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Role of CD64 and myeloperoxidase as biomarkers for early diagnosis of sepsis in pediatric intensive care unit

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Abstract

Globally, sepsis is the primary cause of death for children. While research conducted on adults has a significant impact on the diagnosis and treatment of sepsis in newborns and young children, there are significant factors that are pertinent to pediatrics as well. This prospective case-control study was conducted during the period from August 2020 to October 2022 after approval by the institutional ethical committee. The study included 48 critically ill children admitted at the Pediatric intensive care unit and 30 apparently healthy children as a control group. Laboratory investigations including complete blood picture, C-reactive protein (CRP), blood culture and sensitivity and plasma level of cluster of differentiation 64 (CD64) and myeloperoxidase (MPO) were investigated for all participants. A daily follow up for the signs of systemic inflammatory response syndrome (SIRS) or sepsis was done, and the patients were divided into SIRS and non-SIRS subgroups then patients were divided into two groups according to the presence of severe sepsis. A follow up CD64 and MPO sample were withdrawn from them to assess their prognostic value. SIRS was reported in 39.58 % of patients while severe sepsis was reported in 20.8%. CD64 and MPO were significantly higher in cases than controls (p= 0.003, p<0.001, respectively) and, in patients with SIRs and severe sepsis CD64 was 1559.00± 367.09 pg/ml and 1547.9 4± 436.14 pg/ml, respectively. Also, MPO was significantly higher in patients with SIRS (113.58± 25.19 mU/ml) and severe sepsis (111.70± 26.50 mU/ml). CD64 and MPO significantly increased after development of sepsis in admitted patients. ROC was significantly higher for CD64 and MPO at admission than that for CRP at admission (p=0.123, p=0.014, respectively). In conclusion, plasma level of CD64 and MPO in peripheral blood can be considered an early sensitive marker for the detection and follow up of pediatric sepsis.

Keywords: sepsis, children, Myeloperoxidase, Biomarkers.

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Introduction

Sepsis is a life-threatening condition. Regardless of any underlying medical conditions, many children suffer from the potentially fatal illness known as sepsis. Even in developed countries, sepsis is thought to be one of the main causes of death for children.[1] Many children who are

reported to have died from other underlying diseases actually die from sepsis, even though demographic data does not clearly reveal this.[1] Tachycardia, tachypnea, fever, and leukocytosis are the hallmarks of the clinical illness known as systemic inflammatory response syndrome (SIRS), which develops after inflammation.[2] During their hospital stay, more than 50% of patients in the intensive care unit (ICU) have SIRS symptoms.[3] Sepsis or a non-infectious inflammatory stimulation such as burns, pancreatitis, polytrauma, or surgery can be the cause of SIRS. Differentiating between sepsis and non-infectious SIRS is crucial because, in the case of sepsis, prompt antibiotic treatment can save a patient's life.[4] Clinically, non-infectious SIRS and sepsis are difficult to differentiate from one another, and existing diagnostic methods such as microbiologic cultures are laborious and insensitive.[5] Monocytes, macrophages, neutrophils, and other immune cells have the surface of cluster of differentiation 64 (CD64), a type I high-affinity receptor for the Fc component of immunoglobulin G. It causes monocytes and macrophages to produce cytokines, superoxide anions, and undergo phagocytosis. The presence of interferon gamma (IFN-γ), which is generated during infection, in peripheral blood increases the expression of CD64. The degree of stimulation that inflammatory cytokines get is directly correlated with an increase in its surface density, which is measured in cytometry as the mean fluorescence intensity (MFI). Thus, in response to bacterial infection, various investigations have demonstrated an increase in CD64 expression.[5,6,7] Also, neutrophil CD64 was more accurate than procalcitonin (PCT) for differentiating systemic inflammatory response syndrome from severe sepsis and septic shock in respiratory ICU patients.[7] The Sequential Organ Failure Assessment (SOFA) score was designed to provide population level insights into the acute morbidity of ICU patients.[8]

