A retrospective observational study to estimate use of biomarkers to predict severity of community acquired pneumonia in children

Anam Bashir¹, Raheel Khan², Stephanie Thompson³, and Manuel Caceres³

¹Medical College of Wisconsin

²West Virginia University

³Charleston Area Medical Center

December 31, 2021

Abstract

Purpose: Multiple studies have investigated the role of biomarkers in predicting pneumonia severity in adults but minimal research exists for children. The aim of this study was to determine if the following biomarkers: white blood cell count (WBC), platelet count, C-reactive protein (CRP), procalcitonin (PCT), neutrophil-lymphocyte ratio, neutrophil count, or band count predict community associated pneumonia (CAP) severity in children. **Methods:** A retrospective chart review was conducted on pediatric patients (aged 60 days to 18 years) diagnosed with CAP, admitted to a regional, tertiary hospital. Patients were stratified into two severity cohorts, mild (no ICU care), and moderate /severe (required ICU care). Biomarker values were then compared between the severity cohorts and area under the curve (AUC), cut-off values, performance characteristics were calculated. **Results:** A total of 108 patients met inclusion criteria. Among the biomarkers examined, elevated levels of CRP (51.7 mg/L in mild vs. 104.8 mg/L in moderate/severe, $p = 0.003$, PCT (0.29 ng/ml in mild vs. 4.02 ng/ml in moderate/severe, $p = 0.001$) and band counts (8% in mild vs. 15% moderate/severe, $p = 0.009$) were associated with increased pneumonia severity. In predicting moderate/severe CAP, PCT had the highest AUC of 0.77 ($p = 0.001$) followed by bands AUC of 0.69 ($p = 0.009$) and CRP AUC of 0.67 ($p = 0.003$). The cut-off for PCT of 0.55ng/ml had a sensitivity of 83% and a specificity of 65%. A cut-off level of 53.1 mg/L for CRP had a sensitivity of 79% and specificity of 52%. A cut off level of 12.5% bands had a sensitivity of 61% and specificity of 71%. **Conclusion:** Biomarkers, in particular PCT, obtained early in hospitalization appear to perform as predictors for CAP severity in children and may be beneficial in guiding CAP management

Title Page

Title:

A retrospective observational study to estimate use of biomarkers to predict severity of community acquired pneumonia in children

Authors:

1) Anam Bashir, MD

Department of Pediatrics

West Virginia University-Charleston/ Charleston Area Medical Center, Charleston, WV

2) Raheel Khan, MD

Department of Pediatrics, Division of Infectious Diseases

West Virginia University-Charleston/ Charleston Area Medical Center, Charleston, WV

3) Stephanie Thompson, PhD

Center for Health Services and Outcome Research, Charleston Area Medical Center, Charleston, WV

4) Manuel Caceres, MD

Department of Pediatrics

Pediatric Medical Group/ Charleston Area Medical Center, Charleston, WV

Contribution:

Anam Bashir wrote the first draft of the manuscript. Raheel Khan, Stephanie Thompson and Manuel Caceres edited the draft of the manuscript. No honorarium, grant, or other form of payment was given to anyone to produce the manuscript

Correspondence:

Anam Bashir MD, Department of Pediatrics, Medical College of Wisconsin, 8920 W Connell Ct, Milwaukee, WI 53226 [zehrabash@gmail.com], Ph: (304) 549-176

Financial disclosure:

No funding was received

Conflict of interest:

No potential, perceived, or real conflict of interest.

Support:

No support from any pharmaceutical and any industry was provided.

Sponsor:

There was no sponsor for this study.

Key words:

C-reactive protein, procalcitonin, band count, pneumonia

Short Title:

Predicting severity of pneumonia in children using biomarkers

Publication:

There are no prior publications or submissions with any overlapping information, including studies and patients.

ABSTRACT

Objective

Multiple studies have investigated the role of biomarkers in predicting pneumonia severity in adults but minimal research exists for children. The aim of this study was to assess if the following biomarkers: white blood cell count (WBC), platelet count, C-reactive protein (CRP), procalcitonin (PCT), neutrophil-lymphocyte ratio, neutrophil count, or band count predict community associated pneumonia (CAP) severity in children.

