Significance of Acute Phase Reactants as Prognostic Biomarkers for Pneumonia in Children

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Community acquired pneumonia (CAP) is a major contributing cause for the increased rate of childhood mortalities and morbidities in the developing countries. Thus, we aimed in this study to appraise the significance of acute phase reactant proteins in correlation with the modified pneumonia prognostic score to assess the disease severity and outcome in children. This study included 40 children with pneumonia (age between 6 months 2 years) and other 40 healthy controls (age and sex matched). They were enrolled in the study and a detailed history's obtained, full clinical examination and assessment of AGP, CRP and ferritin in serum in addition to CBC. AGP, CRP and ferritin showed significant higher levels in children with pneumonia than healthy controls. Also, their values were significantly higher in nonsuvivors than survivors. The present investigation provides a distinct evidence for the prominence of acute phase reactants (ferritin, CRP and AGP) in comparison with the clinical scores in predicting early high risk prognosis of pneumonia in children.

Keywords: Pneumonia, Prognosis, Clinical score, Acute phase reactant proteins, Children.

Respiratory tract infections include croup syndromes, bronchitis, bronchiolitis and pneumonia. Pneumonia is an infection that affects the air sacs in one or both lungs. It had been reported that, pneumonia is the single major cause for children mortality in the developing countries [1]. In particular, the Community Acquired Pneumonia (CAP) is the major contributor for the high annual mortalities and morbidities in childhood age [2]. Proper diagnosis and early treatment of CAP are vital for reduction of antibiotic resistance, long-term morbidities, life-threatening health problems

and consequently the economical burden [3]. In Egypt, children under 5 years represent nearly 13.4% of the total population. The incidence of pneumonia in Egypt has been established at 0.11–0.20 pneumonia episodes per year [4].

Clinical signs such as intercostal retraction, desaturation, younger age, immune compromise and low socioeconomic circumstances involving, malnutrition, young mothers low education level and the presence of household air pollutants are considered as risk factors for increased mortality among children diagnosed with CAP [5]. On the

other side, adequate antenatal care and regular immunizations have reduced the risk of mortality. A presumptive clinical diagnosis of pneumonia is usually done when new radiological infiltrates develop in patients with fever, leukocytosis, tracheal secretions as well as the separation of microorganisms from patients airways [6]. Whatever, the microbiologic diagnostic procedure chosen, extra laboratory processing and delaying of about 48-72 hours are required for accurate quantitative microbial culture results. In parallel, physicians often feel uncomfortable about the diagnosis and may give unwanted antibiotics during waiting for laboratory data.

In the past, predictors of treatment failure included very young age, high respiratory rate in correlation for age in addition to severe chest retractions and they could predict increased liability to death. So, there was an urgent need for implementing clinical diagnostic and prognostic tools commonly named as risk scores, severity of disease indices, or high mortality predictive scores that can utilize these risk factors for further segregation of high-risk category for implementing adequate management in order to inhibit adverse outcomes. No universal predictive tools have been broadly adopted for application in children with pneumonia, especially in low-resources countries [7]. Clinicians have endorsed several scoring systems for predicting pneumonia outcome and serving as guidelines for admission to intensive care units, like pneumonia severity index (PSI) [8] and predisposition, insult, response, and organ dysfunction score (PIRO) [9]. Because there is no prognostic score without limitations, there has been an urgency for incorporating these scores in combination of diagnostic and prognostic biomarkers as they are less liable for bias and could provide a reliable diagnostic and predictor tools for disease severity and outcome [10]. Therefore, various biological markers have been investigated in order to improve pneumonia management [11].

Inflammation has been shown to be associated with the alteration in the micronutrient-relevant markers such as ferritin, and retinol [12]. This inflammatory condition also leads to changes in blood concentration of acute phase proteins (APP) like c-reactive protein (CRP) and á1-acid glycoprotein (AGP) which occurs concurrently with the variation in the micronutrient levels [13].

AGP is one of lipocalin family which is also called orosomucoid. It is mainly a plasma protein with different physiological, metabolic regulating and immune modulating actions via binding and transport of basic compounds, including drugs [14].

CRP is a major APPs that increases in bacterial pneumonia although, it can be also increased slightly in severe viral acute respiratory syndromes [15]. The concentrations of CRP increase rapidly in acute insult, reaches peak level with in 48 hours and decreases again within a week with a mean half life around 19 hours [13,16].

Recently, The World Health organization (WHO) recommends the assessment of the levels of both AGP and CRP in addition to micronutrient biomarkers as iron and vitamin A in large scale periodic population surveys and various researches [17].

Consequently, the current study was intended to appraise the significant share of acute phase reactant proteins in correlation with the modified pneumonia prognostic score to assess the disease severity and outcome in the children.