A hemeprotein called myeloperoxidase (MPO) is kept in the neutrophil azurophilic granules. To phagocytose ingested bacteria, MPO produces reactive oxidants such as hypochlorous acid. Consequently, MPO is a crucial part of innate immunity and one of the keystones of the neutrophil attack on bacteria. In the host immunological defense, neutrophils are the first cell type to respond. MPO is released into plasma by activated neutrophils. As a result, CD64 and MPO may be used as diagnostic biomarkers to distinguish between sepsis and SIRS without infection.[9] Consequently, this study aimed to assess the role of MPO and CD64 as biomarkers in early diagnosis of sepsis in pediatric intensive care unit (PICU) and their prognostic value as a biomarker for mortality.

Patients and Methods

This was a prospective case control study, conducted at the pediatric intensive care unit from September 2020 till October 2022. The study included 48 critically ill children, and 30 apparently healthy children as a control group. The inclusion criteria were sepsis patients less than 18 years of both sexes. The duration of admission was less than 48 hours. The exclusion criteria included patients with inflammatory diseases such as rheumatoid arthritis, cardiovascular diseases, neurodegenerative diseases., hepatic diseases, and patients with primary or secondary immunodeficiency.

Definition of study diseases

SIRS: defined by the presence of two or more of the following: alterations in core body temperature (hypothermia or hyperthermia), changes in leukocyte count (leukopenia or leukocytosis), effects in heart rate (age-specific bradycardia or tachycardia), and/or age-specific tachypnea. Sepsis: A patient with a diagnosis of SIRS in the presence of a known or suspected infection. Severe sepsis: is sepsis plus either cardiovascular dysfunction, acute respiratory distress syndrome, or ≥2 organ dysfunction.[10]

All included patients were followed up daily for the development of signs of SIRS or sepsis then were divided into two subgroups, SIRS and non-SIRS subgroups. Patients with sepsis were subsequently divided into two subgroups according to the presence of severe sepsis.

Blood samples were withdrawn from patients with sepsis to assess CD64 and MPO in severe sepsis and to determine their prognostic

value. All included children were subjected to full demographic and history data taking, including name, age, sex, residency, main presenting symptom, history of other diseases as inflammatory diseases, cardiovascular diseases, neurodegenerative diseases, and hepatic diseases.

The full clinical assessment included vital signs (heart rate, respiratory rate, blood pressure and temperature), anthropometric measurements including weight and height, and body mass index (BMI). BMI was calculated by dividing weight in kilograms by the square of height in meters. The general examination included abdominal, cardiac, chest and neurological examination.

Laboratory investigations

Complete blood count (CBC) was assessed using a hematology analyzer (Sysmex XN-530, Japan), according to the manufacturer's instructions. Prothrombin time, concentration and International normalized ratio (INR) were measured by a medical laboratory analyzer (LABiTec PT-R, Germany) according to the manufacturer's instructions. C-reactive protein (CRP), using a diagnostic equipment (Mispa-i2, Agappe, India), according to the manufacturer's instructions.

Microbiological laboratory investigations, including blood culture using a blood culture system (BD BACTEC™ FX40 instrument, USA), and microbial identification and antibiotic susceptibility testing using a microbial identification system (VITEK 2 system, bioMérieux, France), according to the manufacturer's instructions.

Chemical laboratory investigations, including liver function tests, and serum creatinine were assessed using a clinical chemistry analyzer (Beckman Coulter AU480 Chemistry Analyzer, USA), according to the manufacturer's instructions.

Plasma levels of CD64 and MPO were determined using commercial enzyme linked immunosorbent assay (ELISA) kits (Sino.Gene.Clon Biotech Co., Ltd., China), according to the manufacturer's instructions. The optical density of the final ELISA products was measured using a microtiter reader (Stat Fax-2100, Awareness Technology, USA), according to the manufacturer's instructions.

