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Evaluation of serum amyloid-A protein in the diagnosis of sepsis among children at PICU of Al Zahraa University Hospital

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Abstract

The early and efficient diagnosis of sepsis in critically ill children remains a difficult task as the clinical signs are nonspecific. Complete blood count parameters and C-reactive protein have low sensitivity., Also, the difficulty of its diagnosis may be due to decreased positive values of blood culture and the need for longtime to detect blood culture results. The serum Amyloid A (SAA) protein level in the blood increases earlier and up to 1000-fold in response to inflammation. This study aimed to assess the role of SAA as diagnostic and prognostic marker in pediatric sepsis in the first 24 hours after pediatric intensive care unit (PICU) admission. This case-control study included 45 children with sepsis admitted at PICU from May 2023 to March 2024 and 45 children with matched age and sex as controls. We investigated SAA level in the same time with routine laboratory investigations of both groups. SAA level was higher in the patient group, ranged from 0.9 to 47.2 µg/m, with median 4.54 µg/ml, as compared to the control group with median 0.58 µg/ml ranged from 0 to 2.3 µg/ml. ($p \leq 0.001$). Also, SAA level was significantly lower in the survived group with median 13.6 µg/ml, ranged from 5.7 to 20 µg/ml than the non-survived group with a median of 32.3 µg/ml; ranged from 30.3 to 47.2 µg/ml. In conclusion, we found that SAA was extremely high in critical and extremely critical ill patients which can be used as a predictor of mortality in severe sepsis among children.

Keywords: Pediatric intensive care unit, Pediatric Sepsis, Serum Amyloid A.

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Introduction

Sepsis is the leading cause of death worldwide in the pediatric population resulting in an estimated 7.5 million deaths annually. It encompasses the top four causes of childhood mortality as reported by the World Health Organization (WHO): severe pneumonia, severe diarrhea, severe malaria, and severe measles.[1]

The systemic response to infection involves the release of several mediators which has led to suggestion that some of these mediators could be used as markers of sepsis. Several acute phase proteins were used for the diagnosis of sepsis. However, to date, although laboratory markers of infection might aid in the diagnosis, no single laboratory test provided rapid and

reliable identification of early infected neonates and children.[2]

The first steps include early diagnosis of sepsis followed by the appropriate stratification of patients (e.g., admission to a hospital ward for observation or for a more intensive monitoring environment in a critical care unit). This led to great interest for developing diagnostic and stratification biomarkers for sepsis.[3]

Early diagnosis of sepsis is a challenge. Several biomarkers are available for early diagnosis of sepsis. Of course, early diagnosis of sepsis could reduce mortality and hospital stays.[4]

Even though blood culture was considered the most reliable test for the diagnosis of sepsis but it is too slow and has false negative outcomes. As well, C-reactive protein (CRP) is the most commonly used marker for the diagnosis and follow up of sepsis that clinicians depend on in all hospitals. It has a low specificity and comprises a physiological 3-days increase resulting in a low possibility to detect sepsis at an early stage.[5]

Serum amyloid A (SAA), the precursor protein in inflammation- associated reactive amyloidosis, whose level in the blood increases up to 1000 fold in response to inflammation, is synthesized in the liver. SAA is also an acute phase reactant like procalcitonin (PCT) and CRP, which has been proven to be a prognostic marker in late-onset sepsis in preterm infants.[6, 7]

Sharma et al., 2024[7] reported that SAA had an overall better diagnostic accuracy than CRP for predicting early onset sepsis. Also, they showed that SAA was a useful inflammatory marker during late-onset sepsis in preterm infants.

Despite that several studies were conducted about the role of SAA in predicting sepsis in neonatal period, its role in pediatric age group is notclear.[8] Therefore, we conducted our study to assess the accuracy of SAA in sepsis diagnosis in children who will be admitted to the pediatric intensive care unit (PICU) of Al Zahra university hospital.

