

Monocyte Chemoattractant Protein-1 as a predictor of community-acquired pneumonia outcome in children

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Abstract

Community-acquired pneumonia (CAP) exhibits high mortality rates, Biomarkers are useful tools for diagnosis, searching for antibiotic therapy, prognosis, and follow-up treatment. Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine C-C ligand-2 (CCL-2), is expressed by monocytes, macrophages, lymphocytes, and airway epithelial cells in response to inflammation. The objective of this study was to investigate serum levels of MCP-1 in a group of hospitalized children with community-acquired pneumonia, with emphasis on prognostic value of MCP-1 regarding patients' outcome. The present study was conducted at Cairo University Pediatric Hospital and El Tahreer General Hospital in Imbaba-Ministry of Health, Cairo, from April to October 2016. Eighty patients were included as study candidates. They were Egyptian children with community acquired pneumonia admitted to the general wards and intensive care units (ICUs) of the hospitals. The study reached the following: In hospitalized children with community-acquired pneumonia, serum MCP-1 levels were

significantly higher in non-survivors compared to survivors (p value <0.001). □ There was a significant direct correlation between MCP-1 and respiratory distress, ESR and CRP.

□ High Serum MCP-1 was sensitive and specific in predicting mortality outcome compared to ESR and CRP. with sensitivity of 100%, 83.3%, 70.8% and respectively and specificity of 96.4%, 76.8%, 91.1% and respectively.

Key words: MCP-1, community acquired pneumonia (CAP), biomarkers.

Introduction

Community acquired pneumonia (CAP), is defined simply as the presence of signs and symptoms of pneumonia in a previously healthy child caused by an infection that has been acquired outside of the hospital [1]. Community-acquired pneumonia (CAP) continues to be a major cause of death among children aged under-5 years worldwide and almost all of these deaths occur in developing countries [2]. Community acquired pneumonia (CAP) exhibits high mortality rates, up to 50% in severe cases. Biomarkers are useful tools for searching for antibiotic therapy modifications and for CAP diagnosis, prognosis, and follow-up treatment [3]. Monocyte chemoattractant protein-1 (MCP-1/CCL2) is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages. Monocyte chemoattractant protein (MCP-1) increases in the serum of immunocompetent patients with community-acquired pneumonia (CAP). The antibiotic treatment reduced the number of white blood cells (WBCs) and neutrophils as well as the level of C-reactive protein (CRP). MCP-1 levels act in the development of CAP and are involved in the severity of CAP [4].

Patients And Methods

- Study design:

The study was conducted at Cairo University Pediatric Hospital and El Tahreer General Hospital

in Imbaba-Ministry of Health, Cairo. Eighty patients was included. They will be Egyptian children with community-acquired pneumonia admitted to the general wards and intensive care units (ICUs) of the hospitals. The study was held from April to October 2016. Sample size calculation was performed using Clinical's online sample size calculator ([http://clinical.com/Stats/Sample Size.aspx](http://clinical.com/Stats/SampleSize.aspx)).

➤ Inclusion criteria:

- Admitted pediatric patients aged from 2 months to 12 years of age, with a clinical diagnosis of pneumonia confirmed by a chest x-ray. Approval by the parents/caregivers to participate in the study.

➤ Exclusion criteria:

- Children with diseases other than community-acquired pneumonia, Children with ventilator-associated pneumonia, Children hospitalized and treated for two or more days before inclusion
Intake of corticosteroids within 2 weeks prior to inclusion and, Refusal to participate in the study Group.

All candidates will be subjected to history taking, age, gender, residence, history of fever, cough, expectoration, hemoptysis and shortness of breath, history of exposure to a source of infection, history of investigations and treatment given, and history of any complications.

- Physical examination, General examination: with stress on level of consciousness, color, respiratory distress grade, heart rate, respiratory rate, blood pressure, temperature and peripheral perfusion
- Chest examination and radiological investigation(Chest x-ray posteroanterior and lateral views).
- Laboratory investigations:

All cases were undergo complete blood count (CBC), erythrocytic sedimentation rate (ESR), quantitative c-reactive protein (CRP), and sputum cultures.