Statistical Analysis

The computer statistics programs Microsoft Excel (Microsoft Corporation, NY, USA) and the Statistical Package for the Social Science, (SPSS, Inc., Chicago, IL, USA version 20) were used for all statistical computations. When applicable, frequency (number of cases), percentages, and mean ± standard deviation (± SD) were used to statistically describe the data. The T-test and analysis of variance (ANOVA) were used for comparison of mean ±SD of numerical data. Relative Risk (RR) with 95% CI was used to identify the RR of CD64 level in relation to different clinical data. The receiver operating characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity of CD64 and MPO expression for predicting pediatric sepsis. The significance threshold (p-value) for each of the above-mentioned statistical tests was set at the 5% level. A significant outcome was indicated by a p-value of less than 0.05.

Results

The demographic, clinical and laboratory data of the cases and controls are shown in Table 1. The age was not different between the cases and controls, but the weight, height, and BMI were significantly higher in controls than the cases. The case group exhibited significantly lower mean hemoglobin (HB), prothrombin concentration (PC), and serum albumin levels in comparison to the control group. Conversely, the mean total leukocyte count (TLC), INR, alanine aminotransferase (ALT) and serum creatinine were significantly higher in the patient group compared to the control group. The mean Staff/TNC (Band or immature neutrophils/Total Neutrophil Count) CRP, CD64, and MPO on admission were significantly higher in case group compared to control group (p<.0.01).

Table 1. Demographic, clinical and laboratory data of the cases and controls.

Study parameters	Cases (n=46)	Controls (n=30)	*p* value
Age (years)	2.26± 1.10	3.61± 1.03	NS
Weight (kg)	10.45± 5.33	16.03± 8.85	0.007
Height (cm)	81.42± 25.41	95.70± 23.66	0.015
BMI	13.87±3.20	16.35±2.99	0.001
Hemoglobin (g/L)	9.93± 1.07	11.39± 0.82	<0.001
Platelet (10^3/Ul)	263.22± 85.65	297.43± 71.82	NS
TLC (10^3/Ul)	14.29± 7.80	7.44± 2.17	0.001
PT (seconds)	11.76± 1.62	11.00± 0.83	0.020
PC	84.54± 17.12	96.37± 3.77	0.001
INR	1.28± 0.30	1.03± 0.08	0.001
S. albumin (g/L)	3.55± 0.58	3.94± 0.37	0.002
ALT (IU/L)	90.98± 58.81	14.27± 3.40	0.029
Creatinine (mg/dl)	0.70± 0.48	0.38± 0.15	0.001
CRP at admission (mg/L)	24.62± 26.43	3.82± 1.35	<0.001
Staff/TNC	16.80± 9.37	7.10 ± 2.10	<0.001
MPO on admission (mU/ml)	79.75± 34.08	39.99± 5.69	<0.001
CD64 (pg/ml) on admission	1286.87± 470.52	960.68± 439.42	0.003

CRP, c-reactive protein. MPO, myeloperoxidase. HB, hemoglobin. INR, international normalized ratio. ALT, Alanine aminotransferase. BMI, body mass index. TNC, Total neutrophilic count. TLC, total leukocyte count. CD64, Cluster of Differentiation. PT, prothrombin time. PC, prothrombin concentration. $p > 0.05$ is not significant (NS).

The results of blood culture in the patient groups showed no growth as the most obtained result (66.7%). The most common isolated organisms from patients were *Klebsiella* (16.7%), *Pseudomonas* (6.3%), Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 4.2%, Coagulase-negative *staphylococci* (Cons), *Acinetobacter*, and *Enterococci* were reported in 2.1% for each. SIRS was reported in 29 (60%) of patients while sepsis was reported in 15 patients (31.3%), 10 of them had severe sepsis.

