients with bacterial co-infection: 29.5 (IQR 4.0–45.4)  $\mu g/l$  versus 0.5 (IQR 0.12–1.8)  $\mu g/l$  ( $P < 0.001$ ) (Fig. 1). A cut-off of 0.8  $\mu g/l$  or more identified bacterial co-infection with a sensitivity of 91%, a specificity of 68% and a negative predictive value of 91%. The area under the ROC curve (AUROC) for diagnosing bacterial co-infection using PCT was 0.90 (95% CI

**Fig. 1** PCT levels on ICU admission in 52 patients having isolated viral or mixed bacterial and viral pneumonia

0.74–1). A PCT level less than 0.8  $\mu g/l$  combined with the lack of alveolar condensation was strongly associated with the absence of bacterial co-infection (OR 12.9, 95% CI 3.2–51.5;  $P < 0.001$ ). Mortality in the ICU was 11.5% (6/52) among this subgroup; PCT levels of 0.8  $\mu g/l$  or more were associated with a more severe outcome (invasive ventilation and/or death in ICU) (OR 7, 95% CI 1.75–28.4;  $P = 0.001$ ).

In the 54 patients in whom CRP levels were measured (24 with and 30 without bacterial co-infection), median (interquartile range 25–75) values were respectively of 95 (57–161) and 260 (110–347) ( $P = 0.002$ , rank-sum test). For the 32 patients in whom both biomarkers levels were



**Fig. 2** ROC analysis of PCT (solid line) and CRP (dashed line) for predicting bacterial co-infection at ICU admission, in patients in whom measurements of the two biomarkers were obtained simultaneously ( $n = 32$ )

determined simultaneously, median values for CRP were 95 (50–161) in patients with viral infection only ( $n = 21$ ) and 276 (146–435) in those with bacterial co-infection ( $n = 11$ ) ( $P = 0.004$ ). Corresponding values for PCT were 0.4 (0.1–1.4) and 34 (12.2–46.7) ( $P = 0.0002$ ). At a cut-off of 0.8  $\mu\text{g/l}$  and of 230  $\text{mg/l}$  for PCT and CRP, respectively, the AUROC to diagnose bacterial co-infection was 0.90 (95% CI 0.78–1) for PCT, compared to 0.82 (95% CI 0.67–0.97) for CRP ( $P = 0.22$ ) (Fig. 2).

## Discussion

We report on 103 patients with severe A/H1N1 influenzae pneumonia, almost one-half of whom had bacterial co-infection documented in the absence of prior antibiotic administration; most co-infections were caused by *Streptococcus pneumoniae*. In the 52 patients in whom PCT was measured, we found that a PCT level of 0.8  $\mu\text{g/l}$  or more discriminated well between isolated viral and mixed (bacterial and viral) pneumonia.

Experience using biomarkers as an diagnostic adjunct during influenza pneumonia is very limited. Studies describing the ability of PCT or of CRP to discriminate between viral and bacterial infection have included few patients with influenza or severe disease [5]. A meta-analysis concluded that PCT was more accurate than CRP for the distinction between viral and bacterial infection [7]. Ingram et al. suggested that PCT levels assisted in the discrimination between severe lower respiratory tract infections of bacterial or A/H1N1 virus origin [8]. Our results suggest that the combination of low levels of PCT and the lack of alveolar infiltrate on chest radiograph makes bacterial co-infection unlikely in patients presenting with severe viral pneumonia.

PCT has emerged as a diagnostic biomarker for estimating the likelihood for a bacterial infection and tailoring antimicrobial therapy [9]; however, its prognostic value is less clear. In critically ill patients, a high maximum PCT level and a PCT increase over 1 day were independent predictors of 90-day all-cause mortality [10]. In patients with *Legionella pneumoniae*, Haeuptle et al. [11] found a high accuracy of initial and serial PCT levels for prediction of mortality and need for ICU admission. High PCT levels were associated in our series with bacterial co-infection and more severe outcomes such as mechanical ventilation and/or death.

Previous studies on patients with influenza pneumonia reported a rate of bacterial co-infection ranging from 20 to 25% of patients [12]; similar rates were reported during the A/H1N1v2009 pandemic season [1]. This proportion may actually be underestimated because of the common administration of antibiotics prior to hospital or ICU admission. Overall, almost one-half of our patients had a documented bacterial co-infection, after excluding those having received prior antibiotics. This is clearly a much higher rate than that recorded during seasonal influenza [13]. Similarly to experience from Argentina [14], the presence of bacterial co-infection was associated in our series with more severe disease.

Limitations of our study include its relatively small sample size, and the lack of repeated PCT measurements. The ‘true’ proportion of patients with bacterial co-infection during influenza pneumonia is also difficult to ascertain. However, by excluding patients having received prior antibiotics, thus allowing a more accurate diagnosis of bacterial infection, we believe we have come closer to estimating the frequency and influence of bacterial co-infection in patients with severe influenza A/H1N1 pneumonia. PCT levels appear to discriminate well between patients having or not having bacterial co-infection during influenza pneumonia. Despite the small sample of patients in our series in whom both measurements of PCT and CRP were obtained, PCT levels appeared to more accurately discriminate viral from mixed viral and bacterial infection among patients presenting with community-acquired pneumonia of suspected viral origin during the influenza epidemic.

In summary, bacterial co-infection likely affected almost one-half of patients with severe influenza A/H1N1 pneumonia, and was associated with more severe outcomes. Measurements of PCT levels at admission can help discriminate patients having bacterial co-infection from those with isolated viral pneumonia. Clinical trials from different settings have established that PCT can be safely used to help decide upon initiation and duration of antibiotic therapy, and thus potentially help to reduce antibiotic overuse [9, 15]. When combined with clinical judgment during influenza epidemics, a low PCT level may identify a subgroup of patients in whom empiric antibiotic therapy may be withheld or withdrawn early.

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## Appendix: List of contributors

The authors gratefully acknowledge the contribution of the following physicians participating to the PCT study within the REVA-SRLF H1N1 registry: Steering Committee of the H1N1 registry: L. Brochard, C. Brun-Buisson, CHU Henri Mondor, Créteil; A. Mercat, CHU d'Angers, Angers; J.C. Richard, CHU Charles Nicolle, Rouen. Investigators: CHU d'Angers, Angers: A. Kouatchet, A. Mercat; CH d'Auxerre: L. Arroudj, D. Royer; Hospital Santa Creu i San Pau, Barcelona: F. Roche Campo, J. Mancebo; CHU Ambroise Paré, Boulogne: C. Charron, A. Vieillard-Baron; CHU G. Montpied,

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