INFLUENZA

Procalcitonin Identifies Bacterial Co-Infections in Vietnamese Children with Severe Influenza Pneumonia

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ABSTRACT

Aims: **This study assessed the diagnostic value of IL-6, hs-CRP, PCT in differentiating severe pneumonia caused by Influenza virus alone and Influenza virus among Vietnamese children under 5 years old.**

Materials and methods: **A cross-sectional study on 63 children with severe Influenza virus pneumonia was conducted. IL-6, hs-CRP and PCT tests were performed. Receiver operating characteristic (ROC) analysis was employed to measure the diagnostic values of PCT, IL-6 and hs-CRP.**

Results: **Of 63 children, 11 children were confirmed to have bacterial coinfections (17.5%). The most common bacterial coinfection was *Haemophilus influenza* (7 cases). The area under curve was 0.642 for PCT; 0.627 for hs-CRP; and 0.574 for IL-6. The optimal cut-off point for PCT was > 2.75 ng/ml. The optimal cut-off point for hs-CRP was > 23.4 mg/dl. Finally, the optimal cut-off point for IL-6 was > 7.742 pg/ml. The most accurate was for PCT with 79.3%, following by CRP with 70.1%.**

Conclusions: **This study underlined that inflammatory biomarkers such as PCT had a moderate-to-high capability to disseminate severe pneumonia children with Influenza virus alone or Influenza virus and bacterial coinfections. This may support clinicians in administrating appropriate antibiotics to children suffering severe Influenza virus pneumonia.**

KEY WORDS

respiratory syncytial virus, pneumonia, procalcitonin, bacterial coinfections

INTRODUCTION

Influenza virus infections are a substantial contributor to respiratory morbidity and mortality, with the highest burden of severe disease experienced by children (Dawood *et al*., 2010). It is estimated that influenza virus affects 5—10% of the world population annually, resulting in as many as 110 000 influenza-associated deaths in children lower 5 years of age (Lafond *et al*., 2016), (Nair *et al*., 2011).

Evidently, bacterial coinfections could elevate the severity of pneumonia in children compared to viral infections alone (Diederen *et al*., 2009; Jennings *et al*., 2008; Ruuskanen, Lahti, Jennings, & Murdoch, 2011). However, regular laboratory tests and radiographic results have poor ability to differentiate viral pneumonia patients with or without bacterial coinfections (Gendrel *et al*., 1997; Peltola & Jaakkola, 1988; Toikka *et al*., 1999). Thus, identify biology markers to early and effectively discriminate these two circumstances is necessary for deciding whether antibiotics should be used or not. To date, some inflammatory biomarkers such as interleukin (IL)-6, high-sensitivity C-reactive protein (hs-CRP), and more recently, procalcitonin (PCT) are widely mentioned as potential mediators for diagnosing inflammatory illnesses (Lacour *et al*., 2001; F. Moulin *et al*., 2001; Simon, Gauvin, Amre, Saint-Louis, & Lacroix, 2004). PCT has been used in several guidelines to manage antibiotic use in children with respiratory diseases (Kumar, Debata, Ranjan, & Gaind, 2015; Stolz *et al*., 2007) because the high level of serum PCT concentration frequently occurs in those with bacterial or parasitic infections (Assicot *et al*., 1993).

This study aims to assess the diagnostic value of hs-CRP, PCT and IL-6 in differentiating severe pneumonia caused by the Influenza virus alone and the Influenza virus with bacterial coinfections among Vietnamese children under 5 years old.

MATERIALS AND METHODS

Study Designs

We performed a cross-sectional study in 63 children who were confirmedly diagnosed with severe Influenza virus pneumonia treated at the National Hospital of Pediatrics from January 2015 to March 2017 according to WHO-2013 standards (WHO, 2013). Severe cases of pneumonia were defined when having cough or difficulty breathing plus at least one of the following main symptoms: 1) Cyanosis or SpO2 < 90%; 2) Severe respiratory distress (moaning and intercostal muscle external retraction); 3) Could not drink or give up or vomit everything; coma or not awake; or convulsions. We excluded children who 1) were under 1 month of age and older than 5 years; 2) had non-viral pneumonia (for example pneumonia after drowning, chemical pneumonia, aspiration pneumonia); 3) had chronic, associated congenital diseases (for example airway malformation, congenital lung disease, liver failure, kidney failure ...); or 4) were eligible to participate in the study but the parents or guardian did not agree to participate.