▪ Special Investigation: Assay of serum levels of MCP-1(Quantikine ELISA) humanCCL/2MCPcatalog Number(DCP00,SCP00,PDPC00) was done as follows: Three milliliters of venous blood will be withdrawn from each candidate within 48 hours of hospitalization. The blood sample will be centrifuged and serum will be stored in plastic tubes at -20° C till assay is performed after all samples were collected. MCP-1 was analyzed using commercial enzyme-linked immunosorbent assay (ELISA) kit according to manufacturer's specifications. Assay procedure (Abstracted from the kit's manual),all reagents and samples were brought to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. For Serum/Plasma Samples Only: 50 µL of Assay Diluent RD1-83 was added to each well. Assay Diluent RD1-83 may contain a precipitate should mixed well before and during use.

2. 200 µL of Standard, sample*, or control was added per well , covered with the adhesive strip provided,and Incubated for 2 hours at room temperature. A plate layout was provided to record standards and samples assayed.

3. Each well was aspirated and washed, for twice to three washes with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, any remaining was removed by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4.. 200 µL of MCP-1 Conjugate to was added to each well,covered with a new adhesive strip, and Incubated for 2 hours at room temperature.

5..aspiration/wash was repeated as in step 3.

6. 200 μ L of Substrate Solution was added to each well, Incubated for 30 minutes at room temperature and protected from light.

7.. 50 μ L of Stop Solution was added to each well, the color in the wells should change from blue to yellow, If the color in the wells is green or the color change does not appear uniform, the plate was gently tapped to ensure thorough mixing.

8.. optical density of each well was determined within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

- **Statistical methods**

Statistical analysis was performed using SPSS-17. The following tests were used: Frequency distributions, percentage distributions, Means \pm standard deviation, t-tests, chi-square test, and tests of correlation and regression. Tests of diagnostic accuracy (sensitivity, specificity and receiver operating curve [ROC]) was conducted. P-values less than 0.05 will be considered significant. Confidence intervals (95% CI) will be calculated when appropriate.

Results

Eighty hospitalized children with community-acquired pneumonia were enrolled in this study. 46 (57.5%) male and 34 (42.5%) female patients aged from 2 months to 12 years. 28.7% were from urban area and 71.3% were from rural area. Testing of normal distribution of data (using Kolmogorov-Smirnov method) was performed and data was found to be non-normally distributed. Accordingly, non-parametric statistical tests were employed in data analysis.

The number of cases with different degree of respiratory distress in selected cases were shown in figure 1.

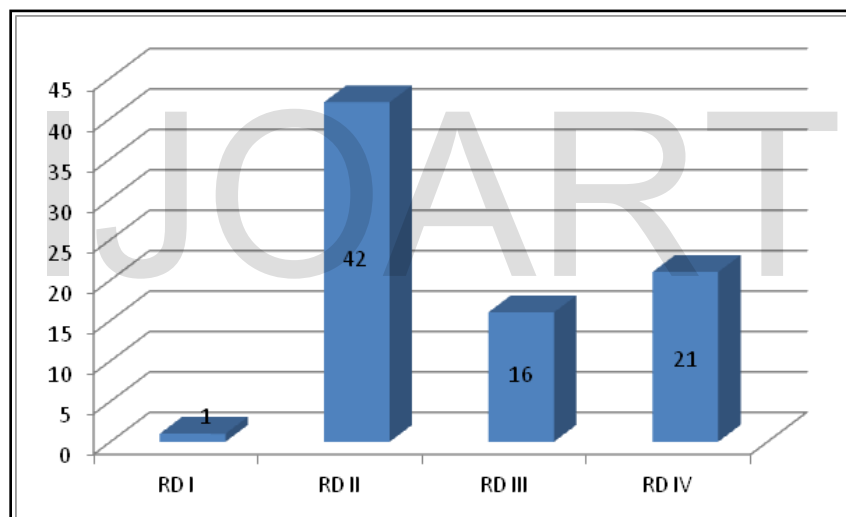


Figure (1): Respiratory distress in cases

According to chest xray community acquired pneumonia in selected cases was classified anatomically into 73 cases with bronchopneumonia, 7 with Lobar pneumonia, 64 cases need

ICU admission and 30 of them were on mechanical ventilation. Table 1 show median

hemoglobin level, total leucocytic count, ESR and CRP of studied patients.

