



ORIGINAL ARTICLE

Evaluation of Neopterin as a Prognostic Marker for Severity of Pneumonia in Pediatrics

Dina Mohamed Shokry¹, Yousif Mohamed Yousif¹, Amal Fathy Ghareeb^{2,3}, Mahmoud Mohamed Rabie¹

¹*Pediatrics Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

²*Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*

³*Clinical Laboratory Sciences Department, Collage of Applied Medical Sciences, Taif University, Taif*

Corresponding author:

Mahmoud Mohamed Rabie
 Pediatrics Department, Faculty
 of Medicine, Zagazig
 University, Zagazig, Egypt

rby61531@gmail.com

Submit Date 2019-04-10

Revise Date 2019-07-17

Accept Date 2019-07-18

ABSTRACT

Background: The aim of this study was to investigate the role of Neopterin in evaluation of severity and outcome of pneumonia in pediatric Department of Zagazig University Hospitals. **Methods:** A prospective case control study was carried out at the pediatric Department of Zagazig University Children Hospitals during the period from November 2017 to December 2018. Sixty infants and children patients participated in the study. 40 were diagnosed as pneumonia admitted to Pediatric Department of Zagazig University as case group. And twenty infants and children of control. Serum level of Neopterin was measured for all patients recruited in this study, by a commercial ELISA assay. **Results:** In our study there was statistically significant difference between case and control groups in Neopterin level, there is statistically significant difference in Neopterin level between cases that need ICU admission and M.V. and cases treated in ward only without need, there was statistically significant difference in Neopterin level between survivors and non-survivors. In this study, there was statistically significant difference in Neopterin level with disease severity in which Neopterin is higher in severe than moderate and mild cases. Our study shows that there was statistically significant positive correlation between Neopterin level and total PRESS score, granulocytes and CRP. In our study we tried to detect the cutoff point of Neopterin to detect severe pneumonia calculated from ROC curves it was > 13.93 (nmol/L), and the area under the curve of Neopterin was 0.866 with sensitivity 92.3% and specificity 77.8%. **Conclusion:** Neopterin is a useful prognostic and diagnostic marker for pneumonia in Pediatrics. The present study suggests that Neopterin is a promising biomarker for reflecting the severity of Pneumonia.

Key words: CAP (community-acquired pneumonia); PRESS (pediatric respiratory severity score); M.V (mechanical ventilation).

INTRODUCTION

Pneumonia in children under 5 years of age is the major cause of mortality and most of the cases involve community-acquired pneumonia (CAP) [1].

Worldwide estimates report about one million deaths every year in this age group, excluding neonates [2].

Pneumonia remains a common reason for the hospitalization of infants and elderly adults. The disease can be caused by a variety of micro-organisms, but the pathogen most often responsible for pneumonia is the bacterium *Streptococcus pneumoniae* [3].

Community acquired pneumonia (CAP) is associated with a high risk of developing

respiratory failure or severe sepsis with organ dysfunction or shock, resulting in high mortality rates in hospitalised patients [4].

Pneumonia is diagnosed by assessing symptoms, making physical examination, and x-ray, its causes include bacteria, viruses, fungi, and parasites, over 100 organisms can cause community acquired pneumonia with most cases caused by streptococcus pneumonia [5].

Neopterin is a catabolic product of guanosin triphosphate, a purine nucleotide and belongs to the group pteridine. When cytokine interferon gamma stimulates the human macrophages they synthesize Neopterin. It's an indicator of the proinflammatory immune status and hence serves as a cellular immune system marker. In renal diseases, leukemia and pulmonary tuberculosis we find that the Neopterin concentrations are usually high [6]. Neopterin is a novel biomarker which is useful in predicting severity and complications in severe pneumonia in adults and few studies have explored the predictive value of this marker in children [7]. The aim of this study was to investigate the role of Neopterin in evaluation of severity and outcome of pneumonia.

METHODS

Subjects:

A prospective case control study was carried out at the pediatric Department at Zagazig University Children Hospitals during the period from November 2017 to December 2018. Sixty infants and children patients participated in the study 40 were with pneumonia admitted to Pediatric Department of Zagazig University as case group and twenty infants and children of control comparable with case group.

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association

(Declaration of Helsinki) for studies involving humans.