Comparison between patients with and without SIRS revealed that males represented 47.4% of SIRS patients and 52.6% females which were not different than non-SIRS, 62.0% males and 38.0% females (p=0.56). Positive blood culture was observed in 78.9% of SIRS versus 3.7% of non-SIRS patients (p<0.001). During the steps of management, 68.4% of SIRS needed inotropic therapy compared to only 7.4% of the non-SIRS patients (p<0.001). Mechanical ventilation was applied to 52.6% of SIRS and for only 3.7% of non-SIRS patients (p<0.001). The study ended with a mortality rate of 42.1% in SIRS and 3.7% in non-SIRS patients (p<0.001).

The platelets count, PC, and albumin level in the SIRS group was considerably lower than in the non-SIRS group (p<0.001). While ALT, PT, and creatinine were significantly higher in SIRS group. The SIRS group had significantly higher mean of staff/TNC, CD64, and MPO levels than the non-SIRS group. However, the CRP level at admission was not significantly different between the two groups (Table 2). On comparing the severe and non-severe sepsis groups, the mean serum albumin was significantly lower in the severe sepsis group compared to the group with non-severe sepsis (p<0.001). The mean Staff/TNC and MPO on admission were significantly higher in severe sepsis group compared to the group with non-severe sepsis (p<0.001). Mean levels of CD64 on admission were significantly higher in the severe sepsis group compared to the group with non-sever sepsis (p=0.007). There was no significant difference in the mean CRP on

admission between both groups (Table 3). There were significant increases in the CRP, CD64 and MPO levels with the development of severe sepsis. CD64 and MPO were significantly higher at follow up (2837.90±913.49 pg/ml and 179.30± 61.31 mU/ml, respectively, *p* =<0.001).

Table 2. Comparison of the demographic, clinical and laboratory data between patients with systemic inflammatory response syndrome (SIRS) and Non-SIRS patients.

	Systemic inflammatory response syndrome (SIRS)		*p* value
	No (n=29)	Yes (n=19)	
HB (g/L)	9.94± 1.10	9.85± 1.14	NS
Platelet (10^3/Ul)	300.34± 76.22	205.79± 60.41	<0.001
TLC (10^3/Ul)	14.31± 8.27	14.12± 7.37	NS
PT (seconds)	10.93± 0.80	13.05± 1.65	<0.001
PC	94.45± 4.19	70.16± 18.32	<0.001
INR	1.11± 0.12	1.54± 0.30	<0.001
S. albumin (g/L)	3.91± 0.39	3.08± 0.48	<0.001
ALT (IU/L)	28.83± 7.52	179.00± 74.06	0.005
Creatinine (mg/dl)	0.58± 0.38	0.86±0.57	0.047
CRP (mg/L)	24.05± 25.19	23.78± 27.13	0.972
Staff/TNC	26.21 ±7.07	10.03 ± 2.89	0.047
MPO (mU/ml)	113.58± 25.19	57.23± 13.91	<0.001
CD64 (pg/ml)	1559.00± 367.09	1089.04± 444.45	<0.001

HB, hemoglobin. TLC, total leukocyte count. PT, prothrombin time. PC, prothrombin concentration. INR, international normalized ratio. ALT, Alanine aminotransferase. CRP, C-reactive protein. CD64, cluster of differentiation 64, TNC, total neutrophilic count. MPO, Myeloperoxidase. *p* > 0.05 is not significant (NS).

Table 3. Comparison of laboratory data between patients with and without severe sepsis.