Subjects and Methods

Study setting and design

This longitudinal case control study included 45 children with sepsis admitted to the pediatric critical care unit, Al Zahraa University hospital during the period from May 2023 to March 2024. Patients included both sexes, their ages ranged from one month to 12 years. In addition, 45 age and sex matched apparently healthy children, attended to the outpatient clinic were included in the study as a control group.

Inclusion criteria included: age range between one month to 12 years. Patients with established diagnosis of sepsis or severe sepsis or septic shock at the time of admission to the PICU, was based on the American college of critical care medicine and international pediatric squamous cell carcinoma (SCC) definitions for sepsis as follows.[9] abnormal heart rate, abnormal temperature, hypotension, oliguria, fast breathing, unexplained metabolic acidosis and mottling.[10]

Exclusion criteria included: patients with malignant tumors, severe malnutrition, heart failure, liver failure, acute kidney injury or chronic kidney disease. Chronic diseases as amyloidosis, atherosclerosis, systemic lupus erythematosus, rheumatoid arthritis, pericarditis, and inflammatory bowel disease that might increase or affect SAA measurements were also excluded.

Methods

All patients and control children were subjected to full clinical history taking of demographic data including sex, age, and residence, socioeconomic standard, and special habits.

Complete clinical examination, included thorough general examination including face, built, decubitus, colors, vital signs (pulse, blood pressure, temperature, and respiratory rate), head, neck, upper limb, lower limb, heart, abdomen, and anthropometric measurement (weight, height, head circumference, and body mass index, calculated by dividing weight by height in meter square.

Pediatric Risk of Mortality score (PRISM).[11] It was calculated within 24hrs of admission for each patient, using the 14th measured clinical and laboratory variables Data were entered on the website: https://devweb.utahdcc.org/cpccrn/calculators/prismivcalculator which calculated the PRISM score automatically and displayed it in the nominated window on the screen. The patient age was entered in months, in a specific window led again to automatic calculation and displaying of the predicted death rate.

Pediatric Sequential Organ Failure Assessment score (pSOFA) for each patient was calculated. Total scores were classified a change in baseline of the total SOFA score of 2 points or more to represent organ dysfunction. Depending on a patient's baseline level of risk, a SOFA score of 2 or greater identified a 2-to-25-fold increased risk of dying compared with patients with a SOFA score less than 2.[12]

Laboratory investigations were performed within 24 hours after admission. General laboratory tests were performed, which included complete blood count (CBC) which was done using fully automated cell counter (Sysmex KX21N, Kobe, Japan), according to the manufacturer's instructions. C- reactive protein (CRP) was done using latex agglutination kits (lot # A3256/1, CRP visilatex–slide assay), blood gases were done by a blood gas analyzer (Gem premier 3000, Werfen, Belgium), according to the manufacturer's instructions. Blood urea nitrogen (BUN), serum creatinine, Sodium and Potassium were done using a fully automated chemistry analyzer (Cobas c 311, Germany), using commercial kits supplied by (Roche Diagnostics, Germany), according to the manufacturer's instructions.

In addition, SAA was determined by enzyme linked immunosorbent assay (ELISA) kits, (Catalogue No. 201-12-1226, SAA ELISA Kits), according to the manufacturer's instructions. An ELISA washer (106ff41412 Bio Tek, USA) was used during the process. The optical densities of the final ELISA products were measured using a microtiter reader (1851 Das, Italy), according to the manufacturer's instructions. Finally, a standard curve was obtained by plotting the concentration of the standards versus their absorbance values.

Statistical Analysis

The Statistical Package of Social Science (SPSS) application for Windows (version 21) was used to analyze data of this study. The Kolmogorov Smirnov test was initially used to determine whether the data were normally distributed. The subsequent tests were utilized, Chi square test to compare qualitative variables; Monte Carlo test and Fisher exact test to compare qualitative variables when expected count less than 5. The Student t test was used to compare two quantitative variables (parametric); Mann Whitney test to compare two quantitative variables (nonparametric); Spearman correlation for comparison of numerical data (non-parametric). The receiver operating characteristic (ROC) curve analysis was used to determine the sensitivity and specificity at different cutoff points. The confidence interval was set at 95%, while the allowed margin of error was set at five %. Therefore, the p-value was considered significant at $p<0.05$.