Table (1): Hematological investigations of cases

Investigation	Median (interquartile range)
Hemoglobin (g/dl)	10.00 (1.97)
Total leucocytes ($\times 10^3$ /cmm)	11.00 (8.22)
ESR (mm 1st hour)	20.00 (23.75)
CRP (mg/L)	18.50 (24.75)

The median level of Serum MCP-1 in the studied patients was 300pg/ml with

median interquartile range of 684. The studied children were classified according to

outcome into survival and non survival as shown on table 2 & figure 2.

Table 2: Mortality outcome of patients within 30 days of hospitalization

Outcome	Frequency(80)	%
Survivors	56	70
Non-survivors	24	30

ANALYTICAL STATISTICS

Table (3): Comparison between survivors and non-survivors.

	Survivors (56) ¹	Non-survivors (24) ¹	95% CI ²	P-value
Age (months)	7.50 (18.00)	3.00 (7.00)	0.001,6.00	0.021*
Sex (male/female)	34/22	12/12	-	
Weight (kg)	6.75 (5.50)	4.55 (3.12)	0.50,3.50	0.007*
Heart rate (/ min.)	130.00 (20.00)	134.50 (21.50)	-15.00,0.00	0.105
Respir. rate (/min.)	30.00 (8.00)	30.00 (8.25)	-5.00,0.00	0.234
Temperature (°C)	37.50 (0.65)	37.70 (0.50)	-0.20,0.00	0.836
Hemoglobin (g/dl)	10.00 (1.55)	10.05 (2.57)	-0.50,1.30	0.429
Leukocytes (10 ³ /cmm)	10.95 (5.65)	13.75 (13.57)	-6.49,0.99	0.151
ESR (mm 1st hour)	15.00 (5.00)	40.00 (34.50)	-25.00,- 14.00	<0.001*
CRP (mg/L)	12.00 (24.00)	57.50 (51.75)	-52.01,-23.01	<0.001*
Serum MCP-1 (pg/ml)	170.00 (148.00)	1660.00 (1500.00)	-1904.0,- 860.0	<0.001*

¹ median (interquartile range); ² confidence interval at 95% level; * significant

Non-survivors were significantly lower in age and weight, again acute phase reactant was significantly higher in them. Serum MCP-1 was significantly higher in non-survivors compared to survivors ($p = < 0.001$). (tab3).

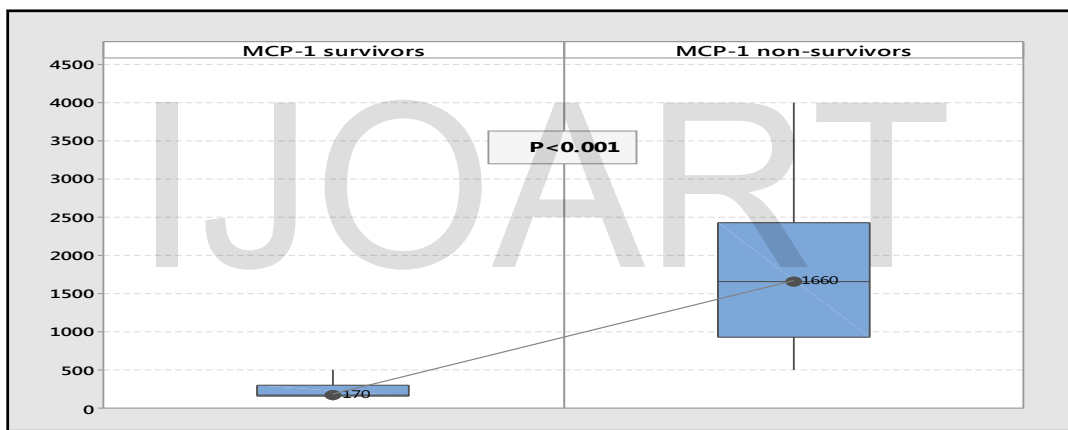


Figure (2): Median MCP-1 levels in cases according to their outcome

($p = < 0.001$)

Table (4): Spearman's correlation between serum MCP-1 and other variables (n=80)