Exclusion criteria were as follows: Terminal stages of disease (malignancy, end-stage Liver, or renal diseases), hospitalized cases within 14 days before onset of Symptoms, cystic fibrosis, active pulmonary tuberculosis, severe immune compromised, coagulopathy, systemic anticoagulant treatment, pretreatment outside hospital and any congenital disease.

Clinical evaluation and laboratory data all patients will be subjected to the following:

- (A) Full history taking including:
 - Personal history
 - Present history:
 - (a) Cough (b) Fever. (c) Difficult breathing.
 - (d) Flu like symptoms.
- III-Past history of previous pneumonia.
 - IV- Vaccination history
 - V- Dietetic history in infants
- VI- Family history of similar illness.
 - Full clinical examination:
 - I-General examination: level of consciousness, complexion (pallor, jaundice, cyanosis).
 - II-Vital signs: Temperature-Respiratory rate - Heart rate and blood pressure.
 - III-Anthropometric measurements (weight, height, head circumference and mid arm circumference).
 - IV-Systemic examination with especial emphasis on cardiac (gallop rhythm, tachycardia, murmurs) and abdominal examination (distention, tenderness, organomegaly).
 - V- Local chest examination by:
 - Inspection for (retractions, chest movements, localized bulge or localized retraction and signs of respiratory distress) was done.
 - b) Palpation for (tracheal shift, palpable bronchi).
 - c) Percussion d) Auscultation for (breath sounds and adventitious sounds).
 - e) Classification of pneumonia severity according to PRESS [8]. The PRESS, with its simple components of respiratory rate, wheezing, retraction, SpO₂, and feeding difficulties, may be useful and applicable to triage and assessment of respiratory status by medical

staff at the initial bedside examinations. It would likely be useful in prehospital settings).

Radiology:-Chest X ray showing (opacity, hyperinflation, exaggerated Broncho vascular markings, pleural effusion, etc.). Abdominal ultrasound to exclude other liver and kidney disease according to examination. CT (if needed). Demographic characteristics, diagnosis, complication and fate of the disease were shown in tables 1-4 and s1,s2).

Laboratory:

Complete blood count (CBC): Hemoglobin concentration. Total leucocytic count neutrophils lymphocytes platelets count capsular reactive protein (CRP) and measure serum Neopterin level by ELISA (Sandwich technique) The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Neopterin in samples. Add Neopterin to monoclonal antibody Enzyme well which is pre-coated with Human Neopterin monoclonal antibody, incubation; then, add Neopterin antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex.

Then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance Neopterin of sample were positively correlated.

Statistical analysis:

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20. Quantitative variables were described using their means and standard deviations. Categorical variables were described using their absolute frequencies. Kolmogorov-Smirnov (distribution-type) and Levene (homogeneity of variances) tests were used to verify assumptions for use in parametric tests. To compare means, independent sample t test was used when appropriate. Nonparametric test (Mann Whitney) was used to compare means when data was not normally distributed and to compare medians in

categorical data. To compare means of more than two groups, one way ANOVA was used for normally distributed data and Kruskal Wallis test was used for not normally distributed data. Categorical data were compared using Chi-square test or Fischer's exact test when appropriate. To assess the best cutoff for study variables, ROC curve analysis was used. The level statistical significance was set at 5% ($P < 0.05$). Highly significant difference was present if $p \leq 0.001$

RESULTS

In our study there was statistically significant difference between case and control groups in Neopterin levels Table 5, Fig 1,2. Also there was statistically significant difference in Neopterin level between cases that need ICU admission and M.V. and cases treated in ward only without need for ICU admission and M.V (Table S3) there was statistically significant difference in Neopterin level between survivors and non-survivors Table (S4). And there was statistically significant difference in Neopterin level with disease severity in which Neopterin is higher in severe than moderate and mild cases Table (S5). And there was statistically significant positive correlation between Neopterin level and total PRESS score Table (S6) so we concluded that Neopterin is a useful diagnostic and prognostic marker for pneumonia in Pediatrics the present study suggests that Neopterin is a promising biomarker for reflecting the severity of Pneumonia. Neopterin in combination with PRESS score may improve the predictive accuracy for severity and outcome of community acquired pneumonia in pediatrics.