SUBJECTS AND METHODS

This case control study was conducted in the Pediatric Department, Ain Shams University Hospital during period from November, 2020 to January, 2021.

Subjects

The study group included forty children having pneumonia (aged between 6 months and 2 years) who were diagnosed according to WHO criteria [18] "children with fever (e" 38.5ᵖ C), paroxysmal dry cough, difficulty of respiration with at least one of the following signs : respiratory rate e" 50 breaths / minute in an infant of 2 - 11 months age, e" 40/ minute in age of 12 - 58 months, or a lower chest retraction " in addition to radiological findings that indicate pneumonia (opacity, hyperinflation, exaggerated broncho vascular markings). Children with other systemic illness including congenital cardiac anomalies, chronic specific lung infection, protein energy malnutrition and those who had already had been treated with antimicrobials or corticosteroids were rolled out from the study. There were also forty apparently healthy children age and sex matched who were involved in the study as controls . This

study was approved by Medical Research Ethical Committee of the National Research Centre, Egypt, with registration No 20 /179. A written informed consent for all participants was collected from their parents after clarifying the aim and methodology of the study.

Methods

All children in the study were enrolled and a detailed history was obtained with further local and general examination. chest x-ray and modified PIRO score were done for children with pneumonia only. Local chest examination included inspection for (retractions, chest movement, localized bulge or localized retraction and signs of respiratory distress) and auscultation for (breaths sounds and adventurous sounds). Modified PIRO score is a simple scoring system for predicting pneumonia outcome. It involved the following parameters: predisposition (age <6 months, comorbidity), insult (hypoxia [O2 saturation <90%], hypotension [according to age] and bacteremia), response (multilobar or complicated pneumonia) and organ dysfunction (kidney failure, liver failure and acute respiratory distress syndrome). Each criterion is given one point (range: 0–10 points). The predictive value of the modiûed PIRO score of death was assessed by dividing sick children into four risk levels: low (0–2 points), moderate (3–4 points), high (5–6 points) and very high risk (7–10 points) according to Araya et al. [9]. A venous blood samples (3ml) were obtained from each child under complete aseptic conditions. An amount of (1.5 ml) was collected in EDTA containing tubes, freezed for 3 hours. The remaining half was collected in 2 tubes without EDTA and further centrifuged using cooling centrifugation for 10 min at 4 °C and then the serum samples were further stored at -20°C till the analysis time.

Blood samples were withdrawn to children with pneumonia on the first day of admission. The endpoint variable was death within 30 days of admission.

The laboratory investigations included

Complete blood count including hemoglobin, RBC, WBC, platelets counts was determined by using an automated analyzer (Cel-dyn.3500; Abbott Diagnostics, Abbott park; IL).

Serum iron level was measured using the colorimetric ferrozine method without deprotinization by using iron assay kit obtained from Japan Institute for the Control of Aging (JaICA) Nikken SEIL Co., Ltd. Japan according to protocol of manufacturer and serum iron stores (ferritin) was estimated by Immunoradiometric assay according to the method of Addison et al. [19]. Serum á-1AGP was assessed by using enzyme linked immunosorbent assay (ELISA) kit purchased from Sunlong Biotech Co., LTD, China according to manufacturer manual.

Semi quantitative CRP was analyzed by latex agglutination. (Omega Diagnositic, Ltd, Alva, UK) following the manufacturer's instructions.

Statistical analysis

The obtained data were manipulated and tabulated by using SPSS software program version 20.0. Data were further analyzed in the form of mean and standard deviation regarding the quantitative data as mean family and room number, crowding index and age in sociodemographic data, weight and laboratory results, while qualitative data as sex, residence, type of feeding within first year, mother education, birth order, family and room numbers in sociodemographic data and scores of risk and treatment among cases were presented by number and percent. Comparisons between cases and controls, also between level of risk among cases were done. Comparison between the quantitative data using t test or ANOVA and Post Hoc tests with calculation of p value to determine the statistically significant difference between groups. Chi-square was used to determine if the association between two qualitative variables is statistically significant. Correlations of clinical data and inflammatory markers among cases and correlation coefficient and p value were computed. A value of p<0.05 is considered statistically significant and a value of p<0.01is considered statistically highly significant. ROC curve and area under curve were done between cases and controls also, survivors and nonsuvivors regarding serum ferritin, CRP and AGP. Accordingly coordinates of the curve were done to calculate the cut off values with specificity and sensitivity

RESULTS

A total number of 80 children were involved in the current study, then were subdivided into two equal groups: case group(children with pneumonia) and control group (healthy children).