	Variable	Spearman's rho	
		Coefficient	-0.295
	Age (months)	P-value	0.008*

MCP-1	Weight (kg)	Coefficient	-0.233
		P-value	0.037*
	Respiratory distress grade	Coefficient	0.411
		P-value	<0.001*
	Heart rate (/min.)	Coefficient	0.130
		P-value	0.252
	Respiratory rate (/min.)	Coefficient	0.155
		P-value	0.170
	Temperature (° C)	Coefficient	0.081
		P-value	0.472
	Hemoglobin (g/dl)	Coefficient	-0.050
		P-value	0.662
	Total leukocytic count (10 ³ /cmm)	Coefficient	0.131
		P-value	0.249
	ESR (mm 1 st hour)	Coefficient	0.501
		P-value	<0.001*
	C-reactive protein (mg/l)	Coefficient	0.604
		P-value	<0.001*

* = Significant

There was a significant negative correlation between serum MCP-1 , age and weight. There

was a significant direct correlation between MCP-1 and respiratory distress, ESR and

CRP.figur4.

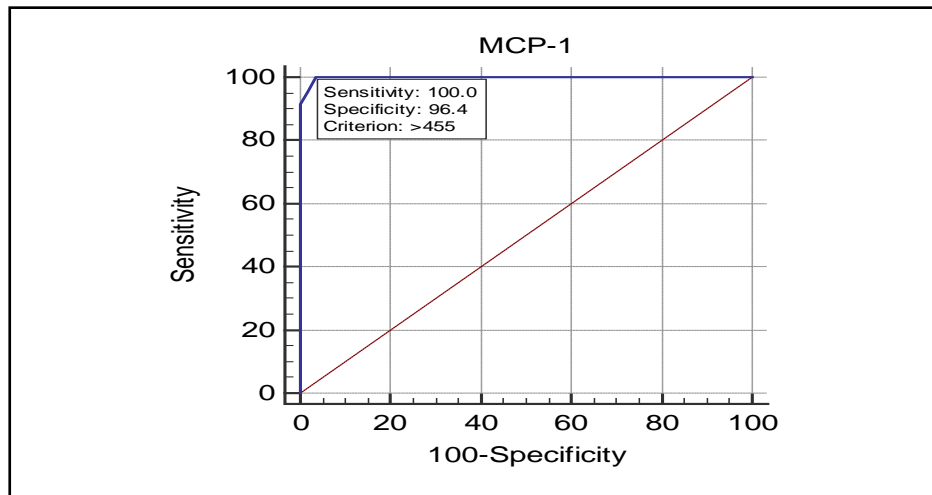


Figure (3): Receiver operating characteristic (ROC) curve of serum MCP-1 in cases.

Serum MCP-1 showed high sensitivity and specificity in predicting unfavorable outcome in pediatric CAP .

Table (4): Predictive value of serum MCP-1 in pediatric CAP.

Results	MCP-1
Area under curve (AUC)	0.99
Best cut-off value	>455 pg/ml
Sensitivity	100%
Specificity	96.4%

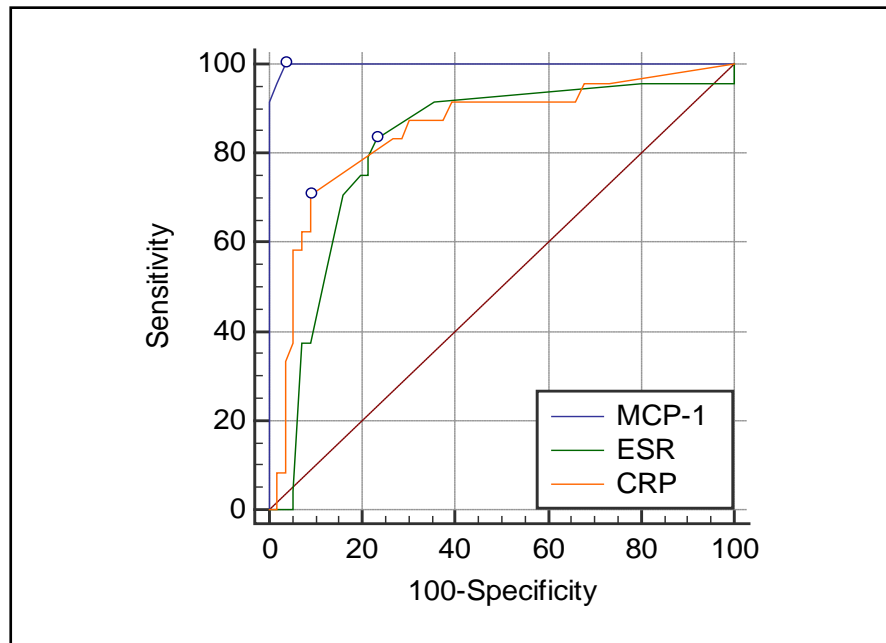


Figure (4): Comparison of ROC curves of ESR, CRP, and MCP-1 in predicting mortality.