Study Design

A retrospective chart review was conducted on pediatric patients (aged 60 days to 18 years) diagnosed with CAP, admitted to a regional, tertiary hospital. Patients were stratified into two severity cohorts, mild (no

ICU care), and moderate /severe (required ICU care). Biomarker values were then compared between the severity cohorts and area under the curve (AUC), cut-off values, performance characteristics were calculated.

Results:

A total of 108 patients met inclusion criteria. Among the biomarkers examined, elevated levels of CRP (51.7 mg/L in mild vs. 104.8 mg/L in moderate/severe, $p = 0.003$, PCT (0.29 ng/ml in mild vs. 4.02 ng/ml in moderate/severe, $p = 0.001$) and band counts (8% in mild vs. 15% moderate/severe, $p = 0.009$) were associated with increased pneumonia severity. In predicting moderate/severe CAP, PCT had the highest AUC of 0.77 ($p = 0.001$) followed by bands AUC of 0.69 ($p = 0.009$) and CRP AUC of 0.67 ($p = 0.003$). The cut-off for PCT of 0.55ng/ml had a sensitivity of 83% and a specificity of 65%. A cut-off level of 53.1 mg/L for CRP had a sensitivity of 79% and specificity of 52%. A cut off level of 12.5% bands had a sensitivity of 61% and specificity of 71%.

Conclusion:

Biomarkers, in particular PCT, obtained early in hospitalization appear to perform as predictors for CAP severity in children and may be beneficial in guiding CAP management.

MANUSCRIPT

ABBREVIATIONS

CAP- Community Acquired Pneumonia

WBC- White blood cell count

CRP- C reactive protein

PCT- Procalcitonin

NLR- Neutrophil to lymphocyte ratio

AUC- Area under curve

PPV- Positive Predictive Value

NPV- Negative Predictive Value

CI- Confidence Interval

BACKGROUND:

Community acquired pneumonia (CAP) is the leading cause of mortality in children worldwide [1] and remains one of the most common causes of pediatric hospitalization in the United States [2]. The percentage of children hospitalized for CAP after presenting to the Emergency Department (ED) ranges widely, from 19% - 69% [3]. CAP severity variation in hospitalized patients suggests a lack of standardized tools for predicting prognosis. Our current study aimed to determine if laboratory values present on admission associate with pneumonia severity. Early identification of poor prognosis of children with pneumonia could help guide patient management in terms of inpatient versus ICU admission, appropriate referrals, and choice of antibiotic therapy.

CAP presenting clinical symptoms have low specificity for diagnosis and the need for antibiotics [4]. In adults, scoring systems to estimate pneumonia severity and prognosis include CURB-65 (Confusion, Uremia, Respiratory rate, Blood pressure, Age > 65), PSI (pneumonia severity index), and SOAR (Systolic blood pressure, oxygenation, age, and respiratory rate). The PIRO (predisposition, insult, response, and organ dysfunction) score is the CAP prognosis sole scoring system developed in adults later examined in a single pediatric study [5]. Biomarkers can be used in deciding the need for antibiotic therapy, prognosis, and follow up treatment for pneumonia [6]. Research has been performed in adults regarding CAP prognostic models

and biomarkers. However, additional research is needed to define the role of biomarkers in pediatrics CAP prognosis and management.

The objective of the study was to examine if the regularly obtained biomarkers of white blood cell count (WBC), platelet count, C-reactive protein (CRP), procalcitonin (PCT), neutrophil-lymphocyte ratio (NLR), neutrophil count, and band count predicted CAP severity in children.