	Sepsis		*p* value
	Severe (n=10)	Non- severe (n=5)	
HB (g/L)	9.64± 1.09	10.01± 1.06	NS
Platelet (10^3/Ul)	222.50± 52.70	274.53± 90.07	NS
TLC (10^3/Ul)	16.16± 8.02	13.78± 7.77	NS
PT (seconds)	13.20±1.81	11.36± 1.33	<0.001
PC	63.90± 13.35	90.28± 13.25	<0.001
INR	1.63± 0.19	1.19± 0.25	<0.001
S. albumin (g/L)	2.86± 0.39	3.74± 0.47	<0.001
ALT (IU/L)	212.90± 168.19	29.33± 7.83	<0.001
Creatinine (mg/dl)	1.24± 0.53	0.55± 0.35	<0.001
CRP (mg/l)	23.50± 18.12	24.93± 28.52	NS
Staff/TNC	26.80± 9.08	14.03± 7.45	<0.001
CD64 (pg/ml)	1547.9 4± 436.14	1075.46±469.85	0.007
MPO (mU/ml)	111.70± 26.50	70.87± 30.66	<0.001

HB, hemoglobin. TLC, total leukocyte count. PT, prothrombin time. PC, prothrombin concentration. INR, international normalized ratio. ALT, Alanine aminotransferase. CRP, C-reactive protein. CD64, cluster of differentiation 64, TNC, total neutrophilic count. MPO, Myeloperoxidase. *p* > 0.05 is not significant (NS).

The ROC curve analysis was performed to determine the diagnostic ability of CD64 and MPO expression in prediction of pediatric sepsis in PICU admitted children (Figures 1 and 2). CD64 at a cutoff value of >1308 (pg./ml) had an area under the curve (AUC) of 0.792, sensitivity of 80%, specificity of 76.9%, with 95% Confidence interval of 0.653 to 0.895 (p<0.001). While MPO at a cutoff value of >85.7 mU/ml, had an area under the curve (AUC) of 0.855, sensitivity of 100%, and specificity 74.36, 95% Confidence interval of 0.725 to 0.939 (p<0.001). The correlations between CD64 and MPO levels and study variables are shown in Table 4.

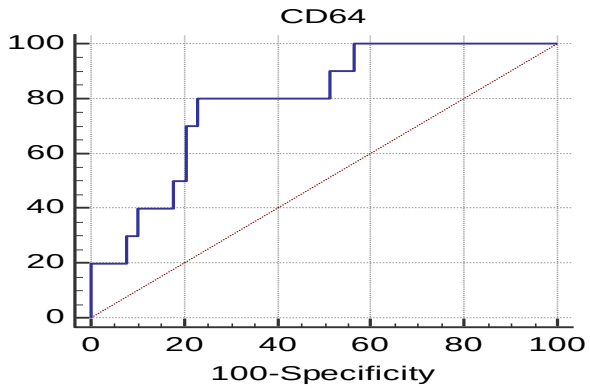

Figure 1. Receiver operating characteristic (ROC) curve for cluster of differentiation 64 (CD64).

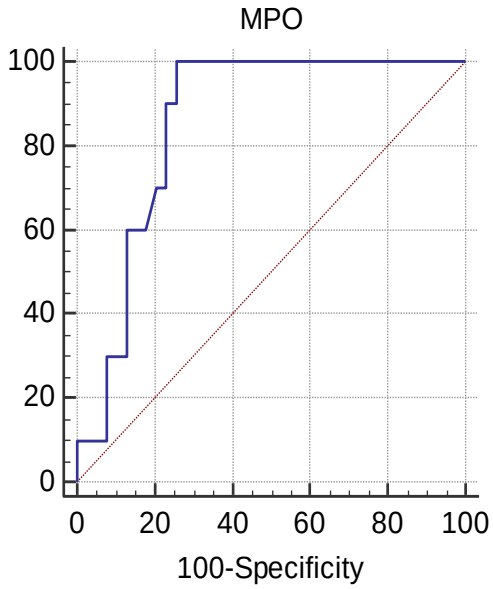

Figure 2. Receiver operating characteristic (ROC) curve for Myeloperoxidase.

Table 4. Correlations between CD64& MPO at admission and other variables.

