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ORIGINAL ARTICLE

New Biomarkers to Diagnose Ventilator Associated Pneumonia: Pentraxin 3 and Surfactant Protein D

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Abstract

Objective To detect the most effective biomarker to confirm ventilator associated pneumonia (VAP).

Methods Fifty patients with VAP suspicious diagnosis and 30 healthy patients were recruited. Suspicion of VAP was established if patients met the modified CPIS score ≥ 6 points. The confirmation of VAP was defined by the quantitative culture of nonbronchoscopic bronchoalveolar lavage (BAL) $>10^5$ CFU/ml of pathogenic microorganism. Serum samples for determination of C-reactive protein (CRP), procalcitonin (PCT), pentraxin 3 (PTX3), surfactant protein D (SPD) were collected on suspected VAP.

Results Twenty seven of 50 patients were accepted as confirmed VAP group whose nonbronchoscopic BAL cultures were positive and rest of them were accepted as unconfirmed VAP group. PTX3, PCT and SPD levels were significantly higher in confirmed VAP group, ($P = 0.021$, $P = 0.007$, $P < 0.001$ respectively). There were no significant differences in CRP levels between the two groups ($P = 0.062$). The most sensitive marker for diagnosing VAP was SPD ($P < 0.001$). Receiver operating characteristic (ROC) curve for modified clinical pulmonary infection score (CPIS) to confirm VAP was evaluated (AUC 0.741 ± 0.07, $P < 0.001$) and the optimal cutoff value was >7 with a sensitivity of 51.85% and a specificity of 91.3%. SPD levels were significantly higher in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infected patients than culture negative patients ($P < 0.001$).

Conclusions The index findings suggest that serum SPD is the most sensitive biomarker in diagnosis of VAP and it can be used as an early and organism specific marker for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Keywords Surfactant protein D · Pentraxin 3 · Ventilator associated pneumonia

Abbreviations

AUC	Area under ROC curve
BAL	Bronchoalveolar lavage
CFU	Colony forming units
CPIS	Clinical pulmonary infection score
CRP	C-reactive protein
ELISA	Enzyme-linked immunosorbent assay
PCT	Procalcitonin
PELOD	Pediatric logistic organ dysfunction
PICU	Pediatric intensive care unit
PRISM	Pediatric risk of mortality
PTX3	Pentraxin 3
ROC	Receiver operating characteristic
SPD	Surfactant protein D
VAP	Ventilator associated pneumonia

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Introduction

Ventilator associated pneumonia (VAP) is a type of health care associated pneumonia that develops after more than 48 h of mechanical ventilation; it accounts for longer duration of mechanical ventilation and pediatric intensive care unit (PICU) stay [1, 2]. Recent literatures have demonstrated that VAP occurs in 3–19% of ventilated pediatric patients and is associated with higher mortality and morbidity rates [3–6]. Pediatric

VAP is a common reason for initiation of empiric antibiotic therapy which includes almost half of all PICU antibiotic days [7].

The accurate diagnosis of VAP in children and adults is still an unsolved problem. Delayed diagnosis of VAP and subsequent delay in initiating appropriate therapy may cause worse outcomes in patients with VAP. On the other hand, an incorrect diagnosis may lead to unnecessary treatment and subsequent complications that are related to the therapy [8]. Several criteria have been proposed for diagnosing VAP in clinical settings, including clinical manifestations, imaging techniques, methods to obtain and interpret bronchoalveolar specimens, and biomarkers of host response [8]. However there is no acceptable gold standard modality yet and the accuracy of these methods in diagnosing VAP is controversial.

The clinical pulmonary infection score (CPIS) was proposed by Pugin et al. based on variables (fever, leukocytosis, tracheal aspirates, oxygenation radiographic infiltrates) [9]. CPIS is helpful to diagnose VAP in pediatric patient but it is not sufficient for definite diagnosis. Also, many biological markers such as C-reactive protein (CRP), procalcitonin (PCT), pentraxin 3 (PTX3) and Surfactant protein D (SPD) have been studied in an effort to improve the rapidity and performance of current diagnostic procedures of VAP [10–13].