The characteristics of the all studied children and their mothers are depicted in Table (1)

There was no significant difference between cases and controls as regards sex and age. The cases were 62.5% males and 37.5% females and the controls were 42.5% females and 57.5% males (p=0.648). The mean age of the two studied groups were 13.85±3.4 months and 15.83±4.4 months respectively (p=0.095) so both groups were sex and age matched .A highly significant difference between both studied groups regarding the residence; as most of the cases were from rural areas (65%), while most of the control group were from the urban areas (80%), (p<0.001). A statistically significant difference was demonstrated between the case and control groups regarding the type of feeding within the first year of life; where the artificial feeding was more in the cases (52.5%) than the control patients (32.5%), (p=0.023). It was

observed that 55% of the control group received breast feeding, while 25% of cases received breast feeding .The mothers of the case group had significantly lower level of education than the control group (p=0.009). There was a significant statistical difference between cases and controls as regards family number, crowding index, birth order and weight (p<0.001).

The results in Table (2) represented the levels of AGP, CRP and ferritin which displayed significant elevation in cases versus healthy controls (p< 0.001). Also, TLC had statistically significant higher value in cases than the healthycontrols (p=0.024) . On the contrary, platelets, iron and Hb% had a statistically higher values in healthy controls than in cases (p<0.001).

The modified PIRO score mean value was 3.46±1.4 and the total deaths were 6 cases (15%).Among patients with modified PIRO score

Table 1. Comparison between the case and control studied groups regarding sociodemographic characteristics

Variable		Cases [N=40 (%)]	Controls [N=40 (%)]	p value
Sex	• Male	25 (62.5)	23 (57.5)	0.648
	• Female	15 (37.5)	17 (42.5)	
Residence	• Rural	26 (65.0)	8 (20.0)	<0.001**
	• Urban	14 (35.0)	32 (80.0)	
Feeding within first year	• Breast feeding	10 (25.0)	22 (55.0)	0.023*
	• Artificial	21 (52.5)	13 (32.5)	
	• Both	9 (22.5)	5 (12.5)	
Mother education	• Illiterate or Read &Write	9 (22.5)	0	0.003**
	• Secondary	16 (40.0)	15 (37.5)	
	• University	15 (37.50)	25 (62.5)	
Birth order	• First	4 (10.0)	23 (57.5)	<0.001**
	• Second	18 (45.0)	17 (42.5)	
	• Third or more	18(45.0)	0	
	(mean±SD)	2.43±0.8	1.43±0.5	<0.001**
Family number	• 3	0	21 (32.5)	<0.001**
	• 4	12 (30.0)	11 (27.5)	
	• 5	21 (52.5)	8 (20.0)	
	• 6 or more	7 (17.5)	0	
	(mean±SD)	4.93±0.8	3.68±0.8	<0.001**
Room number	• 2	35 (87.5)	20 (50.0)	<0.001**
	• 3	5 (12.5)	20 (50.0)	
	(mean±SD)	2.13±0.3	2.5±0.5	<0.001**
Crowding index	(mean±SD)	2.08±0.3	1.47±0.3	<0.001**
Age (months)	(mean±SD)	13.85±3.4	15.83±4.4	0.095
Weight (Kg)	(mean±SD)	10.63±1.6	12.08±1.8	<0.001**

**p < 0.01 highly significant. *p< 0.05 significant

of 0 (n=0), 1 (n=0), 2 (n=11), 3 (n=12), no deaths were observed. Meanwhile, in children with score 4 (n=10) one death occured, 5 (n=2) one death occured, 6 (n=1) one death occured, 7(n=3) three deaths occurred with percentage of mortality 2.5%, 2.5%, 2.5% and 7.5% respectively).There were no cases having scores from 8 to ten within our study. The predictive value of the modiûed PIRO score of mortality was assessed by stratifying patients into four levels of risk: low (0–2 points), moderate (3–4 points), high (5–6 points) and very high risk (7–10 points). In our study, 11 (27.5%) and 22

(55%) of our studied patients were divided in low and moderate risk groups respectively while the high and very high risk categories were comprised by 3 patients each (7.5%). The mortality was one child (2.5%), two children (5%), three children (7.5%) within the moderate, high and very high risk categories respectively (Table 3).