STUDY DESIGN:

The study was a case-control study utilizing retrospective chart review of patients (aged from 60 days to 18 years old) diagnosed with CAP and admitted to Charleston Area Medical Center Women and Children's Hospital (Charleston, WV). All inpatients aged from 60 days to 18 years hospitalized for CAP between October 1, 2015 and October 1, 2018 were included in the study. CAP was defined meeting the following three criteria: 1.) Fever of more than 38° C, 2.) Documented acute respiratory symptoms including cough, dyspnea, tachypnea, pleuritic chest pain and, 3.) Radiographic evidence of pneumonia (lobar consolidation or pleural effusion or new pulmonary infiltrate) [2][5]. Exclusion criteria included discharge from ED without inpatient admission, diagnosis of bronchiolitis, diagnosis of any other infection different from pneumonia at presentation including ear infections, and chronic conditions including immunosuppression, cystic fibrosis, cardiac diseases, renal diseases, sickle cell disease, tuberculosis, or diagnosis of hospital acquired pneumonia. Hospital acquired pneumonia was defined as pneumonia occurring [?] 48hrs after admission to the hospital that was not present at the time of admission. [7]. Records for inclusion were initially identified using ICD-10 discharge diagnosis codes for pneumonia with chart review confirming CAP diagnosis.

Patients were stratified into cohorts based on CAP severity similar to Williams et al. [3]. Severe CAP was defined as requiring mechanical ventilation, shock requiring vasoactive medications (e.g., dopamine, norepinephrine, vasopressin), Glasgow Coma Scale (GCS) < 11 or altered mental status, hospitalizations of [?] 10 days, pleural effusion, or death. Moderate CAP was defined as requiring intensive care unit (ICU) admission for any length of time, with severe and moderate severities combined into a single cohort for analysis. Mild CAP defined as patients requiring non-ICU hospitalization.

Biomarkers of interest included the following when obtained within 72 hours of hospital arrival: WBC count, platelet count, neutrophil count, CRP, PCT, NLR, and band counts. If labs were obtained more than once within the initial 72 hours of hospitalization, the highest value was used. Additionally, the lowest value for WBC and platelet counts during the 72-hour time period were collected. Median time of lab draws used in data analysis was 8 hours (interquartile range: 1-20 hours) post ED arrival. Patient demographics and hospital length of stay were also obtained via chart review. Missing data was handled with complete-case analysis. All aspects of the study were approved by the CAMC/ West Virginia University – Charleston Division Institutional Review Board.

Basic descriptive statistics were performed on each data element. Categorical data were summarized as numbers and percentages. The median patient age and biomarker levels are reported with interquartile ranges (IQR), i.e., 25th and 75th percentiles. Length of stays are reported as median (minimal to maximal) number of days. Demographic characteristics and laboratory values were compared between the two CAP severity cohorts. Comparisons were performed using Chi-square for the categorical variable of gender and Mann Whitney U statistical tests were employed to compare continuous variables of age and biomarker levels.

Diagnostic performances of each biomarker for prediction of moderate/severe CAP were evaluated via receiver operating characteristic (ROC) curves and areas under curve (AUC) with 95% confidence intervals (CIs). For the minimal WBC and platelet variables, smaller values were tested for predicting severe/moderate CAP while for the other biomarkers increasing values were examined. AUC values were interpreted as AUC values of 0.5-0.6 having no discrimination (unable to distinguish moderate/severe CAP from mild CAP), 0.6-0.7 poor discrimination, 0.7 to 0.8 as acceptable, 0.8 to 0.9 as excellent, and more than 0.9 as having outstanding discrimination. [8]

For “optimal” cut-point selection, we identified the biomarker value corresponding to the maximal Youden index, maximizing the difference between true positive rates and false positive rates among all the possible cut-point values. Sensitivity, specificity, positive predictive value (PPV), negative-predictive value (NPV) and accuracy ($[\text{True Positive} + \text{True Negative}] / [\text{True Positive} + \text{True Negative} + \text{False Positive} + \text{False Negative}]$) was calculated for the optimal cut-point for each biomarker. Statistical significance was defined as $p < 0.05$. Data analysis was performed using IBM SPSS 19.

RESULTS:

A total of 108 patients were included in the study and of these 53 were male (49%). The median age at admission was 4 years (range: 62 days to 17 years) with 39 (36%) children less than 2 years of age. The median hospital length of stay was 3.5 days (range: 1-55 days) and for patients requiring intensive care the median ICU length of stay was 8 days (range: 2-55 days). There were no mortalities. The patients were stratified by CAP severity with 58 patients (54%) in the mild CAP cohort versus 50 patients (46%) in moderate/severe CAP cohort (10 patients with moderate CAP plus 40 patients with severe CAP).

