Serum Procalcitonin as A Diagnostic and Prognostic Marker for Bacterial Community - Acquired Pneumonia

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For community acquired pneumonia (CAP), the discrimination between typical and atypical bacterial causes could influence antibiotic choice and outcome of patients. Objective of this study was to evaluate the utility of serum procalcitonin (PCT) level as a diagnostic and prognostic marker for CAP. Typical bacteria were isolated and identified by conventional methods. An indirect immunoflourescence assay was used to diagnose atypical bacteria. Serum level of PCT was measured by ELISA and clinical outcome was evaluated. Out of 240 enrolled CAP patients, 95 (39.6%) had bacterial etiology (30.8 % typical bacterial pneumonia and 8.8% atypical pneumonia). Ninety five bacterial CAP patients were divided into 3 groups; group 1 (mortality, 20.1%), group 2 (complications, 52.6 %) and group 3 (discharge, 26.3 %). Group 1 patients had the highest PCT level in serum compared to other groups with a statistically significant difference (*P*< 0.001). A statistically significant higher serum level of PCT was detected in typical than atypical pneumonia (*P*< 0.001). In conclusion, serum PCT level may serve as a diagnostic and prognostic marker in CAP.

Community-acquired pneumonia (CAP) is a significant cause of mortality and morbidity worldwide. For improving outcome and prognosis of CAP, early proper assessment of pneumonia severity should be done as the mortality due to CAP remains high regardless of recent modalities in antimicrobials and health care services [1].

Unfortunately, clinical, radiological and microbiological differentiation between atypical and typical bacterial causes of CAP is somewhat difficult which necessitated the use of other diagnostic tools. Biomarkers have been arisen as valuable diagnostic and prognostic predictors in CAP. Furthermore, they have been used for follow-up of treatment and for investigating antibiotic modifications during the disease course [2, 3].

Procalcitonin is an important inflammatory biomarker. It is a protein encoded by the *CALC-I* gene on chromosome 11 and is a precursor molecule of the calcium regulating hormone "calcitonin. In bacterial infections, inflammatory cytokines and bacterial endotoxin can induce PCT production by lung, liver, kidney, adipose tissue where its serum level can increase up to 1000 times [2,4].

The valuable role of PCT in guiding antibiotic therapy, recognizing the etiology, discriminating between typical and atypical pneumonia and predicting outcome and prognosis of CAP had been appreciated previously [5, 6].

This study was done to investigate the utility of measuring serum level of procalcitonin in the diagnosis of CAP etiology and in predicting its mortality.

Patients and Methods

Study design: this prospective observational cross sectional study was conducted on 240 clinically and radiologically diagnosed CAP patients in the period

from June 2016 to August 2018 in Departments of Chest and Medical Microbiology & Immunology, Faculty of Medicine, Zagazig University. Institutional Review Board (IRB), Faculty of Medicine, Zagazig University, had approved this study. Informed consent was obtained from all study participants.

Patient enrollment

- Inclusion criteria

Patients with acute illness diagnosed clinically and radiologically as CAP were included in this study. Patients were diagnosed to have CAP if their chest X rays showed new or increasing infiltration in the lung field in addition to one or more of lower respiratory tract infection presentation including (fever, cough, purulent sputum and focal chest signs)[7].Comorbid diseases were documented by patients' medical investigations and previous medical reports.

- Exclusion criteria

These included age below 18 years, refusal of participation, history of hospitalization within 28 days and history of chronic renal disease. History of antimicrobial intake within 2 days and pregnant females were also excluded.

The following was done for patients:

-Clinical examination: Thorough medical history, full clinical examination including general and local. Patients' illness severity and management decision were determined by a validated pneumonia severity index; confusion, urea, respiratory rate, blood pressure and age ≥ 65 years (CURB-65) [8].

-Radiological investigations: Plain chest x-ray (posteroanterior & lateral views); repeated before & after any pleural intervention to exclude complications, pelvi-abdominal ultrasound and contrast enhanced CT to exclude abdominal malignancy.

-Routine Laboratory investigation: Routine hematological investigations including CBC, ESR, CRP, liver function tests, kidney function tests, bleeding profile as well as random level of blood glucose were done for hospital admitted patients.