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Figure 1: Distribution of viral and bacterial pathogens in Influenza virus pneumonia children with bacterial coinfections

Measurement

All patients after hospitalization were carefully examined clinical symptoms by pediatrics. Their parents or guardians were also asked to collect information about demographic characteristics and history of illness. Two milliliters of blood samples were collected and centrifugated for biochemical tests. Hs-CRP quantification was determined by turbidity measurement using Olympus AU 2700 machine. PCT quantification was measured by the luminescent immunization method, running on ADVIA Centaur of Siemens. Specimens put into tubes without anticoagulants or with Li-Heparin and K3-EDTA anticoagulants. After collecting blood samples, they were centrifugated to extract serum or plasma. Meanwhile, IL-6 was quantified with the Bio-Plex Protein Array System of BioRad.

Bacterial testing was performed by the Vitek 2 machine. The colorimetric method was used to identify the chemical, biological properties of bacteria by changing the color of environmental wells. Moreover, an antibiogram method was applied using MIC (minimum inhibitory concentration) in order to measure turbidity, which can monitor the development of microorganisms in the environmental wells. These two methods were performed according to the principle of light intensity reduction: the optical system used visible light to directly monitor the growth of microorganisms through the measurement of the intensity of the blocked light (or attenuation of light intensity) when light passes through a well. The system used 660 nm, 568 nm, 428 nm wavelengths.

Statistical analysis

Clinical and laboratory characteristics were compared between severe pneumonia children with Influenza virus alone and Influenza virus with bacterial coinfections by using Student's t-test (for age, Respiratory rate, Pulse rate, Body temperature and SpO2), Mann-Whitney test (White blood cells, Lymphocyte, Procalcitonin, high-sensitivity C-reactive protein, PaO_2 and Interleukin-6), and Chi-squared test (for gender, clinical features and deaths after hospitalization). Receiver operating characteristic (ROC) analysis was employed to measure the diagnostic values of PCT, IL-6 and hs-CRP. This analysis informed some indices to measure the diagnostic accuracy, including area under the ROC curve, sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Positive Likelihood Ratio (LR+), Negative Likelihood Ratio (LR-) and Accuracy rate. Youden index was calculated to identify the optimal cut-off point. STATA software 15.0 was used to analyze data. Two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS

Of 63 children diagnosed with severe Influenza virus pneumonia during the study period, 11 children were confirmed to have bacterial coinfections (17.5%). The most common bacterial coinfection was *Haemophilus influenza* (7 cases), following by *Klebsiella pneumonia* (2 cases), *Burkholderia Cepacia* and *Streptococcus pneumoniae* (1 case) (Figure 1).

The mean age of patients was 12.7 (SD = 10.5) months, 66.7% were males. There were four children died after hospitalization (8.5%). The median of PCT was 1.0 (IQR = 0.17-2.3) ng/ml, the median of hs-CRP was 9.4 (IQR = 3.5-25.5) mg/dl and the median of white blood cells was 10.8 (IQR = 8.5-14.8) g/dl. Table 1 shows demographic, clinical and laboratory characteristics of Influenza virus pneumonia patients. Patients with coinfections had a significantly higher proportion of the runny nose and a higher proportion of deaths after hospitalization compared to those with the Influenza virus alone ($p < 0.05$).

Table 1: Patients' characteristics between patients with Influenza virus pneumonia alone and Influenza virus pneumonia with viral and bacterial coinfections.

	Influenza virus alone (n = 61)	Influenza virus with bacterial coinfections (n = 12)	p-value
Age (months) (mean ± SD)	13.1 ± 11.0	10.3 ± 7.0	0.42
Male, n (%)	37 (71.2)	5 (45.5)	0.10
Clinical features			
Fever	50 (96.2)	11 (100)	0.51
Rapid pulse rate	37 (71.2)	6 (54.55)	0.28
Runny nose	40 (76.9)	5 (45.45)	**0.04**
Wheezing	37 (71.2)	9 (81.82)	0.47
Diarrhea	14 (26.9)	3 (27.3)	0.98
Vital signs (mean ± SD)			
Respiratory rate (/min)	50.1 ± 8.6	54.7 ± 11.2	0.13
Pulse rate (/min)	149.8 ± 22.7	144.5 ± 20.5	0.47
Body temperature (0C)	38.7 ± 0.7	39.1 ± 0.6	0.12
SpO2	88.7 ± 6.3	88.2 ± 8.1	0.80
Initial laboratory findings (median, IQR)			
WBC (x 10^3/mm^3)	10.3 (8.4 - 14)	12 (9.8 - 19)	0.10
Lymphocyte (%)	3.2 (2.1 - 5.2)	3 (2.1 - 4.2)	0.86
Procalcitonin (ng/ml)	0.9 (0.2 - 2)	1.9 (0.3 - 2.8)	0.14
Hs-CRP (mg/dl)	8.2 (3 - 23.8)	25 (5.2 - 38)	0.19
PaO2	69 (54.5 - 80.5)	78 (53 - 217)	0.15
Interleukin-6 (pg/ml)	12.3 (5.5 - 28.9)	19 (9.2 - 20.4)	0.60
Deaths after hospitalization, n (%)	1 (2.7)	3 (3.0)	**< 0.01**

SD, standard deviation; SpO2, oxygen saturation; WBC, white blood cell

Figure 2 illustrates the distribution of PCT, hs-CRP and IL-6 between severe pneumonia children with Influenza virus alone and Influenza virus combined with viral/bacterial coinfections.