Table 1.Demographic criteria of the studied groups:

	Mild	Moderate	Severe	Test	p
	N (%)	N(%)	N (%)		
Gender					
Male	9 (64.3)	9 (60)	7 (63.6)	0.068 [∞]	0.986
Female	5 (35.7)	6 (40)	4 (36.4)		
Age (months)					
Mean ± SD	22.79 ± 23.51	13.87 ± 16.91	26.36 ± 36.2	0.877 [¥]	0.654
Median	10.5	8	13		
Range	4 - 72	2 - 66	2 - 120		

Table 2. Comparison between study groups regarding physical signs:

	Mild	Moderate	Severe	X2	p
	N (%)	N(%)	N(%)		
Wheezes:					
Absent	12 (85.7)	8 (53.3)	1 (9.1)	14.509	0.001**
Present	2 (14.3)	7 (46.7)	10 (90.9)		
Tachypnia:					
Absent	6 (42.9)	1 (6.7)	0 (0)	9.788	0.007*
Present	8 (57.1)	14 (93.3)	11 (100)		
Retraction:					
Absent	13 (92.9)	9 (60)	0 (0)	21.703	<0.001**
Present	1 (7.1)	6 (40)	11 (100)		
O2 saturation:					
Adequate	14 (100)	14 (93.3)	1 (9.1)	30.759	<0.001**
Inadequate	0 (0)	1 (6.7)	10 (90.9)		
Feeding difficulties:					
Absent	13 (92.9)	7 (46.7)	2 (18.2)	14.552	0.001**
Present	1 (7.1)	8 (53.3)	9 (81.8)		

Table 3.Diagnosis in the studied groups

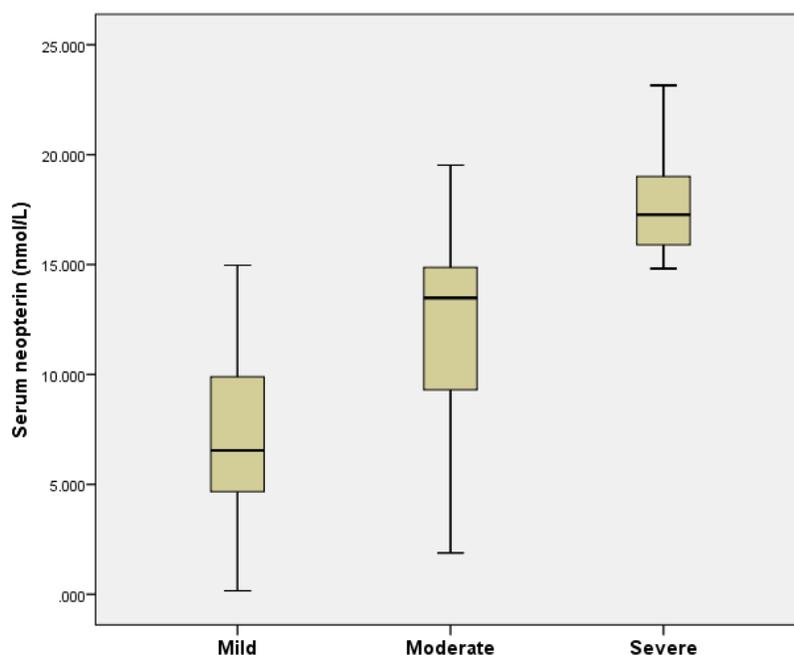
	Mild	Moderate	Severe	χ ²	p
	N (%)	N (%)	N (%)		
Diagnosis					
Lobar pneumonia	7 (50)	2 (13.3)	2 (18.2)	5.544	0.063
Bronchopneumonia	7 (50)	13 (86.7)	9 (81.8)		

Table 4.Fate and complications of the studied groups:

	Mild	Moderate	Severe	χ^2	p
	N (%)	N (%)	N (%)		
Fate:					
Cured	13 (92.9)	14 (93.3)	5 (45.5)	13.826	0.008*
Died	0 (0)	0 (0)	4 (36.4)		
Complicated	1 (7.1)	1 (6.7)	2 (18.2)		

Table 5.Neopterin level in the case control groups

	Case	Control	Test	P
Neopterin				
Mean \pm SD	11.89 \pm 5.88	3.08 \pm 2.74	-5.034 [§]	<0.001**
Range	0.16 – 23.15	0.13 – 7.51		

