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Research article

Individual and combined bioscore model of presepsin, procalcitonin, and high sensitive C - reactive protein as biomarkers for early diagnosis of paediatric sepsis

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ABSTRACT

Background: Paediatric sepsis remains a major public health problem with significant morbidity and mortality especially in developing countries. Clinical symptoms associated with sepsis are unreliable and laboratory parameters unspecific, making an early diagnosis of paediatric sepsis difficult. The lack of definitive biomarker(s) for early diagnosis of sepsis further leads to the misuse of antibiotics. Diagnosis based on a single biomarker does not provide adequate accuracy. Subsequently, combining multiple biomarkers into a single score will help clinicians make a better diagnostic judgment.

Aims: This study for the first time evaluated the individual and combined diagnostic accuracy of procalcitonin (PCT), presepsin (sCD14-ST) and high sensitive C-reactive protein (hs-CRP) using a Bioscore model.

Materials and methods: In a case control study conducted at the Paediatric Emergency Unit (PEU) and the Mother and Baby Unit (MBU) of Komfo Anokye Teaching Hospital (KATH), sixty (60) paediatric subjects aged zero to six (0–6) years, were diagnosed with sepsis using case-definition by the national neonatal bloodstream infection surveillance and Pediatric Sepsis Consensus Congress. Thirty (30) other paediatric subjects, aged and sex matched without sepsis or inflammatory conditions were used as controls. One-time blood sample was taken at the time of admission for blood culture and measurement of PCT, hs-CRP, and presepsin by ELISA. The Statistical Package for Social Sciences (SPSS release 20.0, Copyright ©SPSS Inc.) was used for analysis.

Results: Out of the sixty septic paediatric subjects, 14 patients (23.3%) had positive blood cultures (LCS) and 46 (76%) had negative for blood cultures (CS). *Klebsiella spp*. recorded the highest median levels of PCT, and hs-CRP while *Pseudo. Aeruginasa* recorded the highest of sCD14-ST levels. Significant elevations in PCT, sCD14-ST and hs-CRP levels were observed among septic cases in comparison to controls ($p < 0.0001$). Individually, PCT showed better accuracy (AUC = 78.7%) followed by hs-CRP (AUC = 78.4%) and sCD14-ST (AUC = 74.8%). Combination of PCT + hs-CRP had the highest accuracy (AUC = 80.1%) followed by hs-CRP + sCD14-ST (AUC = 77.2%), PCT + sCD14-ST + hs-CRP (AUC = 77.0%) and PCT + sCD14-ST (AUC = 75.9%).

Conclusion: hs-CRP, PCT, and sCD14-ST are independent predictors of paediatric sepsis due to their high prognostic values. Moreover, Bioscore combination of these biomarkers was significantly associated with increased odds for sepsis. The incorporation of these biomarkers into routine diagnostic tests will aid in prompt diagnosis of paediatric sepsis.

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1. Introduction

Paediatric sepsis remains a major healthcare problem affecting millions of children with high morbidity and mortality especially in Asia and Sub-Sahara Africa [1]. It is characterized by an aberrant host response coupled with multifaceted inflammatory pathophysiologic processes. This disparate of high incidence of sepsis observed in Asia and Sub-Sahara Africa are due to increased bacterial, parasitic and HIV infections [2]. Moreover, poor hygienic standards, widespread malnutrition and lack of resources further aggravate the situation and pose a major challenge of achieving the sustainable development goal (SDG) three; health and well-being [1, 2].

Many investigators have tried to diagnose sepsis among the paediatric population using clinical symptoms and laboratory markers. However, the clinical symptoms related to paediatric sepsis are ambiguous and laboratory parameters may be unspecific, thus making the timely diagnosis of sepsis difficult [3, 4, 5, 6]. The non-existence of definitive biomarker(s) for timely diagnosis of sepsis further leads to misappropriation of antibiotics. It is imperative to address this problem by finding a possible combination of biomarkers that will maximize the area under the receiver operating characteristic (ROC) curves to aid in the early diagnosis of sepsis [7].

Conventional blood cultures remained the gold standard test for the diagnosis of bacterial sepsis [8]. The isolation of the viable organism from blood cultures further helps in antimicrobial susceptibility testing and epidemiological surveys. However, obtaining adequate amounts of blood for culture from paediatric subjects is usually challenging. Besides, it takes more than 48 h for preliminary positive results to be obtained [9, 10]. There could also be possible contamination, especially by skin microorganisms. It is therefore imperative to identify other biomarkers that can help in early diagnosis.