Results of Receiver-operating characteristics (ROC) curve analysis are shown in Figure 3. Overall, the area under curve was 0.642 (95% confidence interval = 0.455 0.830, p = 0.14) for PCT; 0.627 (95% confidence interval = 0.437-0.818, p = 0.19) for hs-CRP; and 0.574 (95% confidence interval = 0.347-0.802, p = 0.52) for IL-6. The difference among areas under the ROC curve for these three biomarkers were insignificant ($p > 0.05$).

Diagnostic value for PCT, hs-CRP and IL-6 are presented in Table 2. The optimal cut-off point for PCT was > 2.75 ng/ml (sensitivity 36%, specificity 90%, positive predictive value 44%, negative predictive value 87%). The optimal cut-off point for hs-CRP was > 23.4 mg/dl (sensitivity 64%, specificity 75%, positive predictive value 35%, negative predictive value 91%). Finally, the optimal cut-off point for IL-6 was > 7.742 pg/ml (sensitivity 100%, specificity 36%, positive predictive value 20%, negative predictive value 100%). The most accurate was for PCT with 79.3%, following by CRP with 70.1%.

DISCUSSION

Identifying biomarkers for the rapid detection of bacterial coinfections in Influenza virus pneumonia among children hospitalized is essential in emergency circumstances. Our study highlights the potential role of serum PCT concentration in detecting and discriminating children with severe Influenza virus pneumonia alone and severe Influenza virus pneumonia with bacterial coinfections.

In this study, the optimal cut-off point for PCT was 2.75 ng/ml, which might be different from prior studies using PCT to discriminate

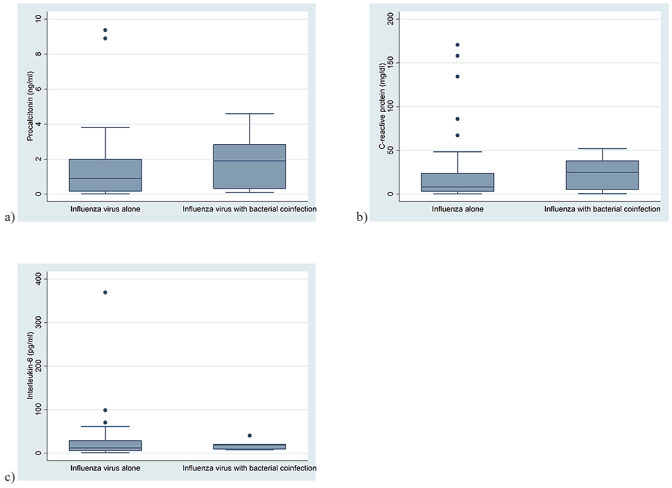

Figure 2: Box plot of a) Procalcitonin (PCT), b) high-sensitivity C-reactive protein (hs-CRP), and c) Interleukin-6 (IL-6) levels on initial hospital visit between Influenza virus pneumonia alone and Influenza virus pneumonia with bacterial coinfections.

Table 2: Accuracy of diagnostic parameters

	PCT > 2.75 ng/ml	Il-6 > 7.742 pg/ml	Hs-CRP > 23.4 mg/dl
Sensitivity	0.36 (0.11-0.70)	1.00 (0.48-NaN)	064 (0.31-0.89)
Specificity	0.90 (0.79-0.97)	0.36 (0.19-0.55)	0.75 (0.60-0.86)
Positive Predictive Value (PPV)	0.44 (0.24-0.76)	0. 20 (0.10-NaN)	0.35 (0.22-0.72)
Negative Predictive Value (NPV)	0.87 (0.59-0.96)	1.00 (0.67-1.00)	0.91 (0.71-0.95)
Positive Likelihood Ratio (LR+)	3.78 (1.21-11.86)	1.55 (1.19-2.01)	2.50 (1.31-4.77)
Negative Likelihood Ratio (LR-)	0.70 (0.45-1.11)	0.00 (0.00-NaN)	0.49 (0.22-1.08)
Accuracy	79.3%	41.7%	70.1%