	CD64 at admission		MPO at admission	
	r	*p* value	r	*p* value
CRP (mg/l) at admission	0.137	NS	0.205	NS
CRP (mg/l) follow-up	0.358	<0.001	0.657	<0.001
HB(g/dl)	-0.369	0.001	-0.369-**	0.001
Platelet(*10^3/μl)	-0.386	<0.001	-0.386-**	<0.001
INR	0.584	<0.001	0.584**	<0.001
S. albumin(g/dl)	-0.555	<0.001	-0.555-**	<0.001
ALT(u/l)	0.497	<0.001	0.497**	<0.001
Creatinine(mg/dl)	0.392	<0.001	0.392**	<0.001

CRP, C-reactive protein. MPO, myeloperoxidase. CD64, Cluster of differentiation. HB, hemoglobin. INR, international normalized ratio. ALT, Alanine aminotransferase. $p > 0.05$ is not significant (NS).

Discussion

The current study was performed to determine the role of CD64 and MPO as a biomarker in early diagnosis of sepsis in pediatric ICU patients and its prognostic value as a biomarker for mortality. The study included 48 infants with sepsis and another 30 normal children as a control group. The patient's group was followed up for manifestations of sepsis, SIRS and sever sepsis. Patients with severe sepsis were assessed by another sample to follow up the plasma level of CD64 and MPO.

In 2022, Gao et al.,[11] performed a retrospective analysis of 100 blood samples, showed for the first time the diagnostic role of neutrophil CD64 in sepsis. Over the last ten years, a number of prospective studies have demonstrated the clinical utility of CD64 in the sepsis diagnosis, Gao et al., 2022,[11] Yin et al., 2020[12] and Cong et al., 2021.[7] In a previous study, Cong et al., 2021,[7] reported that neutrophil CD64 differentiated systemic inflammatory response syndrome from severe sepsis and septic shock more accurately than PCT in respiratory ICU patients.

In our patients, CD64 could differentiate between SIRS without infection and sepsis. Additionally, we observed a significant difference in CD64 levels between patients with sepsis and patients with septic shock. This is in concurrence with findings of the study by of Cid et al., 2010,[13] Seree-Aphinan, 2020,[14] and Yin et al., 2020.[12] Their findings from CD64 studies in sepsis and septic shock patients showed a direct relationship between the severity of these pathological processes and the CD64 blood level. Also, our study findings agreed with those of a study by Gao et al., 2022,[11] who demonstrated a strong correlation between clinical groups and the existence of inflammatory and infectious processes. This also agreed with what was reported by Yin et al., 2020,[12] who demonstrated a greater correlation between neutrophils CD64 in sepsis and SIRS patients than in noninfectious SIRS patients.

In contrast, a previously published study by Gámez-Díaz et al., 2011,[15] revealed lower CD64 levels in patients with septic shock and there was no difference in CD64 levels between non-infectious SIRS and sepsis. Regional variations, patient ages, sample size, disease severity, and testing methodologies could be some of the differences between their study and our study.

In a study by Smyth et al., 2022,[16] showed that MPO was a marker of neutrophil activity measured in plasma and could be used as a marker of neutrophil accumulation in tissue, which agreed with our findings. According to findings of the study by Ulfig & Leichert 2021,[17] raised plasma MPO levels are indicative of human neutrophil degranulation and proliferation since MPO makes up 5% of human poly morphonuclear by weight and thought to be a key mechanism for oxygen-dependent microbicidal activity. We observed a significant difference in MPO levels between our study patients with sepsis and patients with septic

shock, and that MPO could distinguish between SIRS without infection and sepsis. Also, MPO blood level was clearly correlated with the severity of these pathological processes, as shown by the results of studies conducted on patients suffering from sepsis and septic shock. Such findings agreed with those of Morimont et al., 2022[18] and Bonaventura et al., 2020.[19]

The studies by Carr et al., 2020 and Schrijver et al., 2017,[20, 21] found that patients with septic shock had MPO levels that were statistically significantly higher than those in patients with sepsis. In contrast, a previously published study by Ulfig and Leichert 2021,[17] revealed lower MPO levels in patients with septic shock and there was no difference in MPO levels between non-infectious SIRS and sepsis.