-Bacteriological investigations: Sputum sample was collected in sterile container on patient encountering after rinsing mouth with water. Induced sputum using 3% hypertonic saline was done for patients who could not expectorate. Endotracheal tube aspirate was collected from patients with altered consciousness who were admitted to ICU. Mucopurulent part of collected sputum was used for microbiological evaluation including microscopic examination by Ziehl-Neelsen stain to detect acid fast bacilli and Gram stain to suggest pathogens causing CAP. Then, interpretation of Gram stained smear was done and Bartlett's criteria were applied for rejection of improper samples and another sample was collected when possible. Acceptable specimens (score ≥1) were thoroughly reviewed by oil immersion lens to detect bacterial morphotype number, morphotype predominance, association with pus cells, and association or adherence to epithelial cells. Gram stained smears were interpreted and typical and atypical pathogens were suggested [9, 10].

-For typical pathogens suggested by Gram stained smear: Semi-quantitative culture was performed by four quadrant method on 5% blood agar with optochin disk (5 µg) to help identification of *Streptococcus pneumonia* (*S. pneumoniae*), chocolate agar with bacitracin disk (10 U) to improve detection of *Haemophilus influenza* (*H. influenza*) (incubated in 5% CO_2 at 37°C) and Mac Conkey agar (incubated aerobically at 37˚C) for 40-48 h. Bacterial identification was done by conventional methods [11].

-Serological investigation: Five ml blood was collected from each patient on admission. Serum was separated and stored at -20°C till used for detection of specific IgM to atypical pathogens and assessment of serum procalcitonin level.

-For atypical bacterial pneumonia diagnosis: Specific IgM antibodies to pathogens causing atypical pneumonia was detected by indirect immunofluorescene according to manufacture's instructions using Pneumoslide M test (Vircell, Granada, Spain). Briefly, Serum was prepared by 1:1 dilution in phosphate buffered saline (PBS), 30µl of diluted serum was added to 150µl of Antihuman IgG sorbent and centrifuged to avoid interference. 15µl of supernatant was loaded in slide wells, incubated in humid chamber for 90 min at 37°C. Slides were washed by gentle shaking in PBS then 15µl of a fluorescent secondary IgM antibody was added to the wells and incubated for additional 30min at 37°C, wash step was repeated, mount media was added and lastly examined using fluorescence microscope at 400x magnification.

- After detection of CAP etiology the following was done:

Serum procalcitonin level assessment: This was performed for 95 patients with identified bacterial aetiology by Human Procalcitonin ELISA (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, after preparing all reagents, samples and standards, wells were loaded with standard and sample (100 µl each). The plate was covered and incubated at room temperature for 2.5 h. Then, washing was done 4 times followed by adding 100µl biotinylated antibody and incubation at room temperature for 1 h. Then, 100µl of streptavidin-HRP reagent was added to each well followed by incubation at room temperature for 45 min. Then, 100µl 3, 3′,5, 5′-Tetramethylbenzidine (TMB) substrate was added to each well. The plate was left at room temperature in dark for 30min. Finally 50µl of stop solution was added to each well. Absorbance was measured at 450 nm wavelength and results were recorded.

Evaluation of patients' outcome: The site of patient management was decided according to their CURB-65 score whether in ordinary ward or ICU. Outcome of 95 bacterial CAP patients was assessed by recording; length of hospitalization, development of complications, increased morbidity of associated illnesses and mortality. Clinical improvement as regarding clinical wellbeing, improvement of blood biochemical investigations and radiological improvement was also recorded.

Patients were then divided into three groups; group 1(mortality), group 2 (complications) and group 3 (discharge).

Statistical Analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA, 2011). Quantitative variables were described using their means and standard deviations. T-test was used for detection of difference between two different groups and one-way ANOVA for multigroup comparisons.

Results

This study enrolled 240 CAP patients including, 125 Males and 115 females with their age range from 21 to 78 years. Among them, 95.8% had comorbidities with COPD being the most frequent comorbidity reported and 58.3% were current smokers. Lobar pattern pneumonia was diagnosed in 31.6 % of patients in chest X-rays. While bronchopneumonia in chest X-rays was diagnosed in 10.4% of CAP patients. CURB 65 scores of 5, 3 and 2 were recorded in 16.6%, 29.2% and 54.2% of patients, respectively (table 1).