PCT: Procalcitonin; hs-CRP: high-sensitivity C-reactive protein; IL-6: Interleukin-6

these two types of patients in respiratory diseases. A study of Shin Ahn et al. showed that PCT > 1.5 ng/ml had sensitivity 56% and specificity 84% to detect pneumonia patients having mixed bacterial coinfection or not (Ahn *et al*., 2011). Other studies by Chirouze *et al*. in patients with acute fever (Chirouze *et al*., 2002), Ingram *et al*. in H1N1 influenza patients (Ingram, Inglis, Moxon, & Speers, 2010), and Chua *et al*. in severe acute respiratory syndrome (Chua & Lee, 2004) indicated the cut-off point for PCT was 0.4 ng/ml, 0.8 ng/ml and 1.0 ng/ml, respectively. These variances might be attributable to the presence of different viruses and bacteria in each study. Moreover, our study employed children who suffered from severe Influenza virus pneumonia, who actually had a high level of PCT compared to samples in other studies who had a variety of levels of disease severity.

In literature, serum PCT, hs-CRP and IL-6 concentration have been used widely to differentiate patients suffering viral respiratory diseases alone and those with bacterial coinfections (Lacour *et al*., 2001; F. Moulin *et al*., 2001; Simon *et al*., 2004). Most of the studies found that PCT was dominant in differentiating viral and bacterial infections compared to hs-CRP and IL-6 (Lacour *et al*., 2001; F Moulin *et al*., 2001; F. Moulin *et al*., 2001; Simon *et al*., 2004). Our finding in this study was in line with these previous works when we found that the accuracy rate of PCT was higher than that of IL-6 and hs-CRP. In addition, clinical symptoms and other laboratory test measures were not sufficient for diagnosing bacterial coinfections, which aligned with previous findings

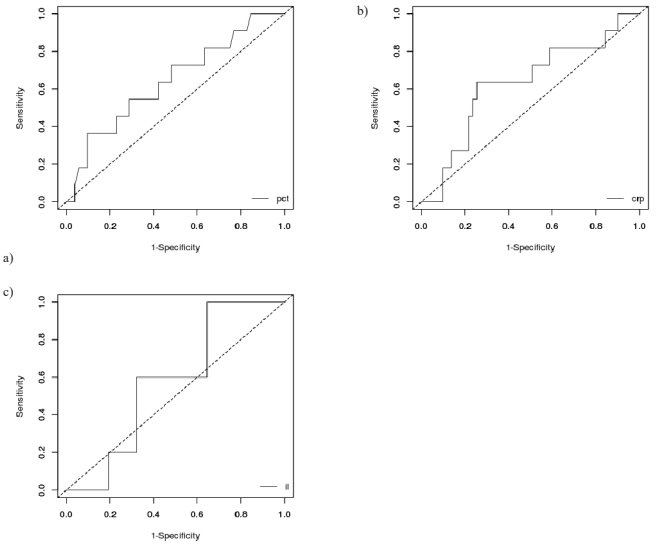

Figure 3: Receiver-operating characteristics curve for discriminating between Influenza virus pneumonia alone and Influenza virus pneumonia with coinfections for a) Procalcitonin (PCT), b) high-sensitivity C-reactive protein (hs-CRP), and c) Interleukin-6 (IL-6) on an initial hospital visit.

(Gendrel *et al*., 1997; Peltola & Jaakkola, 1988; Toikka *et al*., 1999). Serum PCT concentration above 1.5 ng/ml could detect bacterial coinfections more effectively, which can be applied in emergency cases. Although PCT alone cannot be used to decide which antibiotics should be utilized, this is a vital marker that clinicians should require when making a decision rather than based on radiographic or blood cell count findings. Therefore, developing a rapid bedside PCT test is crucial in managing children experiencing severe Influenza virus pneumonia.

This study contained several limitations. First, our sample size was small and conveniently recruited, which thus might reduce our generalizability. Second, our cross-sectional design has its own limitations due to its nature. Several recent studies argued that making decisions related to antibiotics use should be use data of serum PCT concentration overtime via a longitudinal cohort rather than using the initial PCT level (Boussekey *et al*., 2006; Christ-Crain *et al*., 2006). In Vietnam, PCT test has been covered in the health insurance scheme, which facilitates the use of PCT in routine monitoring and controlling bacteria in children with severe Influenza virus pneumonia.

CONCLUSION

This study underlined that inflammatory biomarkers such as PCT had a moderate-to-high capability to disseminate severe pneumonia children with Influenza virus alone or Influenza virus and bacterial coinfections. This may support clinicians in administrating appropriate antibiotics to children suffering severe Influenza virus pneumonia.

CONFLICTS OF INTEREST

The author has no conflicts of interest to declare.

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