Pentraxins are phylogenetically conserved proteins characterized by a multimeric structure and divided into short (CRP and serum amyloid P component) and long pentraxins [14]. PTX3 is the first identified member of the long pentraxin subfamily. It can be rapidly produced and released by mononuclear phagocytes, neutrophils, epithelial and endothelial cells in response to primary inflammatory signals (IL-1 and TNF-α) [15]. Increased level of PTX3 has been seen in acute respiratory distress syndrome and PTX3 levels were found well correlated with the severity of lung injury and it seems to be a useful predictor of survival [16].

SPD is primarily expressed and secreted by type II alveolar cells (Clara cells) but is also detected in the tracheal and bronchial glands of the lower airways. Firstly SPD was identified in respiratory tract, but current studies demonstrate that the expression of SPD is in almost all mucosal surfaces, including epithelial cells in exocrine ducts, the mucosa of gastrointestinal and genitourinary tract and in tear fluid [17]. SPD has a well demonstrated and important role in pulmonary innate immunity, especially in protection against Gram-negative bacteria [18]. Said et al. mentioned that analysis of SPD in bronchoalveolar lavage fluid (BAL) would serve as an early biomarker for VAP [13].

Serum SPD, PTX3 and other biomarker levels have not been studied before together in diagnosis of VAP. The aim of this study was to detect the most effective biomarker to confirm VAP.

Material and Methods

A prospective control-case study was performed in the PICU of a tertiary university hospital (Erciyes University Medical Faculty, Kayseri, Turkey) from January through December 2015. Eighty patients were included in the study. Fifty patients were suspicious VAP group who were older than one year old and were receiving mechanical ventilation >48 h, and 30 patients were healthy control group. Exclusion criteria were age > 18 y, ventilated from tracheostomy, immunosuppression due to medication or disease, active infection at admission, developing health care associated infection except VAP during ventilation period. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki. Written informed consent was obtained from all the participants. The informed consent form was subscribed by the patients themselves or their parents when they were mechanically ventilated.

Suspicion of VAP was established if patients met modified CPIS score ≥ 6 points. The confirmation of VAP was defined by the quantitative culture of nonbronchoscopic bronchoalveolar lavage fluid $>10^5$ colony forming units (CFU)/ml of a potentially pathogenic microorganism. Two expert intensivists blinded to results of CRP, PCT, PTX3 and SPD, reviewed all other available clinical data and assigned the final diagnosis of VAP. In case of disagreement, they reviewed the disputed cases together and reached a consensus.

Patients were enrolled 48 h after the initiation of ventilation. Sex, age, comorbidities, reason of ventilation, duration of ventilation and vital signs were recorded. The Pediatric Risk of Mortality (PRISM) score III and Pediatric Logistic Organ Dysfunction (PELOD) score were calculated within 24 h of admission to PICU. Chest X-ray, arterial blood gases, laboratory examination and physical examination were performed. Modified CPIS [19] was assessed on the basis of the five variables (temperature, blood leukocyte count, PaO_2/FiO_2, tracheal secretions, and pulmonary radiography) by expert intensivist. Empirical antimicrobial therapy was started in patients with suspicion of VAP, and was modified according to the results of microorganism isolated in patients with confirmed VAP or according to the clinical manifestation for patients with unconfirmed VAP. Antibiotics were decided by the critical care team.

Nonbronchoscopic BAL fluid was obtained *via* endotracheal tube. Mini-BAL was performed by Combicath© (Plastimed Division, Prodimed, Saint-Leu-La-Foret Cedex, France) that consisted of an external polyethylene tube (60 cm long 2.7 mm diameter) and internal Teflon tube (65 cm long, 1.7 mm diameter). The catheter was introduced into the endotracheal tube and gently advanced until it met with resistance. Then internal catheter was pushed forward, inner of the external catheter. One ml per kg body weight of physiologic saline was instilled through the catheter and then

suctioned. Microbiological analysis was performed in microbiology laboratory of Erciyes University.