Table (4) illustrated the results of ferritin, AGP ,CRP that revealed significant difference between the four risk groups with the highest level in very high risk group (p<0.001). Also, iron, Hb% and platelets were significantly different

Table 2. Comparison between the case and control studied groups regarding the laboratory investigations

Variable	Cases (N=40) mean±SD	Controls (N=40) mean±SD	p value
Hemoglobin (g /dl)	11.64±0.8	12.85±1.3	<0.001**
Serum iron (ug/d)	82.48±33.3	114.57±2.5	<0.001**
Serum ferritin (ng/ ml)	159.85±102.7	64.73±35.6	<0.001**
TLC	10.59±3.0	9.44±0.8	0.024*
Platelets	191.58±53.1	264.88±72.3	<0.001**
CRP (mg/ dl)	14.78±11.2	3.15±1.3	<0.001**
AGP (ng/ ml)	14.2±9.2	7.6±3.0	<0.001**

* p < 0.05 significant   **p < 0.01 highly significant

Table 3. Descriptive data of modified PIRO score points and risk categories among cases

Modified PIRO score points (mean±SD) 3.64 ±1.4	Cases N=40 No (%)	Deaths N= 6 (15%) No.(%)
zero	-	-
1	-	-
2	11 (27.5)	-
3	12 (30.0)	-
4	10 (25.0)	1(2.5)
5	2 (5.0)	1(2.5)
6	1 (2.5)	1(2.5)
7	3 (7.5)	3 (7.5)
8	-	-
9	-	-
10	-	-
Risk categories		
• Low risk	11 (27.5)	-
• Moderate risk	22 (55.0)	1(2.5)
• High risk	3 (7.5)	2(5)
• Very high risk	3 (7.5)	3(7.5)

between the four risk groups but, with the highest level in low risk group (p =0.036, =0.002, <0.001 respectively). TLC showed insignificant difference between the four risk groups (p =0.19).

The results presented in table (5) displayed that six cases have died (15%) within 30 days of admission. The mean values of AGP, CRP, ferritin and modified PIRO score were significantly different between survivors and non survivors (higher values in non survivors) (p<0.001).While, Hb% and platelets had higher significant values in survivors than non survivors (p =0.008, 0.01 respectively).

As regard to correlations, AGP showed significant positive correlation with CRP, ferritin, modified PIRO score (p<0.001). On the opposite hand, there was significant negative correlation with iron, Hb, platelets (p = 0.011 and< 0.001, < 0.001 respectively). CRP showed significant positive correlation with AGP, ferritin and modified PIRO score (p<0.001). Meanwhile,

it showed significant negative correlation with iron, Hb and platelets (p=0.042, < 0.001, < 0.001 respectively). Ferritin showed significant positive correlation with AGP, CRP and modified PIRO score (p<0.001), whereas, there was significant negative correlation with platelets (p=0.004), as depicted in Table (6).

ROC curve analysis for using AGP in detection of pneumonia clarified that the area under the curve is 0.801 with cutoff level equal to 7.0 (P=0.000), with sensitivity 85% and specificity 65% .While, ROC curve for CRP in pneumonia detection proved that the area under the curve is 0.981 with cutoff level equal to 6.10 (P= 0.000) with sensitivity 92.5% and specificity 95%. ROC curve analysis of ferritin for pneumonia diagnosis

proved that the area under the curve is 0.891 with cutoff level equal to 58.10 (P=0.000) with sensitivity 100% and specificity 72.5% (Fig. 1)

ROC curve analysis of AGP for mortality prediction in children with pneumonia proved that the area under the curve is 0.980 with cutoff level equal to 20.50 (P=0.000) with sensitivity 100% and specificity 94.1%. ROC curve analysis of CRP for predicting mortality in pneumonia proved that the area under the curve is 0.973 with cutoff level equal 16.4 (P= 0.000) with sensitivity 100 % and specificity 88.2%. ROC curve analysis of ferritin for pneumonia mortalitiy prediction proved that the area under the curve is 0.941 with cutoff level equal to 264.0 (P=0.001) with sensitivity 83.3% and specificity 88.2 % (Fig. 2)

Table 4. Comparison between 4 risk categories regarding laboratory investigations

Variable	Low risk 11 (% 27.5) mean±SD	Moderate risk 22 (55.0%) mean±SD	High risk 3 (%7.5) mean±SD	Very high risk 3 (7.5%) mean±SD	p value
Hemoglobin (g/ dl)	12.14±0.9	11.68±0.7	11.27±0.4	10.23±0.6	0.002**
Serum iron (ug/ dl)	78.0±26.7	74.88±32.4	108.1±12.9	126.43±47.7	0.036*
Serum ferritin ng/ ml	100.27±67.3	150.36±89.0	234.0±59.8	372.0±3.5	<0.001**
TLC	9.87±3.3	11.05±2.5	9.4±3.2	13.47±0.5	0.19
Platelets	247.45±50.5	179.73±34.7	140.67±11.0	128.33±30.1	<0.001**
CRP (mg/ dl)	6.73±1.7	13.66±5.6	18.77±4.6	48.03±5.9	<0.001**
AGP (ng/ ml)	7.18±1.2	13.18±5.7	21.33±3.1	39.0±0	<0.001**

