Chapter 5

C-reactive protein and procalcitonin concentrations in bronchoalveolar lavage fluid as a predictor of ventilator-associated pneumonia

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Submitted

Abstract

Objective
Diagnosis of ventilator-associated pneumonia (VAP) is difficult. The usefulness of high-sensitivity PCT (ProCa-S) and – CRP (CRPH) in bronchoalveolar lavage fluid (BALF) and serum in the prediction of VAP was determined.

Study design
The study was conducted over a 28-month period (November 1999 – June 2002) at the University Hospital Maastricht. BALF samples were collected from patients admitted to the intensive care unit. Differential cell-count and quantitative culture of BALF were performed. CRP and PCT on BALF were determined by means of two high sensitivity kits (CRPH and ProCa-S respectively). Since both kits were designed for use on serum, validation for use on BALF was performed.

Results
117 patients were included. 43.6% (51/117) had microbiologically confirmed VAP. Both CRPH as ProCa-S showed good matrix effect, linearity and intra- and interassay variation. No significant differences in PCT and CRP levels in serum and BALF were found between the VAP and the non-VAP group.

Conclusions
Both the ProCa-S as the CRPH kit can be used for assessing the concentration of respectively PCT and CRP in BALF. PCT and CRP levels in BALF appeared to be of no additional value in the diagnosis of VAP.

Introduction

Ventilator associated pneumonia (VAP) is a common complication in intensive care patients. The clinical diagnosis is difficult and a definite microbiological diagnosis is based on quantitative culture of bronchoalveolar lavage fluid (BALF) exceeding 10^4 colony forming units (cfu) per ml and/or the presence of ≥2% cells with phagocytised organisms (infected cells, IC)[1,2]. Routine application of BALF analysis is limited by the fact that this procedure is expensive and time-consuming, and relies upon specialised technicians. In most hospitals facilities for BALF cytology are not available on a 24-hour base, therefore we were interested in a fast method, present in most hospitals, to predict VAP. Several markers to differentiate between inflammation and infection have been described in literature[3,4]. Determination of C-reactive protein (CRP) in serum is a valuable marker of infection in serum[5,6]. Procalcitonin (PCT) has shown great potential as a marker in serum in recent years[7,8]. The value of PCT in serious bacterial infection, mainly sepsis, has been shown[9-15]. and it could also be useful in the diagnosis of other infections like yeast[16] and parasitic infection[17] Few studies have investigated the value of PCT in patients with pneumonia. The studies that are available show discrepant results[15,18,19] and all focused on detection of parameters in serum from patients with a community acquired pneumonia. One of the drawbacks of detecting any parameter in BALF is the dilution (approximately 10-100 times) of the original fluid present in the alveoli. In a preliminary study, we found the commercially available LUMItest unsuitable for the determination of PCT in BALF. Approximately 70% of BALF samples generated a concentration below the detection-limit of the LUMItest. ProCa-S is a novel kit, currently only available for research purposes, which may have a indication in the infectious work-up of BALF due to its low detection limit. Neither ProCa-S nor the high sensitivity CRP have been validated for BALF until now. The aim of the present study is to determine the usefulness of high-sensitivity PCT (ProCa-S) and –CRP (CRPH) in BALF and serum in the prediction of VAP.

Materials and methods

Patients included

This study was conducted at the Intensive Care unit of the University Hospital Maastricht, a 700-bed teaching hospital, during a 28-month period (November 1999 until June 2002). The Intensive Care unit is a 17 bed, mixed surgical/medical unit. Patients suspected of VAP were included. Criteria were those described by Bonten *et al.*[20] *i.e.* rectal temperature >38°C or <35.5°C,

Blood leukocytosis (>10×10^3/mm^3) and/or left shift or blood leukopenia (<3×10^3/mm^3), more than ten leukocytes in Gram stain of tracheal aspirate (in high-power field), positive culture of tracheal aspirate and a new, persistent, or progressive infiltrate on chest radiograph.