Blood samples for determination of CRP, PCT, were collected on suspected VAP. Samples for SPD, PTX3 were frozen at −20 °C after centrifugation. Measurements were performed in the central laboratory of Erciyes University. CRP levels were determined by using immunonephelometry (BN-II system, Siemens, Germany) and Procalcitonin levels were measured by using Enzyme- Linked Fluorescent Assay technique (miniVIDAS, BioMerieux, France). Serum PTX3 levels were measured by using Human PTX3 enzyme-linked immunosorbent assay (ELISA) kit from Boster immunoleader (Boster Biological Technology Co., Ltd., CA, USA). The sensitivity was <10 pg/mL. Serum samples for SPD were performed by a sandwich-type ELISA technique using surfactant Protein D Human ELISA (RD194059101, BioVendor Laboratory Medicine, Inc., Brno Czech Republic) according to the manufacturer's instructions and quality controls.

Data were statistically analyzed with SPSS 22.0 software (IBM, Armonk, NY). Continuous variables are presented as mean ± SD or median (with interquartile range) and categorical variables are expressed as numbers and percentages, where

Table 1 Baseline characteristics of the study group

	Confirmed VAP ($n = 27$)	Unconfirmed VAP ($n = 23$)	P
Age, months	42 (22–132)	27 (32–42)	0.068
Gender, male (%)	15 (55.6)	15 (65.2)	0.685
Co-morbidities, n (%)			
Neurologic	15 (55.6)	7 (30.4)	0.150
Cardiologic	5 (18.5)	6 (26.1)	
Endocrine-Metabolic	1 (3.7)	1 (4.3)	
Gastroenterologic	1 (3.7)	0 (0)	
Other	5 (18.5)	5 (21.7)	
None	0 (0)	4 (17.4)	
Diagnosis, n (%)			
Respiratory	22 (81.5)	15 (65.2)	0.570
Neurologic	2 (7.4)	3 (13.0)	
Cardiologic	2 (7.4)	2 (8.7)	
Other	1 (3.7)	3 (13.0)	
Indication of mechanic ventilation, n (%)			
Type 1 respiratory failure	22 (81.5)	15 (65.2)	0.325
Type 2 respiratory failure	5 (18.5)	8 (34.8)	
PRISM	17 (14–21)	18 (14–26)	0.327
PELOD*	20 (2–33)	20 (11–33)	0.506
CPIS*	8 (6–9)	7 (6–8)	**0.002**
White blood cell, 10^9/l	16.54 (13.23–18.20)	15.18 (13.20–16.94)	0.235
C-reactive protein, mg/l	47.00 (24.90–59.80)	33.4 (19.00–43.20)	0.062
Procalcitonin, ng/ml	3.75 (2.21–4.92)	2.17 (1.14–3.17)	**0.007**
Surfactant protein D, ng/ml	343.79 (221.35–472.61)	106.55 (99.97–137.25)	**<0.001**
Pentraxin 3, ng/ml	6.62 (4.04–10.82)	4.09 (3.09–5.23)	**0.021**
Duration of VAP, days	5 (4–7)	4 (3–6)	0.375
Duration of mechanic ventilation, days	34 (11–59)	27 (5–59)	0.365
Isolated Organisms, n (%)			
Pseudomonas aeruginosa	10 (37.0)		
Acinetobacter baumannii	7 (25.9)		
Coagulase negative *Staphylococcus aureus*	6 (22.2)		
Klebsiella pneumoniae	4 (14.8)		

Variables are presented median (with interquartile range). The statistical significant p values are shown as bold entries

CPIS Clinical pulmonary infection score; *PELOD* Pediatric logistic organ dysfunction; *PRISM* Pediatric risk of mortality; *VAP* Ventilator associated pneumonia