**p <0.01 highly significant, *p< 0.05 significant

Table 5. Comparison between survivors and non survivors regarding laboratory investigations and modified PIRO score

Variables	Non survivor patients N=6 (%15) mean±SD	Survivor patients N=34(%85) mean±SD	p value
Hemoglobin (g/ dl)	10.82±0.8	11.78±0.8	0.008**
Serum iron (ug/ dl)	103.18±54.3	78.83±27.9	0.328
Serum ferritin (ng/ ml)	303.33±83.9	134.53±83.7	<0.001**
TLC	10.78±4.0	10.56±2.9	0.869
Platelets	141.17±30.7	200.47±51.5	0.01*
CRP (mg/dl)	34.53±15.4	11.29±5.4	<0.001**
AGP (ng / ml)	31.17±8.6	11.21±5.2	<0.001**
PIRO score	6.0±1.3	3.0±0.9	<0.001**

** p < 0.01 highly significant *p < 0.05 significant

DISCUSSION

This study was undertaken to clarify the significant role of acute phase reactant proteins in correlation with the modified pneumonia prognostic score to assess the disease severity and outcome in children. To fulfill this purpose, a total of eighty infants were enrolled in this study, 37 males and 43 females with male to female ratio 1:1.16. They further were categorised into two groups: control group (n= 40) and case group (n=40) according to diagnostic criteria of pneumonia. Non survivor patients were representing 6/40 (15%) showing the severity and the morbidity of CAP among Egyptian children. In our study, the cases were 62.5% males and 37.5% females and the controls were 57.5% males and 42.5% females reflecting more affection of males by pneumonia but with no significant difference between the two groups (p= 0.648). A previous meta analysis study recorded that male children are more prone to acquire pneumonia than female children. This could be due to by a stronger immune defense mechanisms in girls and narrower peripheral airways in boys during the first years of life [2]p . No significant difference between age of cases and controls, where; mean age of the cases

and controls were 13.85±3.4 months and 15.83±4.4 months respectively, so both groups were age matched (p=0.09). The mothers of case group in our research had a significant lower level of education than those of control group (p=0.009). Similar findings were confirmed by Abdel Maksoud et al. v , while another study by Jackson et al. [2]p showed that the association between low mother education level and severe respiratory infection is not proved. They considered that risk factors rather than mothers' education such as having past history of illness can affect their behavior and reduce the risk of acquiring pneumonia.

A highly significant difference between the two studied groups regarding the residence; as most of the cases were from rural areas (65%), while most of the control group were from urban areas (80%), (p<0.001). The predominance of pneumonia in rural areas could be explained by poor sanitation, increased family number and overcrowding that increase the risk of exposure to infection [21]. This finding doesn't match with the study by Fadl et al. [22] who found no significant difference between the under five children with pneumonia and healthy controls as regarding residence. There was a significant statistical difference between the cases

Table 6. Correlations of clinical data and laboratory investigations in cases group (n=40)

Variable		Hb % g/dl	Serum iron	Serum ferritin	TLC	platelets	CRP	AGP	PIRO	Crowding index
Serum iron ug / dl	r	.187	1							
	p	.249								
Serum ferritin ng/ ml	r	-.260	.092	1						
	p	.106	.570							
TLC	r	-.123	.041	.080	1					
	p	.448	.803	.622						
Platelets	r	.466**	.249	-.441**	-.194	1				
	p	.002	.121	.004	.229					
CRP mg/d	r	-.587**	-.323*	.655**	.177	-.558**	1			
	p	.000	.042	.000	.274	.000				
AGP ng/ ml	r	-.600**	-.396*	.633**	.137	-.594**	.954**	1		
	p	.000	.011	.000	.398	.000	.000			
PIRO score	r	-.586**	-.378*	.639**	.246	-.631**	.839**	.859**	1	
	p	.000	.018	.000	.131	.000	.000	.000		
Crowding index	r	.118	-.082	.022	.120	.083	-.111	-.127	-.117	1
	P	.469	.616	.895	.462	.609	.495	.433	.478	
Age	r	.115	.227	.128	-.403**	.170	.040	.051	.144	-.160
	p	.479	.158	.432	.010	.294	.807	.753	.383	.325

*. Correlation is significant at the 0.05 level (2-tailed), **. Correlation is significant at the 0.01 level (2-tailed).