Samples and sample preparation

Patients suspected of VAP underwent bronchoscopy with BAL as part of the routine diagnostic work-up. Briefly, a fiberoptic bronchoscope (Pentax FB-15H/FB-15X, Pentax Medicals, Tokyo, Japan) was introduced and "wedged" into the affected segmental or subsegmental bronchus. Sterile saline (0.9% NaCl, room temperature) was instilled in four aliquots of 50 ml, immediately aspirated and recovered. BALF samples were transported to the laboratory within 15 minutes after collection and processed immediately upon arrival. In addition, blood samples were collected immediately after the bronchoscopy.

Microbiologic and cytologic processing of the BALF

Each BALF was processed as previously described, including a total cell count, differential cell count and quantitative culture[21,22]. The percentage of polymorphonuclear neutrophils (PMNs) in BALF was assessed as a marker of inflammation. Bacterial burden of BALF was considered as the logaritmic value of the quantitative culture, i.e. expressed as 10^2, 10^3 10^4,10^5 and >10^5 cfu/ml.

Blood cultures

Blood culture results at the time of BAL (1 day before bronchoscopy until 2 days after) were reviewed.

Clinical chemistry

The following components were measured in BALF: high-sensitivity CRP, high-sensitivity PCT, albumin (as a marker of capillary leak), and urea (as a marker of dilution). In addition, albumin, urea, CRP and PCT levels were determined in serum. Albumin and urea levels were assessed upon arrival at the laboratory, CRP and PCT levels were assessed on serum and on BALF supernatans (centrifugation at 250 g for 10 minutes) that had been stored at – 80°C until the processing date.

BALF CRP was assessed as high-sensitivity CRP with a commercially available kit (CRPH, Beckman Coulter, Fullerton, California, USA). Serum CRP was determined using a commercially available kit (CRP, Beckman Coulter, Fullerton, California, USA). Serum PCT was assessed with the commercially

available LUMItest (Brahms Diagnostica, Norcross, Georgia, USA) (results expressed as μg/l). BALF PCT levels were assessed with a high-sensitivity kit (ProCa-S test PCT, Brahms Diagnostics, Norcross, Georgia, USA), a kit designed for experimental purposes, expressing the results in the high-sensitive ng/l range. Serum albumin and serum and BALF urea levels were assessed using commercially available kits (Albumin (Bromcresol Purple method), Beckman Coulter, Fullerton, California, USA and Urea (Urease method), Beckman Coulter, Fullerton, California, USA). The Bromcresol Purple method for the detection of albumin has a detection limit of 8 g/l which is below the average albumin concentration in BALF[23], therefore BALF albumin levels were detected by means of the BN ProSpec (Dade Behring, Liederbach, Germany) based on particle-enhanced immunonephelometry with a detection limit of 2 mg/l. All PCT measurements were performed in duplicate, and the mean of these duplicate results was considered. CRP and urea in both serum and BALF were measured on a Synchron LX20 analyser (Beckman Coulter, Fullerton, California, USA). Both BALF and serum PCT levels were determined on a Magic Lite Analyzer II (Ciba Corning, Medfield, Massachusetts, USA).
In order to validate the ProCa-S and the CRPH for the use in BALF samples, matrix effect, detection limit, linearity and imprecision were determined for both tests.

Definition of VAP

VAP was defined as presence of ≥2% cells containing phagocitised organisms (infected cells, IC)[2] and/or a quantitative culture result of BALF ≥10^4 cfu/ml.[1]

Rejection criteria

BALF samples were rejected if the recovered volume was less than 20 ml, if the total cell count was less than 60.000 cells/ml, if preparations showed excessive amounts of intercellular debris or damaged nucleated cells or more than 1% squamous epithelial cells.