*Variables are presented median (with minimum- maximum)

appropriate. The comparison between the two groups for data with normal distribution was performed using Student's t-test, and the comparison between groups for data which did not show a normal distribution was performed using Mann-Whitney U test. Categorical variables were compared by means of χ^2 test. For multigroup comparison, One-way ANOVA (for data showing normal distribution) or Kruskal-Wallis (for data which did not show normal distribution) was used. Multiple binary logistic regression test was used to detect the most significant parameter to diagnose VAP. Receiver operating characteristic (ROC) curves were utilized to evaluate the accuracy of modified CPIS, CRP, PCT, PTX3 and SPD to diagnose VAP. The area under ROC curve (AUC) and cut-off values were compared using MedCalc for Windows, version 9.2 (MedCalc Software, Ostend, Belgium). All probabilities were two tailed and $P < 0.05$ was regarded as significant.

Results

Eighty children were enrolled (30 healthy children as control group and 50 patients as study group) in the study. Twenty seven of 50 patients were accepted as confirmed VAP group whose nonbronchoscopic bronchoalveolar lavage cultures were positive and rest of them were accepted as unconfirmed VAP group.

The median age was 42 mo (22–132 mo) in confirmed VAP group and 27 mo (22–42 mo) in unconfirmed VAP group. There were no significant differences in PRISM and PELOD, respectively ($P = 0.327$, $P = 0.506$). Mechanical ventilation duration was observed longer in confirmed VAP than unconfirmed VAP but no significant difference was established ($P = 0.365$) Baseline characteristics and demographic data of the patients are summarized in Table 1. The two groups were compared according to the biomarker levels and it was found that PCT, PTX3, and SPD levels were significantly higher in confirmed VAP group ($P = 0.007$, $P = 0.021$, $P < 0.001$ respectively). However, there was no significant difference in CRP levels between the two groups ($P = 0.062$). Multiple binary logistic regression analysis was performed and showed that SPD was the most significant biomarker for confirming VAP ($P = 0.002$).

When biomarker levels were compared between control and study group, the median serum SPD concentrations (ng/ml) [confirmed, unconfirmed VAP and control group; 343.79 (221.35–472.61), 106.55 (99.97–137.25), 55.26 (29.70–71.89) respectively] and PTX3 concentrations (ng/ml) [confirmed, unconfirmed VAP and control group; 6.62 (4.04–10.82), 4.09 (3.09–5.23), 0.26 (0.13–0.45) respectively] were significantly higher in confirmed and unconfirmed VAP group than control group ($P < 0.001$) (Fig. 1).

ROC curve were drawn to evaluate the value of CRP, PCT, PTX3, SPD and WBC to predict confirmed VAP (Fig. 2). SPD was found to be the most sensitive marker for diagnosing VAP ($P < 0.001$). Comprehensive analyses of diagnostic tests are shown in Table 2. ROC curve for modified CPIS was also drawn to confirm VAP (AUC 0.741 ± 0.07, $P < 0.001$) (Fig. 3). The optimal cut-off value was >7 with a sensitivity of 51.85% and a specificity of 91.3%.