and controls regarding the type of feeding within the first year of life; where the artificial feeding was more in the cases (52.5%) than in the control group (32.5%), (p=0.023). As 55% of the control group received breast feeding, while 25% of cases received breast feeding . Lack of breast feeding is a golden risk factor for acquiring pneumonia and increasing mortality among children younger than five years in the developing countries [23]. The breast milk contains several immune protective and nutritive substances that protect babies as their own immune systems have not developed yet [24]. There was a significant statistical difference between the cases group and control group regarding family number, crowding index and birth order (p<0.001) . These results match with those of Fadl et al. [22],

but they are in contradictory to the study done by Jackson et al. [20]. This reflects the strong and combined association between socioeconomic risk factors and health. Overcrowding and bad home ventilation are strongly linked with higher incidence of pneumonia among children [25] . Having higher birth order (third child or more) increases the likelihood of having pneumonia. Large number of children is associated with maternal inattention to her kids and facilitates the spreading of infection [26]. The present study showed that weight is significantly more in the healthy controls than in the cases (p<0.001). Having smaller weights may predispose to increasing risk of pneumonia due to lower immunity and impaired lung functions [27].
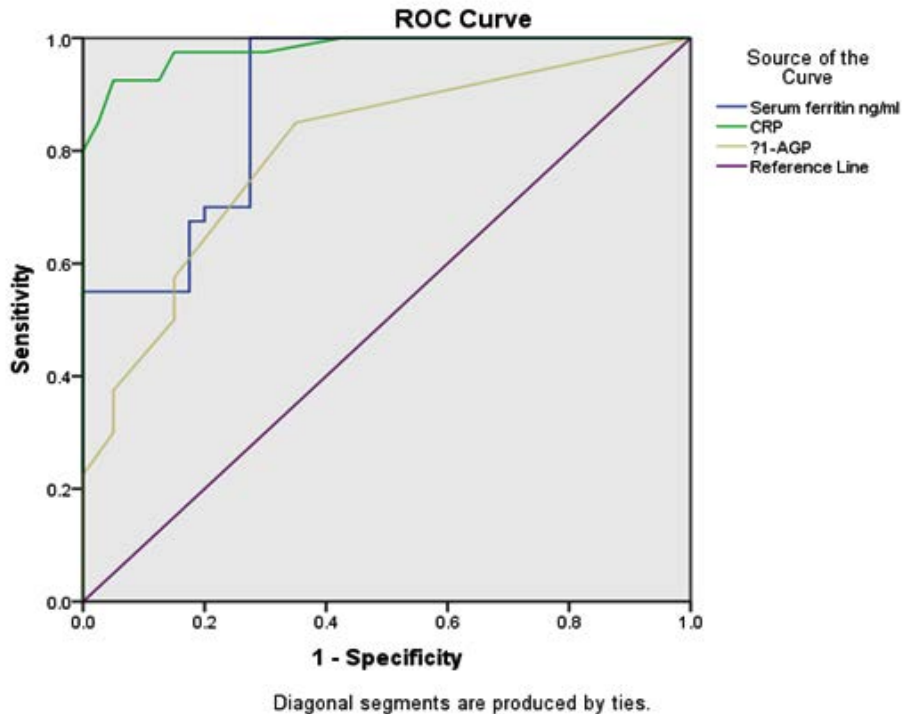
Fig. 1. ROC curve analysis of acute phase reactants for pneumonia detection in children

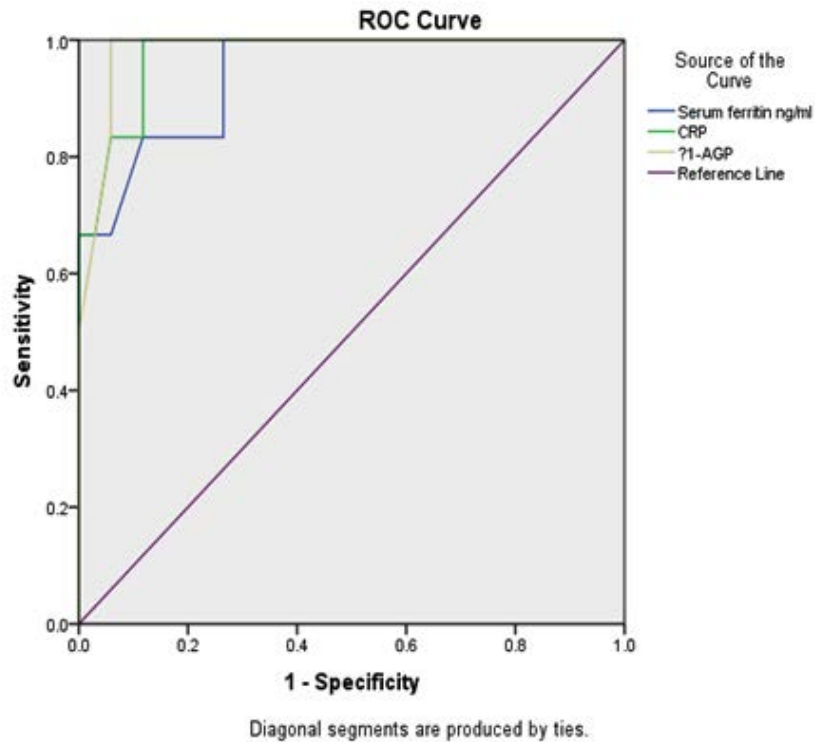| Test Result Variable(s)	AUC	SE	p	Asymptotic 95% CI		Positive if Greater Than or Equal To[a]	Sensitivity %	Specificity %
				Lower Bound	Upper Bound			
Serum ferritin	.891	.035	.000	.822	.960	58.10	100.0	72.5
CRP	.981	.012	.000	.957	1.000	6.10	92.5	95.0
AGP	.801	.050	.000	.703	.898	7.0	85.0	65.0