Statistical analysis

In order to compare the ProCa-S and CRPH concentrations between recovered BALF samples, the levels were converted to concentrations in epithelial lining fluid (ELF) using the concentrations of urea in serum and BALF. The following formula described by Wiedermann and co-workers[24] was used:

$$[X]ELF = ([X]BALF \times \text{urea serum})/\text{urea BALF concentrations}$$

For convenience, we refer to the "concentrations in ELF" as concentrations in BALF in the text.
A Mann-Whitney *U* test was performed to evaluate differences in ProCa-S, CRPH and cellular composition of BALF between the VAP and the non-VAP group (significance was set at 0.05). Correlations between PCT and CRP concentrations in BAL and serum were evaluated using Pearsons correlationcoefficient (r). In order to ascertain the value of ProCa-S and CRPH concentrations in BALF for the diagnosis VAP, Receiver Operation Characteristic (ROC) curves were plotted[25].

Results

In the period November 1999 until June 2002, 133 BALF samples with paired serum samples were obtained from intensive care patients suspected of VAP. A total of 16 BALF samples met the rejection criteria. This amounts to a total of 117 BALF samples, obtained from 117 patients, to be included. Out of these 117 BALF samples, 51 (43.6%) were microbiologically confirmed VAP. Out of these microbiologically confirmed VAP, 28 (54.9%) were caused by Gram-positive micro-organisms, 22 (43.1%) were caused by Gram-negative micro-organisms and one (2.0%) was caused by a yeast. Table 5.1 shows the cellular characteristics of the VAP and the non-VAP group.

Table 5.1 Cellular characteristics of BALF in the VAP group versus the non-VAP group.

	VAP		non-VAP		
	Median	Range	Median	Range	p-value
Total cell count *10^4, in cells/ml	57.0	8.0 - 5479.0	28.0	7.0 - 728.0	<0.01
% PMNs	89.4	9.6 - 99.8	49.4	0.2 - 99.6	<0.01
% Ams	6.8	0.0 - 87	38.6	0.0 - 99.2	<0.01
% Lyms	2.4	0 - 81.2	3.2	0.0 - 90.2	N.S.[a]
% EoS	0.0	0.0 - 1.0	0.0	0.0 - 9.0	N.S.[a]
% MCs	0.0	0.0 - 0.4	0.0	0.0 - 1.0	N.S.[a]
% IC	8.0	0.0 - 68.0	0.0	0.0 - 1.2	<0.01

[a] N.S.: not significant PMNs=polymorphonuclear neutrophil, AMs=alveolar macrophages, Lyms= lymphocytes, EoS=eosinophils, MCs=mast cells, IC=infected cells, BAL=bronchoalveolar lavage, VAP=ventilator-associated pneumonia

Abumin in BALF

Albumin concentrations in BALF varied between 3 mg/l and 4150 mg/l with a mean of 74 mg/l.

Validation of CRPH and ProCa-S determination in BALF

Matrix effect

Since both the CRPH and ProCa-S are designed to use in serum, the effect of measuring in BALF was studied. To a BALF sample with no detectable PCT or CRP, various serum samples with high PCT and CRP concentrations were added and the recoveries were measured. The maximum dilution ratio of serum:BALF was 1:5. Recovery rates varied between 77 and 100% (Table 5.2).

Table 5.2 Matrix effects of CRPH and ProCa-S in BALF.

CRPH			ProCa-S		
Calculated [CRP] mg/l	Measured [CRP] mg/l	Recovery (%)	Calculated [PCT] (ng/l)	Measured [PCT] (ng/l)	Recovery (%)
0.41	0.40	97	1928	1482	77
3.50	3.50	100	2108	1696	80
6.00	5.70	95	2245	2292	102
17.9	17.9	100	2300	2016	88

CRP=C-reactive protein, CRPH = high-sensitivity CRP, PCT=procalcitonin, ProCaS: high-sensitivity PCT, BALF=bronchoalveolar lavage.

Detection limit

We determined the detection limit in BALF, which was 0.18 mg/l and 12 ng/l for CRPH and ProCa-S respectively (data not shown).

Linearity

Both CRPH as the ProCa-S tests showed good linearity in BALF in the investigated ranges. (Slope: 1.05 and 1.00 respectively)

Intra- and interassay variation

To determine the imprecision BALF samples were spiked with either high or low concentrations of CRP or PCT. Table 5.3 summarises the intra- and inter-assay variation for the CRPH as well as for the ProCa-S in BALF. The results of the inter-assay imprecision were obtained during a two week period. As can be concluded from Table 5.2, the results were within acceptable ranges (coefficient of variation <10%).