Table 1 shows patient demographics and biomarker levels stratified by CAP severity. The age of patients and the gender distribution did not differ between the two severity levels. Overall CRP, PCT, and band count median values were found to be significantly higher in the moderate/severe cases of pneumonia compared to mild cases (Table 1). The median CRP value increased from 51.65 mg/L in mild to 104.75 mg/L in moderate/severe cohort (p value = 0.003). PCT showed a similar trend with increasing median values of 0.29 ng/ml in mild, and 4.02 ng/ml in moderate/severe cohort ($p = < 0.001$). Median percentage of band count were 8.0% in mild CAP compared to 15% in moderate/severe cohort ($p = 0.009$). For maximal WBC values, levels in patients with moderate/severe CAP were significantly higher ($p = 0.04$), while for minimal platelet values mild CAP had higher values ($p = 0.01$). The lab values for minimal WBC, maximal platelets, neutrophil, and NLC levels did not significantly differ between the two CAP severities.

Overall, PCT had the highest AUC of 0.77 (95% CI: 0.68-0.86) for predicting moderate/severe CAP. The optimal cutoff value of 0.55 ng/ml for PCT had sensitivity of 83%, specificity of 65% with PPV 69% and NPV 81%. The biomarkers of band counts (AUC = 0.690), CRP (AUC = 0.672), and minimal platelets (AUC = 0.644) exhibited significant but poor discrimination for moderate/severe CAP. For band count, the optimal cut off value of 12.5% was determined to have sensitivity of 61% and specificity of 71% while the optimal cut off value of 53.1 mg/L for CRP had a sensitivity of 79% and specificity of 52%. The other biomarkers were unable to discriminate moderate/severe CAP from mild pneumonia (Table 2 and Table 3)

DISCUSSION:

Major decisions regarding CAP management depend on disease severity. Our study examined frequently collected biomarkers in association with increased CAP severity. PCT, followed by CRP and band count had the highest value in predicting the severity of pneumonia.

The use of standardized scales predicting CAP severity would be ideal to guide disease management and assist decision-making. The lack of pediatric standardized scales leads to wide severity variation in patients admitted for CAP management. The PIRO scale developed in adults was examined in children with the modified scale including the factors of age less than 6 months, hypoxia $< 90\%$, hypotension, bacteremia, response like multilobar or complicated pneumonia, and organ dysfunction like kidney failure, liver failure and acute respiratory distress syndrome as predictors of increased CAP severity. Araya et al found that PIRO scores positively correlated with mortality rates in children with CAP and suggested the PIRO scale could be used to identify patients needing ICU management [5]. However, the authors did not examine outcomes beside mortality. Pediatric mortality from pneumonia is less than 1% in the United States [2] so future research should test PIRO scale’s ability in predicting CAP outcomes besides mortality. William et al. proposed three models to predict severity of pneumonia in children. Out of these three models, a simplified model using nine electronic medical record based predictors (out of 20 variables) was considered the most accurate, but it did not include biomarkers beside WBC count [3]. Thus, additional studies are needed to create and validate pediatric CAP prognostic scales similar to those employed in the adult population.

Results from studies investigating biomarkers' ability to predict pediatric CAP prognosis have been contradictory dependent upon the population and outcome examined. However, studies are increasingly showing a role for PCT for CAP management and prognosis [9], supporting our finding of PCT being able to adequately predict moderate/severe CAP in children. PCT which is undetectable in healthy people, is a precursor of the hormone calcitonin produced by the thyroid gland and by the neuroendocrine cells in lung and intestine. Infections stimulate production of PCT particularly during bacterial infections with PCT mRNA being up-regulated [10]. PCT appears to serve as a diagnostic marker for bacterial (verses viral) pneumonia [9,10,11]. The relationship between PCT and CAP prognosis has been comprehensively examined in geriatric adults [12], and Boussekey et al. demonstrated PCT to have prognostic value in CAP in adults and was associated with septic shock, multiple organ failure and mortality [13]. In children elevated PCT levels associated with poorer CAP outcomes in some studies [10, 11,14] while in others PCT levels failed to associate with CAP severity [15,16].