Bacterial etiology was detected in 39.6% of patients, with 30.8 % diagnosed as typical and 8.8% as atypical pneumonia. Predominant morphotype, polymicrobal and atypical pneumonia were suggested from direct film stained by Gram staining in 71.5%, 7.3% and 22.1% of bacterial pneumonia, respectively. The most frequently isolated bacteria were; *S. pneumoniae* (15%), *K. pneumoniae* (5.8%) and *P. aeruginosa* (4.2%).

Out of 95 hospital admitted CAP patients with identified bacterial etiology, 26.3% improved and discharged (group 3), 52.6% were complicated (group 2) and 21.1 % passed away (group 1).

Serum level of procalcitonin was significantly increased in group 1 ($P<0.001$) when compared to the other groups and in group 2 when compared with group 3 ($P<0.001$) (table 2). Also, it was found to be higher in typical than atypical pneumonia patients with a statistically significant difference ($P<0.001$) (table 3).

Table 1. Demographic, clinical and radiological characters of patients.

Demographic Characteristics	Frequency(No.)	Percent (%)
Age:		
<40	112	46.7
≥ 40	128	53.3
Range	21-78	
Mean±SD	40.9±14.6	
Median	39	
Sex :		
Male	125	52
Female	115	42
Comorbidities :		
Chronic obstructive pulmonary disease	100	41.7
Diabetic	67	28
Hypertensive	45	18.6
Hepatic	18	7.5
No comorbidities	10	4.2
History of aspiration	6	2.5
Smoking:		
Non- smokers	65	27.1
Current smokers	140	58.3
Ex-smokers	35	14.6
Pattern of pneumonia:		
Typical	74	30.8
Atypical	21	8.8
Chest x ray finding:		
Lobar	76	31.6
Multilobar /bronchopneumonia	25	10.4
Pleural effusion	70	29.5
*CURB 65 score:		
CURB 2	130	54.2
CURB 3	70	29.2
CURB 5	40	16.6

*CURB 65: confusion- urea-respiratory rate- blood pressure and age ≥ 65 years.

Table 2. Serum level of procalcitonin and outcome of hospitalized bacterial CAP patients

Outcome	Procalcitonin (mean±SD)	Range	*P* value
Group (1) Mortality (No.=20)	13.2±1.6	11.1-15.1	
Group (2)Complications(No.=50)	8.7±3.3	4.8-14.2	<0.001**
Group (3) Discharge (No.=25)	3.4±1.5	1.2-5.8	

** *P* < 0.05 is significant.

Table 3. Serum level of procalcitonin and bacterial etiology among CAP patients.

Etiology	Procalcitonin (mean±SD)	Range	*P* value
Typical (No=74)	8.07±3	4.8-15.1	<0.0001**
Atypical (No=21)	1.93±0.9	0.23-4	
Total (No.=95)	4.49±3.7	0.23-15.1	

** *P* < 0.05 is significant.

Discussion

Understanding management and outcome of CAP is of a significant interest. Several biomarkers were demonstrated to be independently associated with long-term mortality [12].This study was done to evaluate the value of measuring serum level of procalcitonin for diagnosis of CAP etiology and for predicting long-term mortality outcome.

A total of 240 CAP patients diagnosed clinically and radiologically were enrolled in this study. Males represented 52% of patients with 58.3% current smokers. Severe pneumonia had been found in 10.2% of patients with a CURB 65 score (5). Chronic obstructive pulmonary disease (COPD) was the most frequent comorbidity in those patients. This comes in agreement with Torres *et al.,* 2013 who reported that CAP was more prevalent in males, smokers and those with chronic respiratory diseases [13]. Almirall *et al.,* 2015, highlighted the importance of proper treatment of COPD and stopping smoking because both COPD and smoking were clear risk factors for development of CAP [14].

In this study, Bacterial etiology was detected in 39.6 % of CAP patients. Higher frequencies were recorded previously in Egypt by El Seify *et al.* 2016 (47.8%) and El-Sokkary *et al.,* 2018 (50.4%) [15, 16]. Meanwhile, a lower frequency (34.12%) was reported in a study in Jeddah Clinic Hospital on 296 hospitalized CAP children [17]. The discrepancy of results might be attributed to different patients' age groups, comorbidities and different CAP etiology detection techniques used as cultivation of sputum, blood culture, rapid antigen detection tests and polymerase chain reaction [17].