**Figure 1.** Boxplot showing serum Neopterin level of studied cases

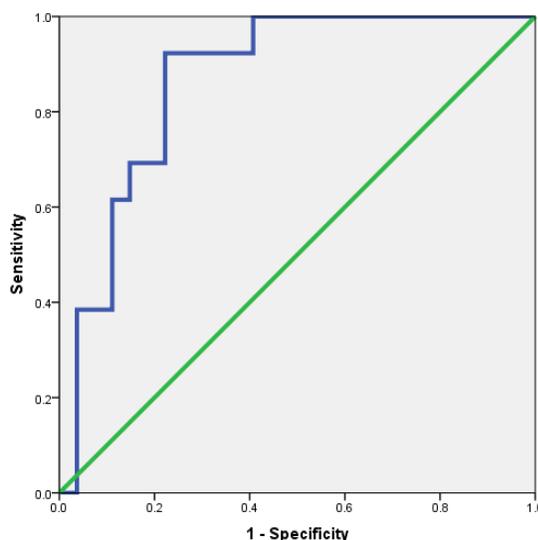


Figure 2. ROC curve showing the performance of serum Neopterin in diagnosis of poor prognosis in pneumonic patients

DISCUSSION

Pneumonia remains an important cause of morbidity and mortality in both industrialized and developing countries. Pneumonia was the single most important disease of all the children who died before their fifth birthday in 2013 [9]. Neopterin has been extensively used as a clinical marker of immune activation during inflammation in a wide range of conditions and stresses. Many studies dealt the role of Neopterin as marker in HIV , hepatitis , arthritis , and diabetes [10] .

Neopterin has also been investigated as a marker to lower respiratory tract infections [11].

The aim of the present study was to investigate and compare the serum levels of Neopterin in the three groups (mild , moderate and severe) pneumonia and to assess usefulness of Neopterin measurement in children with pneumonia and it's correlation with severity and course of disease.

In our study we found level of serum Neopterin in control (3.08 ± 2.74 nmol / L) ranged from 0.13 – 7.51nmol / L and in cases (11.89 ± 5.88) ranged from 0.16 – 23.15 (table 2) . Our result agreed with Saleh et al [12] who found the same result,so it can be used as adiagnostic marker for pneumonia.

According to the present study, fever was highly prominent through out the three groups as the severe the disease the higher body temperature (fever is almost 40°C in children with severe form of pneumonia). Also during clinical examination of children, tachypnea was clearly evident among the three groups (the severer the disease the more the respiratory rate (Table 3). In fact both tachypnea and fever are good indicators the degree of severity of pneumonia in pediatric age group as both were slightly elevated in the mild form , moderately elevated in moderate form and highly elevated in the sever form . The same findings were reported by Schot et al [13].

While hemoglobin content in children wasn't significantly difference, TLC exhibited statically significantly increase in the severe form of pneumonia however TLC wasn't significantly difference in both mild and moderate form this finding indicated that neutrophilia should be considered an indicator for severe types of pneumonia Table (6) . The previous results agreed with Naga et al. [14] , but Gardner et al. [15] found that a very low WBC count was associated with death,while Shaaban and Ahmed [16] concluded that TLC has no role in prognosis, although thrombocytopenia and thrombocytosis were

associated with severe cases and high mortality rate .

In our study, there was a significant difference among the three groups regarding CRP Table (6) . This comes in agreement with Çolak et al. [17], González et al. [18] and Xiao et al. [19] who found the same result.

The present study detected elevated levels of serum Neopterin in mild pneumonic patients (7.09 ± 3.64 nmol / L) ranged from 0.16 – 14.97 nmol / L and Neopterin level (11.89 ± 5.12 nmol / L) ranged from 1.879 – 19.52 nmol / L in moderate pneumonic patients and Neopterin level (17.98 ± 2.78 nmol / L) ranged from 14.82 – 23.15 nmol / L in severe pneumonic patients. There is statistically significant difference in Neopterin level among the three groups. The subsequent measurement of the concentrations of the Neopterin can provide further insight into the progression of the disease course.

There was statistically significant difference in Neopterin level between cases that need ICU admission and M.V. and cases treated in ward only without need for ICU admission and M.V. This agreed with Gómez et al [20] who found the same result .

Regarding Neopterin level in survivors and non survivors, There is statistically significant difference in Neopterin level between survivors and non-survivors . This agreed with Girgin et al [21] who found lower Neopterin level in survivors than non survivors.