Results

This case-control study included 90 children, their age ranged from one month to 12 years. There was no difference in age and sex between the sepsis patients and the control group. (Table 1)

Table 1. Comparison of age and gender between the patient and control groups.

Variable	Control group (n=45)	Patient group(n=45)	*P* value
Age (Month)			
Median (Min-Max)	48.00 (3.0- 144)	72.00 (3.0-120)	[k]NS
Sex			
Male	24 (53.3%)	33 (73.3%)	x^2 NS
Female	21 (46.7%)	12 (26.7%)	

K: Kruskal-wallis rank sum test, X^2: Pearson's chi-squared test. $p > 0.05$ is not significant (NS).

There was a significant increase in CRP, white blood cells (WBCs), aspartate transaminase (AST), alanine aminotransferase (ALT) and serum creatinine in patients than controls. While there was a significant decrease in hemoglobin and platelets count in patients compared to controls (p<0.001). (Table 2)

Table 2. Comparison of laboratory characteristics parameters between the patient and control groups.

Parameters	Control group (N=45)	Patient group (N=45)	[W]*p* value
Hemoglobin (gm/dl)			
Mean (SD)	15.7 (22.0)	10.7 (2.6)	< 0.001
Median (IQR)	11.7 (10.9-12.8)	10.6 (9.6- 12.2)	
WBC (1000/µl)			
Mean (SD)	8.3 (1.4)	16.9 (12.2)	< 0.001
Median (IQR)	8.1 (7.2-9.2)	13.2 (8.0- 23.0)	
Platelets (1000/µl)			
Mean (SD)	335.9 (58.5)	136.7 (70.9)	< 0.001
Median (IQR)	342.0 (302.5- 382.5)	124.0 (92.0- 200.0)	
CRP (mg/dl)			
Mean (SD)	1.9 (2.2)	45.3 (58.7)	< 0.001
Median (IQR)	2.0 (2.0- 3.0)	26.0 (6.0-75.0)	
Creatinine (mg/dl)			
Mean (SD)	0.08 (0.12)	1.07(0.698)	< 0.001
Median (IQR)	0 (0 - 0.4)	0.6 (0-2.9)	
AST (U/l)			
Mean (SD)	18.1 (3.5)	67.3 (105.8)	< 0.001
Median (IQR)	17.0 (16.0- 20.0)	29.0 (20.0- 46.0)	
ALT (U/l)			
Mean (SD)	12.5 (3.3)	53.3 (91.8)	< 0.001
Median (IQR)	12.0 (10.5- 14.0)	21.0(12.0- 45.0)	

W: Wilcoxon rank sum CRP: C-reactive protein, WBCs: White blood cells, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase. $p \leq 0.05$ is significant. $p \leq 0.05$ is significant.

The cases group was classified according to the PRISM III and pSOFA into sepsis, severe sepsis, and septic shock sub-groups. In addition, according to their outcome, the patients group was further classified into surviving and non-surviving groups. (Table 3)

Table 3. PRISM III and pSOFA risk factors and sepsis outcome in the 45 study patients.

PRISM III and pSOFA	Patient group
Sepsis	25 (55.6%)
Severe sepsis	10 (22.2%)
Septic shock	10 (22.2%)
Outcome	
Survived	3 (6.7%)
Non- survived	42 (93.3%)

SAA level was higher in the sepsis patient group as compared to the control group (p<0.001). SAA levels were significantly higher in the non-survived group than the survived group (p=0.022), (Table 4). A positive association was observed between SAA levels and pSOFA as shown in (Figure 1).