In a recent study examining biomarkers' ability to predict CAP severity in children, Florin et al found no statistical difference in PCT or CRP levels across four levels of CAP severity ranging from mild (discharged from ED) to severe (intensive care, vasoactive infusions, chest drainage, severe sepsis) [16]. Furthermore, none of the examined biomarkers adequately discriminated between severe and non-severe CAP. CRP and PCT levels were significantly elevated in patients having specific indicators of severe CAP including empyema requiring chest-drainage and sepsis-related vasoactive infusions. However, occurrence of the outcomes was rare (~2% of patients) and the findings should be replicated.

CRP is an acute phase reactant produced by the liver following stimulation by interleukin 6 and tumor necrosis factor- α due to injury or inflammation [17]. Chalmers et al. found CRP of less than 100 mg/L associated with a low 30-day mortality rate, decreased mechanical ventilation and low rates of complications in adult with CAP, describing CRP as an indicator of CAP prognosis [18]. In children, higher CRP levels correlate with bacterial etiology and severe CAP [15, 17]. CRP's predictive ability for severe CAP was greatly enhanced with the incorporation of the factors of neutrophil proportion, temperature, sputum production, age, and dyspnea [17], highlighting the beneficial effect of combining biomarkers with clinical features for prognosis prediction.

Our study found significant higher WBC counts and lower platelet levels in patients with moderate/severe CAP, however the biomarkers failed to adequately discriminate moderate/severe CAP from mild. WBC counts are expected to rise in infections and inflammatory conditions. In adult patients with pneumococcal pneumonia, WBC count of less than 6000 had a 5-fold higher chance of mortality while WBC counts of more than 25,000 had a 3-fold increase in mortality risk compared to patients with WBC levels between 10,000 and 25,000 [19]. Arava et al. in their study in children with pneumonia found that WBC count < 4000 associated with increased incidence of death [5]. Conversely, another pediatric study showed poor utility of WBC count to predict CAP outcomes. However, this study did not have enough patients with leukopenia to produce meaningful analysis [20]. Platelets are being increasingly described to be involved in the immunological response to bacterial infections [21]. ElMaraghy et al. found a significant correlation between thrombocytosis, thrombocytopenia, and mortality in adult patients with community-acquired pneumonia [22]. A study conducted in pediatric patients with pneumonia showed thrombocytosis in patients with lower respiratory tract infections associated with longer duration of stay [23]. The bio-marker, NLR has been described as a predictor of bacteremia in patients with infections [24]. NLR predicted 30-day mortality in adults with CAP [25]. There is scarcity of literature in children looking at utility of NLR in predicting CAP. However, NLR has been demonstrated to predict sepsis in children with pneumonia, an indirect measure of severity [26]. We did not find an association between NLR and CAP severity.

One limitation of our study is that we did not examine biomarkers' ability to differentiate bacterial versus viral pneumonia as this has been examined in children previously and recently reviewed by Gunaratnam et al. [27] However, the differentiation of bacterial versus viral pneumonia could assist in decision-making for antibiotic use. Additionally, other biomarkers like IL-6, cytokines 7, 10 11, and tumor necrosis factor Alpha (TNF- α) which may hold promise in predicting severity of pneumonia [28,29,30] were not evaluated in the

study due to not being routinely testing in the ED. Lastly, we only included patients who were hospitalized and did not examine patients discharged directly from the ED. Confounders that could not be assessed included etiology viral vs bacterial, age of patients that we could not subgroup because of the small sample size. Since the study was done in a tertiary care hospital, the applicability of biomarkers in outpatient setting could not be assessed.

We found that biomarkers, in particular PCT, can serve as a predictor for disease severity in children admitted with CAP. The inclusion of these biomarkers in the development of scales predicting CAP prognosis in children should potentially increase the scales' sensitivity and specificity for CAP severity and is an area of future research. However, biomarkers in pneumonia should be interpreted in conjunction with clinical presentation with patient management not solely dependent on biomarkers levels.