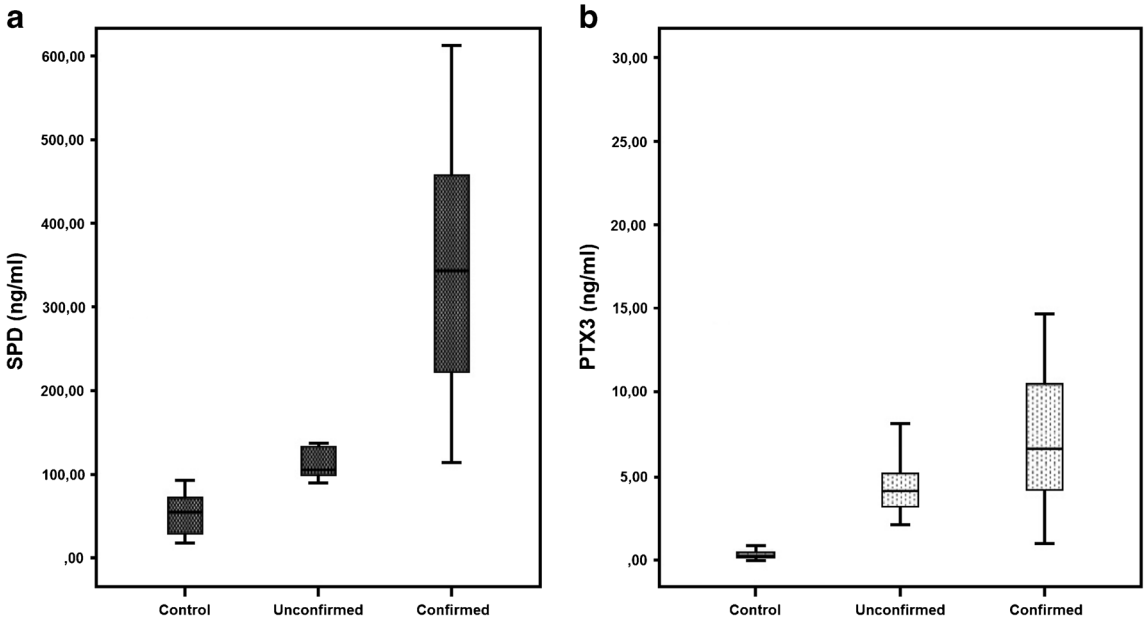

Fig. 1 **a** Comparison of serum SPD concentration between patients with ventilator associated pneumonia group including confirmed, unconfirmed and control group. **b** Comparison of serum PTX3 concentration between patients with ventilator associated pneumonia group including confirmed, unconfirmed and control group

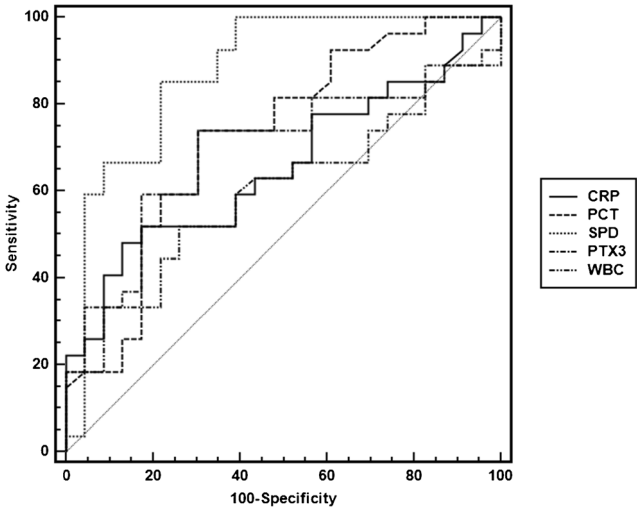

Fig. 2 Comparison of receiver operating characteristic curves of diagnostic tests. *CRP* C-reactive protein; *PTX3* Pentraxin 3; *PCT* Procalcitonin; *SPD* Surfactant protein D; *WBC* White blood cell

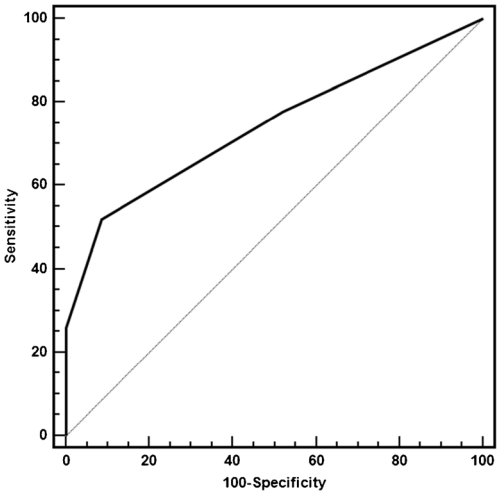

Fig. 3 Receiver operating characteristic curve of clinical pulmonary infection score