Table 5.3 Intra- and inter assay variation of the CRPH and the ProCa-S in BALF, determined on a sample with a low concentration and a sample with a high concentration of respectively CRP and PCT.

	CRPH (mg/l)				ProCa-S (ng/l)			
	Intra assay		Inter assay		Intra assay		Inter assay	
	Sample low	Sample high	Sample low	Sample high	Sample low	Sample high	Sample low	Sample high
x[a]	4.20	11.30	3.50	10.05	44.50	950.0	46.10	975.5
SD[b]	0.11	0.24	0.07	0.29	0.43	8.60	3.80	36.80
CV[c](%)	2.60	2.12	2.05	2.76	0.97	0.90	7.70	3.80

[a] x is the mean of seven determinations; [b] SD is the standard deviation; [c] CV is the coefficient of variation; CRP=C-reactive protein, CRPH=high-sensitivity CRP, PCT=procalcitonin, ProCaS: high-sensitivity PCT, BALF=bronchoalveolar lavage fluid.

CRP and PCT concentrations in BALF and serum samples as a prediction of VAP

Table 5.4 shows the mean, standard deviation (SD), median, range and p-values for PCT and CRP in BALF samples obtained from patients with and without VAP. No significant differences were found between both groups. From this table, it is clear that there was a large overlap between the VAP and the non-VAP group for both serum and BALF concentrations. ProCa-S and CRPH levels in BALF samples tended to be higher in the non-VAP group. For PCT and CRP in serum the opposite was seen. However, none of the differences reached statistical significance.

With regard to the CRPH concentrations in BALF, one outlier was identified (concentration 204.4 mg/l), this belonged to the non-VAP group and also had a high CRP value in the serum together with a slightly increased PCT concentration in serum (124 mg/l and 5.6 μg/l respectively). Both ProCa-S and albumin were low in the BALF of this patient. With regard to ProCa-S concentrations in BALF, four outliers were noted (values >1.0 μg/l), one in the VAP group and three in the non-VAP group. Three of them also had elevated concentrations of PCT in serum and albumin (307, 831 and 4150 mg/l) in BALF (all non-VAP group). The remaining patient had an elevated serum PCT concentration but a BALF albumin level within normal limits.

BALF and serum CRP and PCT in the prediction of VAP

Table 5.5 lists the ROC curves of CRP and PCT in BALF and serum. The AUC of both CRP and PCT in BALF and serum did not exceed 0.5 indicating that both CRP and PCT were not suitable for discriminating between patients with and without VAP in BALF as well as in serum.

Table 5.4 Mean, standard deviation (SD), median and range for PCT and CRP in BALF and serum. None of the differences was statistically significant (p>0.05)

	VAP (n=51)	non-VAP (n=66)
CRP BALF (CRPH, mg/l)		
Mean	13.9	59.3
SD	36.0	243.9
Median	4.1	4.4
Range	1.0 - 204.4	0.0 - 2223.0
PCT BALF (ProCa-S, μg/l)		
Mean	0.3	1.2
SD	0.7	4.9
Median	0.1	0.1
Range	0.0 - 4.1	0.0 - 33.9
CRP serum (CRP, mg/l)		
Mean	155.4	160.8
SD	98.9	87.8
Median	127.0	165.0
Range	9.8 - 363.0	0.9 - 373.0
PCT serum (LUMItest, μg/l)		
Mean	6.4	5.0
SD	17.1	8.8
Median	1.0	1.6
Range	0.1-98.5	0.1-48.1

CRP=C-reactive protein, CRPH=high-sensitivity CRP, PCT=procalcitonin, ProCaS: high-sensitivity PCT, BALF=bronchoalveolar lavage fluid.

Table 5.5 AUC for CRP and PCT in BALF and serum.