REFERENCES:

1. Rodrigues CMC, Groves H. Community-Acquired Pneumonia in Children: the Challenges of Microbiological Diagnosis. *J Clin Microbiol.* 2018;56(3):e01318-17.
2. Jain S, Williams DJ, Arnold SR, Fakhra S, Balk R, Bramley AM, et al. Community-acquired pneumonia requiring hospitalization among U.S. children. *N Engl J Med.* 2015;372(9):835-845.
3. Williams DJ, Zhu Y, Grijalva CG, Self WH, Harrell FE Jr, Reed C et al. Predicting Severe Pneumonia Outcomes in Children. *Pediatrics.* 2016;138(4):e20161019.
4. Krüger S, Welte T. Biomarkers in community-acquired pneumonia. *Expert Rev Respir Med.* 2012;6(2):203-214.
5. Araya S, Lovera D, Zarate C, Apodaca S, Acuña J, Sanabria G et al. Application of a Prognostic Scale to Estimate the Mortality of Children Hospitalized with Community-acquired Pneumonia. *Pediatr Infect Dis J.* 2016;35(4):369-373.
6. Seligman R, Ramos-Lima LF, Oliveira Vdo A, Sanvicente C, Pacheco EF, Dalla Rosa K. Biomarkers in community-acquired pneumonia: a state-of-the-art review. *Clinics (Sao Paulo).* 2012;67(11):1321-1325.
7. Zar HJ, Cotton MF. Nosocomial pneumonia in pediatric patients: practical problems and rational solutions. *Paediatr Drugs.* 2002;4(2):73-83. doi:10.2165/00128072-200204020-00001
8. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. *J Thorac Oncol.* 2010;5(9):1315-1316.
9. Giulia B, Luisa A, Concetta S, Bruna LS, Chiara B, Marcello C. Procalcitonin and community-acquired pneumonia (CAP) in children. *Clin Chim Acta.* 2015;451(Pt B):215-218.
10. Stockmann C, Ampofo K, Killpack J, Williams DJ, Edwards KM, Grijalva CG, et al. Procalcitonin Accurately Identifies Hospitalized Children With Low Risk of Bacterial Community-Acquired Pneumonia. *J Pediatric Infect Dis Soc.* 2018;7(1):46-53.
11. Esposito S, Di Gangi M, Cardinale F, Baraldi E, Corsini I, Da Dalt L, et al. Sensitivity and Specificity of Soluble Triggering Receptor Expressed on Myeloid Cells-1, Midregional Proatrial Natriuretic Peptide and Midregional Proadrenomedullin for Distinguishing Etiology and to Assess Severity in Community-Acquired Pneumonia. *PLoS One.* 2016;11(11):e0163262.
12. Berg P, Lindhardt BØ. The role of procalcitonin in adult patients with community-acquired pneumonia—a systematic review. *Dan Med J.* 2012;59(3):A4357.
13. Boussekey N, Leroy O, Georges H, Devos P, d'Escrivan T, Guery B. Diagnostic and prognostic values of admission procalcitonin levels in community-acquired pneumonia in an intensive care unit. *Infection.* 2005;33(4):257-263.
14. Khan DA, Rahman A, Khan FA. Is procalcitonin better than C-reactive protein for early diagnosis of bacterial pneumonia in children?. *J Clin Lab Anal.* 2010;24(1):1-5
15. Agnello L, Bellia C, Di Gangi M, Lo Sasso B, Calvaruso L, Bivona G, et al. Utility of serum procalcitonin and C-reactive protein in severity assessment of community-acquired pneumonia in children. *Clin Biochem.* 2016;49(1-2):47-50.
16. Florin TA, Ambroggio L, Brokamp C, Zhang Y, Rattan M, Crotty E, et al. Biomarkers and Disease Severity in Children With Community-Acquired Pneumonia. *Pediatrics.* 2020;146(3):e20193728.
17. Wu J, Jin YU, Li H, Xie Z, Li J, Ao Y, et al. Evaluation and significance of C-reactive protein in the