Pseudomonas aeruginosa was the most common organism that was isolated from nonbronchoscopic BAL samples. PCT levels were significantly higher in *Acinetobacter baumannii* infected patients than cultured negative patients and Coagulase negative *Staphylococcus aureus* infected patients ($P = 0.003$). In addition, SPD levels were significantly higher in *Acinetobacter baumannii and Pseudomonas aeruginosa* infected patients than culture negative patients ($P < 0.001$). PTX3 levels were higher in *Klebsiella pneumoniae* infected patients than *Pseudomonas aeruginosa* infected patients ($P = 0.002$) (Table 3). There was no statistically significant relation between CRP levels and microorganisms that were isolated from the samples.

Discussion

Hospital acquired infections contribute to inpatient morbidity and mortality as well as increase health care resource utilization. VAP is a leading cause of health care associated infection in the PICU and accounts for longer duration of PICU length of stay. Gupta et al. showed that there was a three-fold increase in severity adjusted PICU mortality in a multicenter study of pediatric VAP. Many researchers have been studying to find various methods and markers for diagnosis of VAP [3]. Pugin et al. combined fever, leukocyte counts, amount and character of tracheal secretions, appearance of new or persistence of radiographic infiltrates, oxygenation (PaO_2/FiO_2) and formed the CPIS as a diagnostic tool for VAP with 93% sensitivity and 100% specificity [9]. CPIS has been studied in many adult studies and in these studies, sensitivity and specificity were found to have a wide range (7%–72%, 42%–85%, respectively) in comparison with the pathology [20]. Sachdev et al. evaluated the diagnosis of VAP in pediatric patients, which was assessed by CPIS and confirmed by bronchoscopic BAL culture as the reference standard. The value of CPIS 8 was found the best accuracy with a sensitivity of 80% and specificity of 80% [21]. In the index study, the optimal cut-off value was >7 with a sensitivity of 51.85% and a specificity of 91.3%. CPIS can be used as an early diagnostic tool to identify patients with high probability of pneumonia, but requires definite diagnostic methods. It can also be used to evaluate the clinical response to the therapy [21].

In children, an optimal diagnostic technique for VAP is controversial. Because of the invasive nature and cost of bronchoscopy, investigators have evaluated the nonbronchoscopic techniques. Nonbronchoscopic and bronchoscopic methods in diagnosis of VAP were compared and authors reported that nonbronchoscopic bronchial sampling methods were most reliable for the diagnosis of VAP [22]. In consequence, authors prefered nonbronchoscopic BAL sampling because of easy practional, less risk of complications and reliability of the method in the index study.

Several studies have assessed the value of SPD serum levels as a disease marker for human lung disease such as interstitial lung disease, acute and chronic lung injury [17]. Several studies have shown that SPD interacts with a number

Table 2 Comparisons of the performance of tests for the definite diagnosis of ventilatory associated pneumonia

	AUC	Cut-off value	Sensitivity	Specificity	*P*
CRP (mg/l)	0.655	47.9	48.1	87	0.054
PCT (ng/ml)	0.724	2.41	74.1	69.6	0.002
SPD (ng/ml)	0.874	137.248	85.2	78.3	<0.001
PTX3 (ng/ml)	0.691	4.1968	74.1	69.6	0.010
WBC (10^9/l)	0.598	16.46	51.85	73.91	0.620

CRP C-reactive protein; *PTX3* Pentraxin 3; *PCT* Procalcitonin; *SPD* Surfactant protein D; *WBC* White blood cell

Table 3 The relationship between biomarker levels and isolated organisms in nonbronchoscopic bronchoalveolar lavage fluid