Previous studies had demonstrated a positive correlation between inflammatory response intensity, bacterial load and infection severity and PCT level. Accordingly, severe infections with bad sequalae might be predicted by measuring PCT level [4].

Concerning serum PCT level and CAP outcome in the current study, the highest level of PCT was detected in mortality group when compared to other groups. Also, there were statistically significant differences among the 3 groups; mortality, complications and discharge. It was proved that PCT helps predicting outcome and complication of infectious diseases [18]. Similar results reported that a high level of PCT was a marker of poor outcome of infections [19]. A study on 96 hospitalized CAP patients with age range (50-95 ys) also found that a fatal outcome was correlated with higher level of admission PCT in patients [20]. Also, it was reported that PCT was a highly accurate predictor for CAP-associated serious adverse events [21].

It was reported that high PCT level was associated with a bacterial cause of pneumonia and a bad prognosis in a two year prospective observational study done on CAP and ventilator-associated pneumonia patients in India [22]. In the current study, PCT level showed a statistically significant higher level in patients with typical bacterial pneumonia than those with atypical pneumonia. Patients with bacterial pneumonia had mean PCT 8.07±3 ng/ml. This comes similar to the results reported in a previous study that admission PCT level detection could discriminate between typical and atypical CAP, and subsequently could guide initial proper antibiotics treatment [21]. Morever, higher PCT was reported by Musher and coworkers in their study in patients with bacterial pneumonia when compared to viral, fungal or undetected etiology of CAP [23]. Moreover, another study reported that median PCT concentrations were higher in CAP children with typical bacterial pneumonia compared with those with atypical bacterial pneumonia and PCT measurement might discriminate between children who would get benefit from antibiotic treatment and those who would not [24]. Although, Abdelsadek and coworkers found that PCT level failed to discriminate the etiological element in patient with severe CAP due to severe systemic inflammatory response in both typical and atypical agents and thus they used radiological findings in discriminating typical from atypical etiology in severe CAP, they showed that PCT level was much higher in patients with mild and moderate typical pneumonia than those with atypical pneumonia [25].

In conclusion, PCT is an appropriate diagnostic and prognostic biomarker for bacterial CAP.

References

1. Sibila, O. and Restrepo, MI. Biomarkers in community-acquired pneumonia: still searching for the one. Euro Res J 2019 53(2): 1-3.
2. Seligman, R., Ramos-Lima, L. F., Oliveira, V., Sanvicente, C., Pacheco, E. F., Dalla Rosa, K. Biomarkers in community-acquired pneumonia: a state-of-the-art review. Clinics (Sao Paulo, Brazil), 2012; 67(11), 1321–1325.
3. Karakioulaki, M., Stolz, D. Biomarkers in Pneumonia-Beyond Procalcitonin. Inter. J Mol. Sci, 2019 Apr; 20 (8): e2004, 1-18.
4. Mehanic, S. and Baljic, R. The Importance of Serum Procalcitonin in Diagnosis and Treatment of Serious Bacterial Infections and Sepsis. Mater Sociomed. 2013 Dec; 25(4): 277-281.
5. Masiá, M., Padilla, S., Ortiz, de la Tabla, V., González, M., Bas, C., Gutiérrez, F. Procalcitonin for selecting the antibiotic regimen in outpatients with low-risk community-acquired pneumonia

using a rapid point-of-care testing: A single-arm clinical trial. PLoS One. 2017; 12 (4): 1-28. e0175634.