Table 4. Comparison of serum amyloid A (SAA) level between the different study groups.

Biomarker SAA (µg/ml)	Mean (SD)	Median (IQR)	^{K}p
Control group (N=45)	1.33 (1.67)	1.2 (0- 2.3)	0.004
Patient group (N=45)	12.2 (7.4)	9.7 (0.9-47.2)	
Sepsis subgroups			
Sepsis	1.81(1.66)	0.97(0-5.13)	0.001
Severe sepsis	7.45(0.99)	7.24(6.1-39.03)	
Septic shock	23.24(10.96)	19.10(12.5-47.2)	
Survivor	5.77(6.26)	3.85(0.9-26.7)	0.003
Non- survivor	33.1 (15.2)	31.5 (22.8-47.2)	

K: Kruskal-wallis rank sum test, X^2: Pearson's chi-squared test, $p \leq 0.05$ is significant.

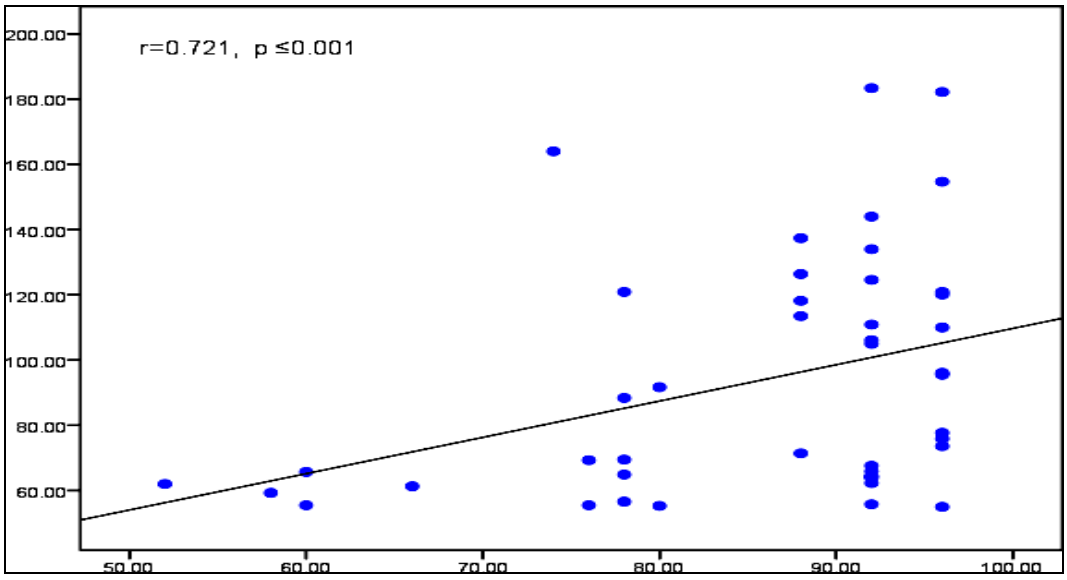

Figure 1. A scatter diagram showing the positive correlation between SAA level and Pediatric Sequential Organ Failure Assessment score (pSOFA).

At an area under the curve (AUC) of 0.978 with 95 % confidence interval (CI) between 0.95 and 1.0, a cutoff point of 145.25 µg /ml can be used as a predictor for the sepsis patient group. And, the sensitivity was 97.8%, specificity 91.1 %, positive predictive value (PPV) 97.6%, negative predictive value (NPV) 91.7% and accuracy 94.4% as shown in (Figure 2).