In our study, There was a statistically significant positive correlation between Neopterin level and total leucocytic count (TLC), granulocyte, lymphocyte , CRP and total PRESS score of the studied groups. Otherwise, there was no statistically significant correlation between Neopterin level and Hemoglobin (Hb) and Platelet count (Plt). Cesur et al [22] found that Neopterin has a positive correlation with total leucocytic count . Diagnostic performance (accuracy) of Neopterin was evaluated in our study. Serum Neopterin had specificity (77.8%). Serum Neopterin exhibited sensitivity (92.3%) .Receiver operating characteristic (ROC) curve

of Neopterin showed that area under curve (AUC) was 0.866 and a p-value of < 0.001 . This favors Neopterin as a prognostic rather than diagnostic utility regarding pediatric pneumonia.

We also concluded that Neopterin is a useful prognostic marker for pneumonia in pediatrics.

Our results were in agreement with results obtained by Pizzini et al [23]. They conducted a study to examine the predictive value of the Neopterin in comparison to clinical scores and inflammatory markers in Pneumonia, COPD, and acute exacerbations. They found that Neopterin levels can further provide important prognostic information with regard to duration of hospitalization and short-term clinical prognosis.

Our results agreed with results obtained by Prat et al [24], they conducted a study to examine the predictive value of the new biomarkers Neopterin and Procalcitonin in comparison to clinical scores and inflammatory markers to predict early high risk prognosis in pneumonia. They found that Procalcitonin and Neopterin levels vary depending on age, aetiology and severity of pneumonia.

Another study was done in Hong Kong university by Rainer et al [25] found that CRP to Neopterin ratio was higher 10 times in bacterial than viral pneumonia and 42 times than healthy controls.

It seems that further studies on a larger scale are warranted to delineate the actual clinical utility of serum Neopterin in prognosis of pediatric pneumonia.

STUDY LIMITATION

Relatively small sample size due high cost concerning measurement of Neopterin .It was a single-center study (Zagazig University).

Serial measurement of the biomarkers studied could not be done to detect the level after time of diagnosis.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information: None declared

Table S1 is shown in online supplement

Tables S1-S5 are shown in online supplement.

REFERENCES

- {1}- Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H (2013): Epidemiology and etiology of childhood pneumonia. *Bull World Health Organ* 86:408–416 .
- {2}- Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE, (2012) :Child Health Epidemiology Reference Group of WHO, UNICEF .Global, regional and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 379:2151–2161.
- {3}- Jokinen C, Heiskanen L, Juvonen H, Kallinen S, Kleemola M, Koskela M, Leinonen M, Rönberg PR, Saikku P, Stén M, et al.(2001): Microbial etiology of community-acquired pneumonia in the adult population of 4 municipalities in eastern Finland. *Clin Infect Dis.* 32:1141–1154.
- {4}- Chalmers JD, Singanayagam A, Akram AR, Mandal P, Short PM, Choudhury G, et al.(2010): Severity assessment tools for predicting mortality in hospitalised patients with community-acquired pneumonia. *Systematic review and meta-analysis. Thorax* 65:878-83 .
- {5}- Claessens, Y.E.; Debray, M.P.; Tubach, F.; Brun, A.L.; Rammaert, B.; Hausfater, P.; Naccache, J.M.; Ray, P.; Choquet, C.; Carette, M.F.; et al.(2015): Early chest computed tomography scan to assist diagnosis and guide treatment decision for suspected community-acquired pneumonia. *Am. J. Respir. Crit. Care Med.* 192, 974–982.
- {6}- Wachiez H Fuchs Warmer ER(1989). Neopterin as amarker for activation of cellular immunity. immunologic basis and clinical application *adv Clin Chem*;27:81.141.
- {7}- Lippi G , Meschi T ,Gevelline G.(2011):inflammatory biomarker for diagnosis ,monitoring and follow up of community acquired pneumonia:clinical evidence and prospectives *Eur/interm Med.*2011;22:460.5.
- {8}-Yumiko Miyajil, Kazuko Sugai, Asako Nozawa, Miho Kobayashi, Shoichi Niwa, Pediatric Respiratory Severity Score (PRESS) for Respiratory Tract Infections in Children July 10, 2015Department of Pediatrics, National Hospital Organization Yokohama Medical Center, JapanDepartment of Pediatrics, Yokohama City University Graduate School of Medicine, Japan.
- {9}- Rodrigues C., & Groves H. (2018). Community-acquired pneumonia in children; the challenges of microbiological diagnosis. *Journal of Clinical Microbiology*;56(3), 1318-17.
- {10}- Unüvar, S., Tanrıverdi, Z., & Aslanhan, H. (2018). Potential Prognostic Role of Immune System Activation Marker Neopterin in Patients with Type 2 Diabetes. *Journal of Medical Biochemistry*; 1
- {11}- Eisenhut, M. (2013): Neopterin in diagnosis and monitoring of infectious diseases. *Journal of Biomarkers*; 2013.
- {12}- Saleh, M. A., van de Garde, E. M., & van Hasselt, J. C. (2019): Host-response biomarkers for the diagnosis of bacterial respiratory tract infections. *Clinical Chemistry and Laboratory Medicine (CCLM)*; 57(4), 442-451
- {13}- Schot J., Dekker R., Giorgi G , et al. (2018) : Diagnostic value of signs, symptoms and diagnostic tests for diagnosing pneumonia in ambulant children in developed countries: a systematic review. *NPJ primary care respiratory medicine*; 28(1), 40.
- {14}- Naga A,Hussein S,Said N,Wahdan O.(2017):evaluation of copeptin as adignostic and prognostic marker for pneumonia in pediatrics in zagazig university hospitals.
- {15}- Gardner G , Bhamidipati R , Rueda M , et al. ((2016): The white blood cell count and prognosis in pneumococcal pneumonia. *In Open Forum Infectious Diseases*; (Vol. 3, No. suppl_1).
- {16}- Shaaban H. and Ahmed Y. (2015). Hemogram values in community acquired pneumonia. *Egyptian Journal of Chest Diseases and Tuberculosis*; 64(3), 617-623.
- {17}- Çolak A., Yılmaz C., Toprak B., & Aktoğu S. (2017): Procalcitonin and CRP as biomarkers in discrimination of community-acquired pneumonia and exacerbation of COPD. *Journal of Medical Biochemistry*; 36(2), 122-126.
- {18}- González A , Utrillo L , Bielsa S , Falguera M and Porcel M (2016): The diagnostic value of serum C-reactive protein for identifying pneumonia in hospitalized patients with acute