Parameter	AUC
CRP in BALF	0.477
CRP in serum	0.453
PCT in BALF	0.448
PCT in serum	0.373

CRP=C-reactive protein, CRPH=high-sensitivity CRP, PCT=procalcitonin, ProCaS: high-sensitivity PCT, BALF=bronchoalveolar lavage fluid, AUC=area under the curve.

Correlations between blood and BALF

There was no correlation between BALF CRPH and serum CRP concentrations (r=0.27) There was a correlation between concentration of ProCa-S in BALF and PCT in serum (r=0.63). No correlation was found between BALF albumin levels and either ProCa-S and CRPH in BALF. However, there was a tendency towards higher albumin levels in BALF samples with either high ProCa-S or CRPH in BALF.

Correlations with PMNs and bacterial load

No correlation ($r<0.1$, $p>0.1$) was found between the percentage of polymorphonuclear neutrophils (%PMNs) and PCT or CRP levels in BALF and serum. No correlation was found between the quantity of micro-organisms cultured in the BALF and the concentration of ProCa-S in BALF. When the bacterial burden in the BALF was considered, a large overlap was noted between the different categories (results not shown). No significant difference was found in the concentrations of BALF ProCa-S in samples from patients with pneumonia caused by Gram-positive micro-organisms versus patients with pneumonia caused by Gram-negative micro-organisms. One outlier (very high concentration of ProCa-S) was identified, this was a sample with Gram-positive bacteria (*Staphylococcus aureus*) present in a quantity of $>10^5$ cfu/ml.

Presence of bacteremia

In all 117 patients, blood cultures were drawn at time of BAL. Micro-organisms were recovered in 10 (8.5%) patients: *Escherichia coli*, *Enterobacter cloacae*, *Streptococcus pneumoniae*, *Salmonella enteritidis*, *Serratia marsescens* (1 isolate each), *Staphylococcus epidermidis* (n=2) and *Candida albicans* (n=3). The focus of the bacteremia could be established in 8/10 patients, most frequent were intravenous catheter related bacteremia (n=6), followed by urinary tract infection (n=1) and gastroenteritis (n=1) In 4/10 patients VAP was present, however the bacteria recovered in BALF were not the same as recovered in the blood culture, making it unlikely that the pneumonia was the focus of the bacteremia. No significant differences were found, in PCT or CRP levels in BALF and serum, between the group of patients with and without proven bacteremia.

Discussion

In this study, ProCa S kit and the CRPH kit were validated for BALF. Both tests performed good when assessed for matrix-effect, linearity and reproducibility. There was a large overlap in ProCa-S and CRPH concentrations in BALF samples between patients with and without VAP. Both ProCa-S and CRPH concentrations in BALF resulted in a small AUC indicating both parameters to be unsuitable as a predictor for VAP.

In the diagnosis VAP, quantitative culture exceeding 10^4 cfu/ml, is regarded as a definite microbiological diagnosis[1, 2]. In earlier studies[26], we found that PMNs and lactate dehydrogenase activity, particularly its isoenzymes, in BALF appeared to be of important practical value in distinguishing between infection and non-infectious pulmonary disorders.

Most other studies dealing with the subject of determining parameters in BALF do not take into consideration the 10-100 times dilution of BALF compared to the alveolar lining fluid[24]. This makes it difficult to compare those data with ours.

In this study, a reasonable correlation was found between serum PCT levels and ProCa-S in BALF. This is in line with Stiletto and co-workers[27] who also found a correlation between PCT in BALF and serum in patients with severe pulmonary contusion.

Our findings indicate PCT in both serum and BALF to be unreliable in the diagnosis VAP. This is in contradiction with the results found by Oppert and co-workers[8]. They found elevations in serum PCT in patients with VAP after cardiopulmonary resuscitation. However they defined their diagnosis VAP according to the presence of radiographic signs and a micro-organism isolated from either blood or bronchial aspirate. Our diagnosis VAP relies upon a quantitative culture result of BALF $\geq 10^4$ cfu/ml making the diagnosis infection more evident. Since Oppert *et al.* may have included patients who do not reach our criteria for VAP, these groups might not be comparable.