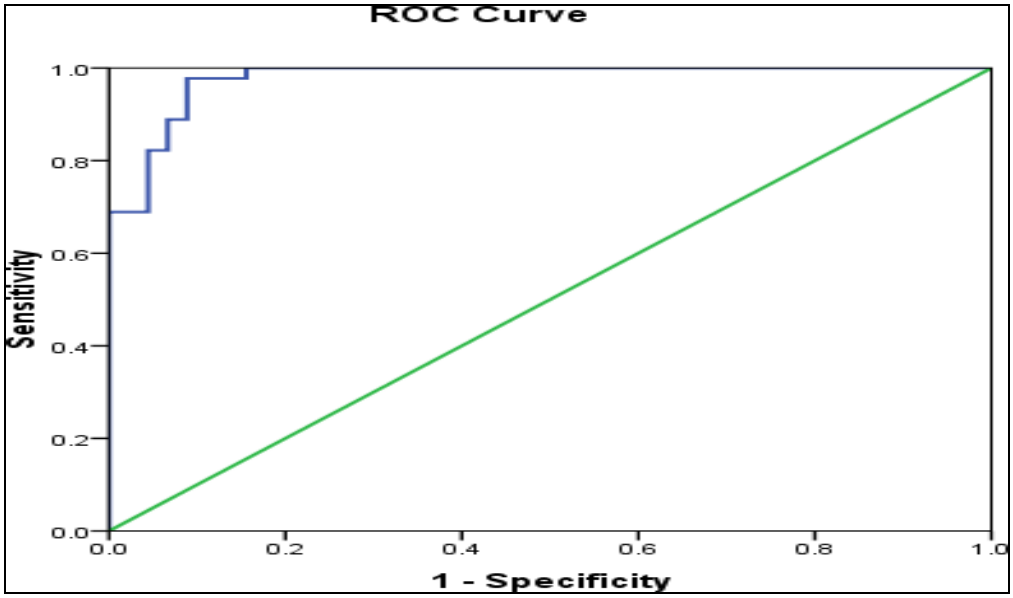

Figure 2. Receiver operating characteristic (ROC) curve comparing the predictive performance of SAA inprediction of sepsis risk.

At AUC of 0.834, with a 95% CI between 0.711 and 0.958, a cutoff value of 93.60 µg /ml can be used as a predictor for mortality. The sensitivity, specificity, PPV, NPV, and accuracy were 93.3%, 63.3%, 56%, and 95%, respectively (Figure 3).

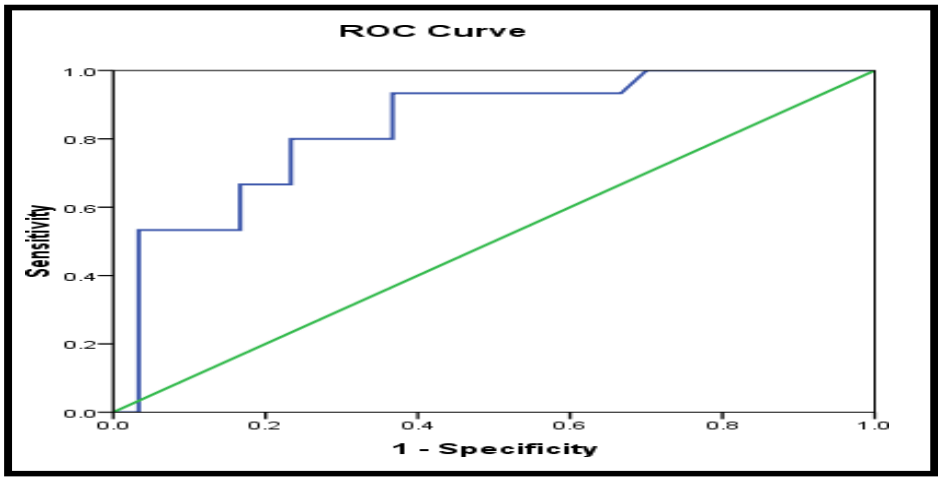

Figure 3. Receiver operating characteristic (ROC) curve comparing the predictive performance of serum amyloid A (SAA) inprediction of mortality.