Biomarker	Negative	*Acinetobacter baummanni*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	CNSA	*P*
CRP (mg/l)	33.4 (19.00–43.20)[a]	47.00 (39.40–59.80)[a]	35.10 (21.00–49.91)[a]	62.00 (28.50–89.50)[a]	38.78 (15.47–64.67)[a]	0.155
PCT (ng/ml)	2.17 (1.14–3.17)[a]	7.13 (3.75–10.60)[b]	3.64 (2.67–4.81)[ab]	4.37 (2.36–7.20)[ab]	2.21 (1.14–2.70)[a]	**0.003**
SPD (ng/ml)	106.55 (99.97–137.25)[a]	486.77 (345.00–563.24)[b]	430.42 (153.08–498.15)[b]	201.08 (132.67–225.30)[ab]	322.15 (203.84–343.79)[ab]	**<0.001**
PTX3 (ng/ml)	4.09 (3.09–5.23)[a]	7.39 (4.62–10.11)[a]	3.41 (1.87–5.84)[ab]	10.82 (7.67–32.87)[ac]	7.56 (5.21–16.76)[a]	**0.002**
WBC (10^9/l)	15.18 (13.20–16.94)[a]	17.28 (15.68–18.78)[a]	16.23 (14.06–17.97)[a]	15.92 (13.22–19.65)[a]	12.54 (11.86–18.38)[a]	0.191

[a,b,c] Significantly different from each other. The statistical significant *p* values are shown as bold entries. *CNSA* Coagulase negative *Staphylococcus aureus; CRP* C-reactive protein; *PTX3* Pentraxin 3; *PCT* Procalcitonin; *SPD* Surfactant protein D; *WBC* White blood cell

of viruses, bacteria and fungi [13]. Kawasaki et al. reported a study about serum SPD levels in bronchiolitis. They emphasized that serum SPD concentrations in children with respiratory syncytial virus (RSV) bronchiolitis were higher than those in the control group, and that mean serum SPD concentration was associated with the severity of RSV bronchiolitis [23]. Said et al. found that BAL fluid SPD levels showed a robust early response to presumed nosocomial inoculation and subsequent established infection. They concluded that analysis of SPD in BAL fluid is an early biomarker for VAP. They also noted that this response can be organism specific; and suggested a relationship between pulmonary Pseudomonas infection and BAL fluid SPD content and functionality [13]. The authors evaluated biomarkers with regard to their efficiency for the diagnosis of VAP and serum SPD level was found to be the most significant biomarker. In the index study authors found that SPD levels were significantly higher in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infected patients than culture negative patients and therefore, the authors interpreted that serum SPD levels could be used to predict the organism. Another biomarker to anticipate the agent is PCT because serum PCT levels were significantly higher in *Acinetobacter baumannii* infected patients than culture negative patients. The authors think that empirical antimicrobial therapy should be started according to the levels of biomarkers which point to organisms causing VAP.

Increased levels of PTX3 have been reported in acute respiratory distress syndrome recently and the PTX3 levels are well correlated with the severity of lung injury and are useful predictor of survival [16]. Lin et al. emphasized that in VAP patients, PTX3 is an early marker which correlates with severity of sepsis and mortality. They also found PTX3 is not superior to CRP as a biomarker to diagnose VAP but PTX3 has a better prognostic value to predict 28-day mortality than CRP [12]. In the index study, serum PTX3 levels were more sensitive than CRP to diagnose VAP. The authors also found that serum PTX3 levels were significantly higher in *Klebsiella pneumoniae* infected patients than *Pseudomonas aeruginosa* infected patients.

There are several limitations in the index study. First; this study was a single center study with a limited number of cases. The authors were only able to study initial value of biomarkers. A more detailed study including the levels of different biomarkers during the process of VAP with larger number of patients is warranted.

Conclusions

Thus, to conclude, the present findings suggest that serum SPD is the most sensitive biomarker in diagnose of VAP which can be used as an early and organism specific marker for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

Contributions NUT and BNA conceptualized the study and its design. NUT and BNA participated in data collection and diagnostic work-up of study participants. BDE and SM analyzed and interpreted the data. NUT drafted the manuscript, which was revised after critical inputs from BNA, SM, BDE. All authors approved the final version of the manuscript, as submitted. BNA is consultant of incharge and will act as guarantor for this paper.

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Compliance with Ethical Standards

Conflict of Interest None.

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