Table (5): Performance of ESR, CRP, and MCP-1 in mortality predication

Results	ESR	CRP	MCP-1
Area under curve (AUC)	0.82	0.85	0.99
Best cut-off value	>20 mm1 st hour	>24 mg/l	> 455 pg/ml
Sensitivity	83.3%	70.8%	100%
Specificity	76.8%	91.1%	96.4%

High Serum MCP-1 was more sensitive and specific in predicting mortality outcome, compared to ESR and CRP.table(5)

Table (6): Independent factors predicting mortality outcome (Binary logistic regression):

Variable	Odds ratio	95% CI ¹	P-value
Age younger than 12 months	8.01	1.16 to 55.11	0.034*
High ESR (> 20)	8.07	1.61 to 40.30	0.010*
Positive CRP (> 6)	2.05	0.15 to 26.49	0.582

Mechanical ventilation	23.47	4.78 to 115.05	< 0.001*
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¹ 95% CI: confidence intervals at 95% level.

Young age, high ESR, and need for mechanical ventilation were significantly able to predict mortality. Serum MCP-1 could not fit into the logistic regression model due to relatively small sample size.

Discussion

Community acquired pneumonia (CAP) is the presence of signs and symptoms including cough, dyspnea, sputum production, and pleuritic chest pain in a previously healthy child caused by an infection that has been acquired outside of the hospital [1]. CAP is a major cause of death among children aged under-5 years worldwide and almost all of these deaths occur in developing countries [2]. Viruses and bacteria, either alone or in combination, are the primary causes of CAP [5]. The majority of children with CAP have multiple pathogens (bacteria and viruses). The diagnosis of CAP should be considered in any child who has an acute onset of respiratory symptoms, particularly cough, fast breathing. Diagnosis includes clinical evaluation, radiographic evaluation, and laboratory investigations to establish

whether pneumonia is present, assesses the severity of pneumonia and determine the causative organism [6]. Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine C-C motif ligand-2 (CCL-2), is expressed by monocytes, macrophages, lymphocytes, and airway epithelial cells in response to inflammation [7]. MCP-1 is a potent monocyte and macrophage, neutrophil, and T-cell chemoattractant in bacterial infections; this is due to MCP-1 binding to its receptor chemokine C-C motif receptor- 2 (CCR-2) [8]. MCP-1 is a protein encoded by the *CCL2* gene in humans. CCL2 is a small cytokine belonging to the CC chemokine family and a potent chemotactic factor for monocytes [9]. In this study males constituted 57.5% of cases compared to females who constituted 42.5%. The male predominance agrees with literature of Boyer et al., 2009 and Farha and Thomson., 2005 who assessed the risk factors of childhood pneumonia in the developing world, and reported that there was strong male predominance in those aged less than 5 years. The majority of patients came from rural areas (57.7%), while only (23.3%) lived in urban areas [10&11]. This is in spite of the fact that the study was conducted in the capital of Egypt; it is because

Abu El Reish hospital is a large tertiary- level center with many cases of pneumonia being referred to it from almost all governorates of Egypt. In addition, El Tahreer General Hospital receives many patients from rural parts of Greater Cairo. In our study, we found that non-survivors were significantly lower in weight compared to survivors. This is comparable to the findings of previous study by **Banajeh, 1998** which reported that low weight for age and malnutrition were significant factors for mortality [12]. Other studies by **Teiwsoh et al., 2009** & **Coles et al., 2005** reported that children with CAP associated with history of prematurity and low birth weight had increased risk of mortality [13 & 14]. We detected that non-survivors were significantly younger in age compared to survivors. **Djelantik et al., 2003** conducted a population-based hospital cohort study among children less than 2 years of age admitted for pneumonia during 2000-2001 at one of three major hospitals in Lombok Island, Indonesia. They found that laboratory, physical examination and radiological findings were not associated with mortality prediction [15]. Among children hospitalized for pneumonia, age less than 4 months and hypoxia were identified with those at high risk of death. However,