TLC was significantly higher in the case group when compared with the control group (p=0.024). These results come in concordance with those of Abdel Maksoud et al. [6] whereas, in the study of Hussain et al. [28] there was no significant difference between the cases and controls regarding TLC.

In the current study, hemoglobin level showed a significant decrease in the cases when compared to the controls reflecting that anemia may be a predisposing factor to infection susceptibility in this young age. These findings paralleled with those of Mourad et al. [29]. A study done by Malla et al. [30] found that reduced hemoglobin is the main cause of acute lower respiratory tract infection.

Serum iron also revealed significant depletion in children with pneumonia than controls. Similar findings were recorded by Hussain et al. [28]. Iron seems to share in immunity components causing lung injury [28].

The mean ferritin level in our research displayed significant increase in the cases in comparison to the controls. The same findings were registered by Choi et al. [31]. However, Abdel Maksoud et al. [6] observed that ferritin level is significantly lower in children with pneumonia than in controls. This could be attributed to that 70% of their cases had iron deficiency anemia.

Serum ferritin level was negatively correlated with the mean platelets count (p=0.004),

Diagonal segments are produced by ties.

Fig. 2. ROC curve analysis of acute phase reactants for mortality prediction in children with pneumonia

Test Result Variable(s)	AUC	SE	p	Asymptotic 95% CI Lower Bound	Upper Bound	Positive if Greater Than or Equal To[a]	Sensitivity %	Specificity %
Serum ferritin	.941	.046	.001	.852	1.000	264.0	83.3	88.2
CRP	.973	.024	.000	.926	1.000	16.4	100.0	88.2
AGP	.980	.019	.000	.944	1.000	20.50	100.0	94.1

while there was positive correlation with CRP, AGP and modified PIRO score (P<0.001). It didn't correlate with sex and age. This was confirmed by Thurnham et al. [12] who reported that age and sex don't affect the ferritin level associated with the inflammatory status. This indicated that the increase in ferritin in association with inflammation is proportional to the baseline ferritin concentration within the group of participants. Also, Thurnham et al. [32] supported the correlation among ferritin, CRP and AGP and confirmed that at least two acute phase proteins must be measured during inflammation to cover the whole phase of the disease. These investigators found that in patients with covert infection as indicated by elevated CRP and/or AGP, an increase in ferritin concentration is recorded as ferritin increased rapidly at the onset of inflammation [33]. Also as recommended by the WHO working group; the measurement of ferritin must be accompanied by the analysis of one or more acute phase reactants to detect the presence of infection or inflammation, because high ferritin thresholds in the presence of inflammation may mask underlying iron deficiency anemia [34].

In the present study, CRP achieved a significant statistical difference between the cases and the control groups being higher among the patients. Joeng et al. [35] stated that children affected by pneumonia have higher CRP level than the healthy controls. A study conducted by Xiao et al. [36] concluded that serum CRP is higher in children with pneumonia than healthy subjects denoting that the elevated CRP levels may be an essential risk factor for pneumonia occurence in children. On the opposite side, other studies didn't support the significant difference regarding the CRP measurement between the two studied groups (P > 0.05) although it was higher in the cases than controls [37]. It has been found that CRP level is constantly high in bacterial pneumonia, but only slightly increases in severe acute respiratory syndrome [38]. Koster et al. t [1] demonstrated that serum CRP levels are positively correlated with pneumonia and also the study of Mintegi et al. [40] that was done on 188 children lower than three years presented to hospital emergency rooms proved that CRP serum levels may be useful in prediction of the risk of pneumonia in children with high fever.

CRP was positively correlated with iron,

ferritin, AGP and modified PIRO score (p<0.001) while it was negatively correlated with Hb and platelets (p<0.001) .In concordance with our results, CRP was positively correlated with AGP [37] and ferritin [32].