Our study results are partly in line with the results of Duflo *et al.*[28] who found an increase in serum PCT in patients with VAP, but no increase in alveolar PCT. The difference between both studies lies in the fact that we used the high-sensitivity kit (ProCa-S) which enabled us to detect even the slightest increase in PCT levels. In 72.6% (85/117) of cases PCT concentration in BALF were below the detection-limit of the LUMItest, this could explain the findings of Duflo *et al*.

The literature on PCT in patients with VAP is limited, however a few studies have been published concerning the use of PCT in patients with pneumonia. Werra *et al.*[15] investigated patients with septic or cardiogenic shock or severe pneumonia and found a significant increase in the concentration of PCT in blood in patients with septic shock only. Karzai *et al.*[18] investigated patients with community acquired pneumonia and did not find an increase in the PCT level in the serum of these patients. Luyt *et al.*[29] evaluated PCT kinetics as a prognostic marker in VAP and found a high PCT level to be associated with a more severe disease. They also used ROC curves to illustrate the usefulness of serum PCT in the prediction of the outcome of VAP and found AUCs exceeding 0.8.

The cellular origin of PCT in infection is not clear yet, however some evidence shows an important role for peripheral blood-monocytes[30]. We found the concentration range of PCT in BALF to be narrower than the concentration range of PCT in serum. Since there was no correlation between ProCa-S and/or CRPH and %PMNs in BALF we believe that there is no local production of PCT within the alveoli. All BALF samples with extreme high concentrations of PCT were from patients who also had high concentrations of PCT in their

serum, and the majority also had high albumin in their BALF. This could indicate alveolar leakage and therefore diffusion of PCT and CRP from the serum to the alveolar space.

No significant difference was found in ProCa-S levels in BALF and serum of patients with VAP versus patients with non-VAP. This may be because ICU patients are prone to several (nosocomial) infections such as bacteremia, which can account for the elevation of PCT in these patients.

No correlation was found between the organism causing the pneumonia and the concentration of ProCa-S in BALF, even though lipopolysaccherid (LPS) is recognised as the major stimulus of PCT production. This may be due to the fact that PCT is not locally produced, the presence of LPS from Gram-negative bacteria in the lung therefore do not stimulate PCT production, in contrast to (Gram-negative) bacteria in blood[14,30].

Since the study was conducted retrospectively we were unable to follow PCT levels in serum (and/or BAL). Boussekey *et al*. investigated PCT kinetics in patients with severe community-acquired pneumonia admitted to the intensive care unit and found PCT to be an independent risk factor of mortality.[31] Therefore it would be interesting to see if this can also be applied to patients with VAP. Due to the retrospective nature of the study, the amount of clinical data was limited. Blood cultures at the time of lavage were available and yielded a micro-organism in 10 out of 117 patients. No significant differences in PCT and CRP levels were found between the patients with and without bacteremia. On the other hand, the retrospective analysis did not allow to assess localised infections other than VAP, which could have influenced PCT and CRP values.

The aim of our study was to evaluate the value of PCT and/or CRP levels in serum and BALF samples in the discrimination between VAP and non-VAP. Unfortunately neither PCT nor CRP, in both serum as BALF, could be used to predict the presence of VAP.

Since the literature on PCT in BALF and serum as a predictor of VAP is contradicting, a large prospective study should be conducted to further assess the value of this parameter, taking into account the dilution of BALF compared to epithelial lining fluid.

In conclusion, the ProCa-S kit and the CRPH kit are useful in assessing the concentration of respectively PCT and CRP in BALF. As the PCT and CRP levels in BALF did not discriminate between patients with and without VAP, they appeared to be of no additional value in the diagnosis of VAP. Therefore, we stress that BALF analysis should at least include depicting the infected cells and a differential cell count to establish a VAP and distinguish it from other diagnoses like drug-induced pneumonitis, acute interstitial pneumonia or diffuse alveolar damage.

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