some studies Pinto et al., 2004 – Lahti et al., 2007 found that age was not related to complications and mortality in CAP [16]. It is suggested that a meta-analysis conducted for all studies published in this area would produce sufficient evidence to whether young age is a risk factor for CAP mortality or not. We defined patients who were still alive after at least 1 month from the date of hospitalization as survivors, the rest were defined as non-survivors. In the study, survivors constituted 70% of cases while non-survivors constituted 30% of cases. A previous study at the same center detected a mortality rate of 31.7% [17]. These mortality rates appear to be high in comparison with other studies, Mortality rate was 8.6% in a study in India ([18] Again in Paraguay, mortality rate was 6.5% [19]. However, differences in study design could have led to that contradiction; some studies excluded children who necessitated ICU admission, whereas in our study, about 80% of children admitted in ICU and about 37.5% were mechanically ventilated. Nevertheless, the authors believe that this mortality rate is still high and appropriate countermeasures must be taken to lower it in the future. Our study showed that, acute phase reactants were significantly higher in non-survivors compared

to survivors. It was reported that there was significant correlation between serum high sensitivity CRP (Hs-CRP) level and outcome of CAP cases (died or survived), and the results showed a significant correlation between serum Hs-CRP level and need of ICU admission, need of oxygen therapy, need of mechanical ventilation as well as a significant positive correlation between serum Hs-CRP level and length of hospital stay among survived patients. Hs-CRP had a significant prognostic value in CAP. Another study showed that patients with very severe pneumonia had significantly higher median CRP compared to patients with severe [20] On the other hand, **Pierrakos and Vincent, (2010)** reported that total leukocytic count and CRP were not reliable predictors for assessing disease severity and mortality risk [21]. Our study showed that, Serum MCP-1 was significantly higher in non-survivors compared to survivors (median = 1660 pg/ml versus 170 pg/ml, respectively, $p < 0.001$). In Taiwan, a study was conducted by **Yong et al., (2016)** which enrolled 137 adult patients with CAP and 74 healthy controls to investigate differential changes in the plasma MCP-1 levels of patients with CAP before and after an antibiotic treatment and further

analyzed the association between the CAP severity and MCP-1 levels. It found that plasma MCP-1 level was significantly elevated in patients with CAP before they received treatment (803.3 ± 74.1 pg/ml) compared with the controls (163.3 ± 17.7 pg/ml) and significantly decreased in patients with CAP after treatment (359.4 ± 40.5 pg/ml, $p < 0.001$) and that plasma MCP-1 levels correlated with the CAP severity [4]. Our study showed that, There was a significant direct correlation between MCP-1 and respiratory distress, ESR and CRP. Up to the authors' knowledge, there were no previous published studies reporting this neither in children nor in adults. Regarding diagnostic accuracy of MCP-1, our study showed that serum MCP-1 had high sensitivity and specificity in predicting unfavorable outcome in pediatric CAP. High Serum MCP-1 was more sensitive and specific in predicting mortality outcome, compared to ESR and CRP. Up to author's knowledge, there were no previously published papers studying prognostic value of MCP-1 levels in pediatric CAP. In accordance with our findings, previous studies have reported that CRP levels may contribute to establishing a CAP diagnosis [22]. However, inconsistent results have been reported by studies evaluating

the use of CRP as a prognostic factor for CAP. For instance, some studies have indicated that CRP is a promising diagnostic and prognostic tool for managing CAP [23]. However, other studies have indicated that elevated CRP levels in patients with CAP have no prognostic relevance [24]. Our study showed that young age was significantly able to predict mortality. This agrees with Pratheepamornkull et al., (2015) who found that young and preterm infants with CAP should be monitored closely due to their high risk for developing serious complications including mortality [25]. The need for mechanical ventilation was an independent factor predicting mortality outcome in our patients. This agrees with Ramachandran et al., (2012) who found that the need for assisted ventilation alone was found to be an independent risk factor for mortality in children with pneumonia [26]. Our study was one of the few works which was conducted on children with CAP for evaluating the prognostic value of MCP-1. However, it had its limitations; mainly the small sample size and the cross-sectional design with lack of the serial measurements of MCP-1.

Recomendation

It is recommended that further research works on larger scale and with longitudinal design could be carried out in order to build enough body of literature to conduct meta-analysis and come up with evidence. The present study might be considered as a step towards that goal.

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