Discussion

Despite of all efforts, to date there is neither solitary biomarker nor combination of biomarkers available for correctly discriminating sepsis from trauma, and tissue damage. Levels of the readily available sepsis biomarkers either rise too slowly or drop too fast for clinicians to be certain about catching a sepsis during development or for clinicians to be able to eliminate sepsis as a reason for deterioration.[13] Therefore, this study aimed to assess the role of SAA as diagnostic and prognostic marker in pediatric sepsis during the first 24 hours after PICU admission.

In the present study, our patients have a median age of 17 months ranged from 1 month and 120 months and there was a male predominance (55.5%), Weiss et al., 2020,[14] reported that the included septic cases had a mean age of 3 (0.7 – 11) years. They also reported that the majority of their cases were males. Balayan et al., 2022[15] found that 55.0% of cases were males; the majority of the included patients (62.7%) were belonged to the age group 0-3 years. They also reported that the male patients are more vulnerable to infections and the possibility could be that the testosterone suppressing the immune response.

In the present study, laboratory investigation showed significant differences between patients and controls as there was a significant increase in CRP, WBCs, AST and ALT, while a significant decrease in hemoglobin and platelet count in patients than control group, also both sepsis severity counts. Such findings are also in agreement with the results of El Nemer et al., 2021[16] and Lubis, 2012[17] and mortality were negatively correlated with platelets.

Also, Balayan et al., 2022,[15] and Cheng et al., 2023[18] found that there was a significant difference in the platelet count between septic and control groups. The cause of thrombocytopenia may be due to toxic destruction of the infants' platelets, megakaryocytic suppression, increased peripheral consumption as in disseminated intravascular coagulopathy, or the presence of immune component because of increased levels of platelet-associated immunoglobulins.

In our study there were significant differences in score of pSOFA between sepsis subgroups. These scores were more increased in septic shock (8.1) than severe sepsis (5.6) and sepsis groups (4.7) consistent with the severity of sepsis. These are in agreement with other results of Liu et al., 2022[19] reported that pSOFA score in severe group and septic shock group were much higher than these in the mild disease group. Also, Lalitha et al., 2021[20] observed that pSOFA score increased in septic shock than severe sepsis and sepsis groups consistent with the severity of the disease.

In our study we found that there was a significant increase in SAA level in patients than controls (p <0.004). Also, there were significant increase in SAA between sepsis subgroups, gradually increased from sepsis to septic shock (p = 0.038). In a study by Habib and Ansar, 2021[21] to assess the accuracy of SAA in sepsis diagnosis in children admitted to PICU, Mansoura, Egypt. They found that SAA was elevated in sepsis and in non-survivors than survivors among critically ill children (p<0.001). Also, Zou et al., 2021,[22] observed significant differences in SAA between the control group and critically ill children, with significant rise of SAA level in ventilation acquired pneumonia.

In addition, a study by Hashem et al., 2020,[23] included 100 adult patients demonstrated that serum SAA level in patients with septic shock was significantly increased compared to patients with non-septic shock at admission (p< 0.05). Regarding sepsis-related mortality, SAA was positively correlated with an increased mortality among adults. However, there are limited studies to assess such relation among critically ill children.[21]

The current study showed that there was a statistically significant positive correlation between SAA level and pSOFA score. Comparing biomarker levels among different strata of pSOFA score revealed that there was an increase of SAA level with an increase of pSOFA score. Such findings are in agreement with those of a study by Liu and Qiu, 2020[24] that reported correlation between severity of sepsis with increase score of pSOFA and noted that a

significance between pSOFA and increased level of serum amyloid A.

In conclusion, we found that among severely and extremely critically ill sepsis patients, serum SAA levels can be suggested as useful markers to predict mortality in children with sepsis.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by GDIAE, and SIM. The first draft of the manuscript was written by SMKI and FAED and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript**.**

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was ethically reviewed and approved by the Research Ethics Committee of the Faculty of Medicine for Girls Al Azhar University, Egypt (approval date: April 2023).

Informed consent

The importance of the study was explained to the parents of the participating children. An informed written consent was taken from the parents before enrolling the children in this study.

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