- clinical diagnosis of severe pneumonia. *Exp Ther Med.* 2015;10(1):175-180.
18. Chalmers JD, Singanayagam A, Hill AT. C-reactive protein is an independent predictor of severity in community-acquired pneumonia. *Am J Med.* 2008;121(3):219-225.
19. Gardner JG, Bhamidipati DR, Rueda AM, Nguyen DTM, Graviss EA, Musher DM. White Blood Cell Counts, Alcoholism, and Cirrhosis in Pneumococcal Pneumonia. *Open Forum Infect Dis.* 2017;4(2):ofx034
20. Williams DJ, Hall M, Auger KA, Tieder JS, Jerardi KE, Queen MA, et al. Association of White Blood Cell Count and C-Reactive Protein with Outcomes in Children Hospitalized for Community-acquired Pneumonia. *Pediatr Infect Dis J.* 2015;34(7):792-793.
21. Fitzgerald JR, Foster TJ, Cox D. The interaction of bacterial pathogens with platelets. *Nat Rev Microbiol.* 2006;4(6):445-457.
22. ElMaraghy AA, AbdelFattah EB, Ahmed MS. Platelet count: Is it a possible marker for severity and outcome of community acquired pneumonia. *Egyptian Journal of Chest Diseases and Tuberculosis.* 2016;2(65):499-504.
23. Sreenivasa; GV, Kumar; B, Manjunatha. (2017). Study of significance of thrombocytosis in lower respiratory tract infections in children. *International Journal of Contemporary Pediatrics.* 2015;2(2), 103-107.
24. de Jager CP, van Wijk PT, Mathoera RB, de Jongh-Leuvenink J, van der Poll T, Wever PC, et al. Lymphocytopenia and neutrophil-lymphocyte count ratio predict bacteremia better than conventional infection markers in an emergency care unit. *Crit Care.* 2010;14(5):R192.
25. Cataudella E, Giraffa CM, Di Marca S, Pulvirenti A, Alaimo S, Pisano M, et al. Neutrophil-To-Lymphocyte Ratio: An Emerging Marker Predicting Prognosis in Elderly Adults with Community-Acquired Pneumonia. *J Am Geriatr Soc.* 2017;65(8):1796-1801.
26. Dursun A, Ozsoylu S, Akyildiz BN. Neutrophil-to-lymphocyte ratio and mean platelet volume can be useful markers to predict sepsis in children. *Pak J Med Sci.* 2018;34(4):918-922.
27. Gunaratnam LC, Robinson JL, Hawkes MT. Systematic Review and Meta-Analysis of Diagnostic Biomarkers for Pediatric Pneumonia [published online ahead of print, 2021 Jul 2]. *J Pediatric Infect Dis Soc.* 2021;piab043.
28. Menéndez R, Martínez R, Reyes S, Mensa J, Filella X, Marcos MA, et al. Biomarkers improve mortality prediction by prognostic scales in community-acquired pneumonia. *Thorax.* 2009;64(7):587-591.
29. Fernandes CD, Arriaga MB, Costa MCM, Costa MCM, Costa MHM, Vinhaes CL, et al. Host Inflammatory Biomarkers of Disease Severity in Pediatric Community-Acquired Pneumonia: A Systematic Review and Meta-analysis. *Open Forum Infect Dis.* 2019;6(12):ofz520
30. Karakioulaki M, Stolz D. Biomarkers in Pneumonia-Beyond Procalcitonin. *Int J Mol Sci.* 2019;20(8):2004. Published 2019 Apr 24. doi:10.3390/ijms20082004

Hosted file

Table 1.docx available at <https://authorea.com/users/453287/articles/551181-a-retrospective-observational-study-to-estimate-use-of-biomarkers-to-predict-severity-of-community-acquired-pneumonia-in-children>

Hosted file

Table 2.docx available at <https://authorea.com/users/453287/articles/551181-a-retrospective-observational-study-to-estimate-use-of-biomarkers-to-predict-severity-of-community-acquired-pneumonia-in-children>

Hosted file

Table 3.docx available at <https://authorea.com/users/453287/articles/551181-a-retrospective-observational-study-to-estimate-use-of-biomarkers-to-predict-severity-of-community-acquired-pneumonia-in-children>