The study by Yeh et al., 2019,[22] stated that the concentrations of CD64 and MPO were higher in patients with septic syndrome and had positive correlation with CRP. This also goes in agreement with that observed by Eichberger et al., 2022,[23] who reported that the production of macrophages, T-cells, or adipocytes causes a rise in CRP, suggesting that the CRP level should not be used as the only indicator of sepsis. It can rather be used as a component of a sepsis workup or as a follow-up during sepsis infection to gauge the response to antibiotics. This may also explaine the need for a biomarker with high sensitivity and specificity to lower the diagnostic uncertainty and effectively identify septic patients. The commonly used SIRS criteria lack enough specificity and sensitivity, as reported by Cantey & Lee 2021,[24] and Shannon et al., 2018.[3] A clinical diagnostic test with low specificity and high sensitivity will produce a high number of false positive results, leading to the needless use of antibiotics. Conversely, a clinical diagnostic test with a high specificity and low sensitivity (e.g. microbial culture) will result in incorrect diagnosis. Therefore, combining various biomarkers may help in the diagnosis of sepsis more accurately. A study by Bauer et al., 2016,[25] demonstrated that a model with CRP, PCT, neutrophil CD64, and MPO in addition to CRP performed better than CRP alone (AUC 0.90 vs. 0.86, p=0.03). Currently, the differential analysis and white blood cell count are nonspecific markers as reported by Sulochana and Viswanath 2017.[26]

Laboratory markers as CRP, TLC, MPO and others that are not elevated in inflammatory conditions only but also in non inflammatory conditions. Furthermore, CRP level is an acute-phase protein whose concentration rises in response to non-specific inflammatory processes. It is recognized as an objective diagnostic assay as reported by Cid et al., 2010.[13]

In our study, the overall mortality rate was 42.1% of patients with SIRS and 80.0% of patients with severe sepsis. This high mortality rate emphasizes the risk facing Egyptian ICUs and indicates the need for immediate control measures as indicated by Fouda et al., 2016.[27] In agreement with our study, the overall mortality in other pediatric intensive care units ranged between 10%-53.6 % as reported by Markwart et al., 2020,[28] Wösten-van Asperen 2019[29] and Tyagi et al., 2018.[30] Similar high mortality rates were previously reported in Egypt, Mahmoud et al., 2023[31] and other developing countries such as Tanzania 39%, Kayange et al., 2010[32] and Cameroon 34.7%, Chiabi et al., 2011.[33] In contrast, very low mortality rates were reported in the developed countries, Li et al., 2013,[34] which was explained by the high standards of living, medical care, and hospital services found in these countries, Shehab El-Din et al., 2015.[35]

In our study, there was a significant correlation between CD64 and MPO levels and HB, platelet, INR, ALT, and creatinine. In our study CD64 and MPO appeared to have a good diagnostic accuracy for sepsis detection since, ROC curve and AUC showed that the best cut off point for CD64 and MPO to prove sepsis in PICU admitted children was found to be >1308 (mu/ml) and, >85.7 (mu/ml), respectively with a sensitivity of 80% and 100%, specificity of 76.92% and 74.36% with 95% confidence interval 0.653 to 0.895 and 0.725 to 0.939, respectively. Such findings indicated the diagnostic accuracy of CD64 and MPO expression on neutrophils for the detection of patients with sepsis infection.

In our study, clinical presentation in patients with SIRS varied from tachypnea in 19 (100%), fever in 19 (100%), manifestations of chronic

liver failure in 13 (68.4%) and manifestations of acute kidney injury (AKI) in 7 (25.9%). Another Egyptian study by Shehab El-Din et al., 2015,[35] reported similar findings, they found that respiratory distress was the most common clinical feature. The study by Hoste et al., 2015[36] found that, for patients in the ICU, AKI was found in about 40% to 50% of patients with sepsis. In a retrospective study across China by Xu et al., 2015,[37] included 146,148 patients found AKI in 47.1% of sepsis cases.