6. El-dib, A.S. and El-Srougy, HA. Diagnostic and prognostic role of procalcitonin in CAP. Egyp J Chest Dis & Tubercul. 2015; 64, 871–875.
7. Lepper, P. M., Ott, S., Nüesch, E., von Eynatten, M., Schumann, C., Pletz, M. W., Jüni, P. Serum glucose levels for predicting death in patients admitted to hospital for community acquired pneumonia: prospective cohort study. Bmj, 2012; 344, e3397.
8. Lim, W. S., van der Eerden, M. M., Laing, R., Boersma, W. G., Karalus, N., Town, G. I., Lewis S.A., Macfarlane, J. T. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax, 2003; 58(5), 377–382.
9. Sharp, S. E., A. Robinson, M. Saubolle, M. Santa Cruz, K. Carroll, and V. Baselski. Cumitech 7B, Lower Respiratory Tract Infections. Coordinating ed., S. E. Sharp.ASM Press, Washington, D. C, 2004.
10. Bhattacharya, A. K. Role of sputum cultures in diagnosis of respiratory tract infections. Lung India, 2006; 23(1), 20.
11. Cheesbrough, M (editor). (2006): District Laboratory Practice in Tropical Countries Part-2.2nd Edition. Cambridge, UK: Cambridge University Press. ISBN 978-0-521-67631-1.
12. Restrepo, MI., Faverio, P., Anzueto, A. Long-term prognosis in community-acquired pneumonia. Curr Opin Infect Dis. 2013; Apr; 26(2):151-158.
13. Torres, A., Peetermans, WE., Viegi, G., Blasi, F. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013; 68:1057–1065.
14. Almirall, J., Serra-Prat, M., Bolibar, I. Risk factors for community-acquired pneumonia in adults: Recommendations for its prevention. Community Acquir Infect.2015; 2:32-37.
15. El Seify, M.Y., Fouda, E.M., Ibrahim, H.M., Fathy, M.M., Husseiny Ahmed, A.A., Khater, W.S., El Deen, N.N., Abouzeid, H.G., Hegazy, N.R., Elbanna, H.S. Microbial Etiology of Community-Acquired Pneumonia Among Infants and Children Admitted to the Pediatric Hospital, Ain Shams University. Eur J Microbiol Immunol *(*Bp). 2016 Aug 5; 6 (3):206-214.
16. El-Sokkary, R.H., Ramadan R.A, El-Shabrawy, M., El-Korashi, L.A., Elhawary, A., Embarak, S., Tash, R.M.E., Elantouny, N.G. Community acquired pneumonia among adult patients at an Egyptian university hospital: bacterial etiology, susceptibility profile and evaluation of the response to initial empiric antibiotic therapy. Infect Drug Resist. 2018 Nov 2;11:2141-2150.
17. Mansour, M.M., Al Hadidi, K.M., Hamed, M.S. Epidemiology and antimicrobial resistance of community-acquired pneumonia in children. J Health Res Rev 2017; 4:122-129.
18. Hoeboer, S., and Groeneveld, J. Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients. PLoS One, 2013; 8(6):e65564.
19. Castelli, G. P., Pognani, C., Meisner, M., Stuani, A., Bellomi, D., &Sgarbi, L. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. Critical care, 2004; 8(4), R234.
20. Schuetz, P., Suter-Widmer, I., Chaudri, A., Christ-Crain, M., Zimmerli, W., & Mueller, B. Prognostic value of procalcitonin in community-acquired pneumonia. European Respiratory Journal, 2011; 37(2), 384-392.
21. Hedlund, J., and Hansson, L. O.Procalcitonin and C-reactive protein levels in community-acquired pneumonia: correlation with etiology and prognosis. Infection, 2000; 28(2), 68-73.
22. Bansal, P., and Sudhir, U. Diagnostic and prognostic value of serum Procalcitonin in pneumonia - A prospective observational study. J. Adv. Res. Med. 2018; 5(4): 8-13.
23. Musher, D. M., Roig, I. L., Cazares, G., Stager, C. E., Logan, N., Safar, H. Can an etiologic agent be identified in adults who are hospitalized for community-acquired pneumonia: results of a one-year study. Journal of Infection, 2013; 67(1), 11-18.
24. Stockmann, C., Ampofo, K., Killpack, J., Williams, D.J., Edwards, K.M., Grijalva, C.G., Arnold, S.R., McCullers, J.A., Anderson, E.J., Wunderink, R.G., Self, W.H., Bramley, A., Jain, S., Pavia, A.T., Blaschke, A.J. Procalcitonin

Accurately Identifies Hospitalized Children With Low Risk of Bacterial Community-Acquired Pneumonia. J Pediatric Infect Dis Soc. 2018 Feb 19; 7(1):46-53.

25. Abdelsadek, A., Naga, O. A., Shams, M. A., Eltelbany, E., &Hamd, A. Procalcitonin as a Predictor for Severity and Etiology of Community Acquired Pneumonia (CAP). Br J Med Med Res, 2017; 19(1), 1-8.