AGP, is a 41–43 kDa protein of immunoglobin family and is considered an important acute-phase proteins [41]. It has the ability to bind and transfer several inflammatory ligands. AGP is considered to be a sensitive diagnostic of inflammaton or acute infection [42]. In the current research, the mean value of AGP in children with pneumonia was statistically higher than the healthy controls (p<0.001) . This finding comes in line with a recent study by Huanying et al. [37]. Our study also showed that as a risk factor of pneumonia, AGP has a diagnostic value whereas a combination of AGP with other phase reactants could improve the efficiency of differential diagnosis.

As regarding AGP in the present study, it correlated positively with ferritin and CRP (p<0.001). On the contrary, it correlated negatively with Hb, iron and platelets (p<0.001, p=0.011, p<0.001 respectively) and it did not correlate with TLC. Huanying et al. [37] recorded significant positive correlation between AGP and CRP levels but, no significant correlation between AGP concentrations, gender distribution and WBC.

In the current approach, AGP is positively correlated with the modified PIRO severity score. To the best of our knowledge, our study is the first one to correlate clinical severity score with AGP. Modified PIRO score, a simple scoring system for predicting pneumonia outcome, was also used to evaluate our patients. In our study, 11 and 22 of our cases were stratified into low and moderate risk groups respectively while the high and very high risk categories were comprised by 3 patients each. The mortality was 3 children (7.5%) from the very high modified PIRO, 2 children (5%) from the high score and one child (2.5%) from the moderate risk score. A study of Araya et al. [9] documented mortality rate of 0% for a low PIRO score (0/708 patients), 18% (20/112 patients) for a moderate score, 83% (25/30 patients) for a high score and 100% (10/10 patients) for a very high modified PIRO score (P < 0.001). This research group utilized this score to discriminate the risk of mortality in children admitted with CAP and thus it could be a reliable tool for patients selection for

ICU admission [43]. Whereas this tool is modiûed from that designed for CAP in adults, it looks to be a good prognostic tool [10].

On comparing the four risk groups of the modified PIRO score regarding CBC parameters, we noted high statistically significant lower mean value of the haemoglobin and platelets in the group of patients of the very high risk of mortality as compared to the other three groups of patients. TLC mean value was lower in the group of the high risk in comparison with the other three risk groups, but there was no significant statistical difference. Our three inflammatory markers; CRP, AGP and ferritin values showed the highest significant levels in the very high risk group of patients .So, modified PIRO score correlates well with the inflammatory markers.

In this study, we proved that modified PIRO score is a good predictor of mortality, as only one patient died from moderate risk group, two patients died from high risk group and another three patients died from very high risk. These findings are in the harmony with the 2011 Pediatric Infectious Diseases Society/ Infectious Diseases Society of America (PIDS/IDSA) guidelines used in the treatment of community acquired pneumonia in children that recommended consideration of acute phase reactants such as CRP to validate severity and mortality scores. The inclusion of these acute phase reactants could lead to modest improvement in predicting outcome enabling physicians to hospitalize risky children early in order to reduce mortality [2].

In this study, we confirmed that the initial values of acute phase reactants (AGP, CRP and ferritin) are significantly higher in non-survivors (children with 30-day mortality) than survivors which are similar to the conclusions of Kellum et al. [44] and Menendez et al. [45]. The diagnostic value of 30-day mortality prediction was assessed and evaluated by ROC curve analysis for acute phase reactants. ROC curve analysis of AGP for mortality prediction in children with pneumonia identified that the area under the curve is 0.980 (more close to one better diagnostic value), (p=0.000) with cutoff level equal to 20.50, with sensitivity 100% and specificity 94.1%. ROC curve analysis of using CRP in mortality prediction showed that the area under the curve is 0.973, (p= 0.000) with cutoff level equal to 16.4, with sensitivity 100 % and specificity 88.2% . ROC curve analysis of ferritin for pneumonia mortalitiy prediction showed that the area under the curve is 0.941, (p= 0.001) with cutoff level equal to 264.0, with sensitivity 83.3% and specificity 88.2 %. AGP showed the best area under the curve, sensitivity and specificity .These results are in concordance with those of Huanying et al. [37] which presented that ROC curve analysis for using AGP in pneumonia mortality prediction was better than that of CRP.

In conclusion, Acute phase reactants (AGP, CRP and ferritin) displayed statistically significant higher levels in the cases of pneumonia than in the healthy controls, non-survivors than survivors and positive correlation with the modified PIRO score, so the present investigation provides a distinct evidence for the prominence of acute phase reactants in comparison with the clinical scores in predicting early high risk prognosis of pneumonia in children.

Further researches having larger sample size with serial measuring AGP, CRP and ferritin levels are needed to establish their reliability in early prediction of pneumonia prognosis and outcome in children.

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