In our research, positive blood culture was reported in 15 (78.9%) patients with SIRS compared to one (3.7%) patient of those without SIRS and in 9 (90.0%) of patients with severe sepsis compared to 7 (18.2%) of patients with sepsis. The study by Nejad et al., 2011,[38] reported that higher infection rates are typically observed by university/teaching hospitals, which typically serve as referral hospitals. Compared to developed countries, PICUs in developing countries tend to admit a higher number of critically ill children, who have medical conditions rather than surgical ones, and who are typically younger and from lower socioeconomic backgrounds as reported by Verlinden et al., 2022.[39] In our study *Klebsiella* species were the most common Gram-negative organism and even the most common isolated organism. Other studies conducted in Egypt also reported the predominance of *Klebsiella* among the Gram-negative pathogens responsible for the infection, as reported by Farag et al., 2020[40] and Fahmey 2013[41] and in other different countries, as reported by Kapoor et al., 2005,[42] Macharashvili et al., 2009,[43] Kohli-Kochhar et al., 2011,[44] Chiabi et al., 2011,[33] Leal et al., 2012[45] and Li et al., 2013.[35]

In our study, 68.4% of patients with SIRS and 100% of patients with severe sepsis needed adding of inotropes therapy in the strategy of treatment. This agreed with those of Scheeren et al., 2021[46] who documented the application of inotrope therapy to patients suffering from circulatory shock and sepsis in order to increase regional blood flow, improve tissue perfusion and maintaine the peripheral circulation.

In our study, the mean PT in non-SIRS group was 10.93±0.80 seconds and in SIRS group 13.05± 1.65 seconds (p<.001). The mean PC in non-SIRS group was 94.45± 4.19 and in SIRS group 70.16±18.32 (p<.001). The mean INR in non-SIRS group was 1.11±0.12 and in SIRS group 1.54±0.30 (p<.001). These results demonstrated the coagulopathy developed with SIRS. As reported by Esmon et al., 2011,[47] complex interactions regulate the relationship and balance between the fibrinolysis and coagulation systems. During sepsis, microbial products trigger the production and release of inflammatory mediators known as pathogen-associated molecular patterns. Damage-associated molecular patterns are pro-inflammatory substances, released from activated or damaged host cells in addition to these pathogen-associated molecular patterns as reported by Dwyer et al., 2021.[48] Additionally, the study by Yamakawa et al., 2016[49] documented a correlation between the use of anticoagulant medication and a drop in mortality in sepsis patients who were at high risk of death (SOFA score of 13–17).

In our study, the mean CRP on admission was significantly greater in the case group than in the control group (p<0.001). This agreed with the finding of a study by Fabbri et al., 2013[50] who found that CRP level was positive in all sepsis cases while Shehab El-Din et al., 2015,[35] found that in 326 of the 344 patients who were admitted with suspected sepsis, the CRP level was positive (>6mg/L) in 278 (85.3%) cases.

In our study, all patients showed higher levels of CD64 and MPO than the normal levels and in comparison, with the control group. These results are in concurrence with those reported by Cid et al., 2010,[13] Tran et al., 2020[51] and Musich et al., 2018,[52] they recorded higher levels of CD64 and MPO in cases than in controls. Consequently, we may conclude that plasma level of CD64 and MPO in peripheral blood can be considered an early sensitive marker for the detection and follow up of pediatric sepsis.

Author Contributions

HMA; made the statistical analysis, EMA, KSA; samples collection, DSM; supervision of whole work, NMKT; performed laboratory work, MH; examined patients, All authors participated in writing the paper.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethical Committee of the Faculty of Medicine, Beni-Suef University (approval no: FM BSU REC/07/06/2020).

Informed consent

A written consent from a parent of the included children was taken prior to including them in the research study.

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