- respiratory symptoms. *Journal of biomarkers*; 2016.
- {19}- Xiao X., Xue L., Sheng H. L., and Xiao Z. H. (2015). Correlation between serum levels of C-reactive protein and infant pneumonia: A meta-analysis. *Experimental and Therapeutic Medicine*; 9(6), 2331-2338.
- {20}- Gil-Gómez R., Blasco J., Sánchez Y ., et al. (2017) : Neopterin levels and systemic inflammatory response syndrome in pediatric critically ill patients. *Anales de Pediatría (English Edition)*; 87(6), 343-349.
- {21}- Girgin, G., Sahin, T. T., Fuchs, et al . (2011). Tryptophan degradation and serum Neopterin concentrations in intensive care unit patients. *Toxicology Mechanisms and Methods*; 21(3), 231-235.
- {22}- Cesur S., Aslan T., Hoca T. , et al. (2014): Clinical importance of serum neopterin level in patients with pulmonary tuberculosis. *International Journal of Mycobacteriology*; 3(1), 5-8.
- {23}- Pizzini A., Lunger F., Sahanic A., Nemati N. , Fuchs, D. et al.(2017): Diagnostic and prognostic value of inflammatory parameters including Neopterin in the setting of pneumonia, COPD, and acute exacerbations. *COPD: Journal of Chronic Obstructive Pulmonary Disease*;14(3) 298-303.
- {24}- Prat, C., Domínguez, J., Andreo, F., Blanco, S. et al.(2006): Procalcitonin and neopterin correlation with aetiology and severity of pneumonia. *Journal of Infection*; 52(3), 169-177.
- {25}- Rainer TH, Chan CP, M. F. Leung et al., (2009) : “Diagnostic utility of CRP to neopterin ratio in patientswith acute respiratory tract infections,” *Journal of Infection*; vol. 58, no. 2, pp. 123–130.

To Cite This Article: Dina MS, Yousif MY, Amal FG, Mahmoud MR.Evaluation of Neopterin as a Prognostic Marker for Severity of Pneumonia in Pediatrics.ZUMJ 2020;26(1);156-164.DOI: 10.21608/zumj.2019.11318.1178