Clinically, three inflammatory biomarkers have been applied in sepsis diagnosis: procalcitonin (PCT), C-reactive protein (CRP), and presepsin (sCD14) [11,12]. PCT is a prohormone of calcitonin and it is known to be elevated in bacterial sepsis and has shown mortality prediction value in a meta-analysis. However, PCT elevation may also occur in non-septic inflammatory conditions [13, 14, 15, 16, 17]. There are worries about the dependability of CRP alone as a marker for early diagnosis of paediatric sepsis [16, 17]. Presepsin is an effective adjunct biomarker for the diagnosis of sepsis but is inadequate to detect or rule out sepsis when used alone. Meta-analysis shows some mortality prediction value of presepsin in patients with sepsis [18, 19].

The measurements of these individual biomarkers have been studied but are often of marginal usefulness due to inconsistency in results [7]. Consequently, it has been established by medical investigators that diagnosis centered on a single biomarker might not offer adequate accuracy [7]. Subsequently, it is becoming common that various biomarker tests are done on each individual, and the corresponding measurements combined into a single score to support clinicians make improved diagnostic decision [20, 21, 22]. It is imperative therefore to harness the Bioscore models of these biomarkers to address the difficulty of finding the ideal mixture of biomarkers and subsequently to make the most of the area under ROC curves. This necessitates a possible combination of these biomarkers to aid in the early diagnosis of sepsis. It is against this background that for the first time we evaluated the individual and combined diagnostic accuracies of PCT, CRP, and presepsin using Bioscore model to predict early and accurate diagnosis of sepsis in Ghana.

2. Materials and methods

2.1. Study design and site

This case-control study was conducted at the Paediatric Emergency Unit (PEU) and the Mother and Baby Unit (MBU) of the Komfo Anokye Teaching Hospital (KATH). KATH is the second major tertiary hospital in Ghana located in the capital of the Ashanti Region. With a thousand bed capacity, it serves as the main referral center that offers health care services to the middle and southern sector of Ghana.

2.2. Ethical consideration

The study protocol was reviewed and ethical clearance granted by the Committee for Human Research, Publication and Ethics (CHRPE) of the Kwame Nkrumah University of Science & Technology (KNUST) and the Research and Development Unit of KATH (CHPRE/AP/011/15). Study participants or guardians were adequately informed of the procedures, nature, risk and the purpose of the study. Parents of qualified patients were made to fill or thumbprint a consent form with the help of the research team before their wards were recruited for the study.

2.3. Defining sepsis

Defining sepsis in paediatric subjects is challenging due to age specific vital signs, it therefore requires continuous reevaluation and modification [23]. Subsequently, the Pediatric Sepsis Consensus Congress (PSCC), definition of paediatric sepsis took into account age specific vital signs as well as age specific risk factors for invasive infections which in turn affect antibiotic coverage guidelines [23]. PSCC defined paediatric sepsis as;

1. Two or more systemic inflammatory response syndrome criteria.
2. Confirmed or suspected invasive infection.
3. Cardiovascular dysfunction, acute respiratory distress syndrome, or two or more organ dysfunctions (Supplementary and Tables 1 and 2).

2.4. Study population and recruitment

Sixty (60) paediatric subjects aged zero to six (0–6) years, diagnosed with sepsis by two independent paediatricians were recruited. The diagnosis of sepsis was done according to case-definition by the national neonatal bloodstream infection and PSCC. Out of the sixty septic paediatric subjects, 14 patients (23.3%) had positive blood culture isolate and were classified as laboratory confirmed sepsis (LCS) and 46 (76%) tested negative for blood cultures and were classified as clinical sepsis (CS). Thirty [30] other paediatric subjects, aged and sex matched without sepsis or inflammatory conditions were used as controls. CS was considered by the occurrence of symptoms of infection and as to whether antibiotics were initiated on admission day and sustained for more than 48 h in spite of negative culture, as well as acute onset of ventilatory support, increased apnea; hypotension; glucose intolerance; impaired peripheral perfusion; lethargy; temperature instability; ileus/onset of feed intolerance; increase in serum bilirubin; fall in urine output; metabolic acidosis/base deficit > -10 mmol/l; and anticonvulsant therapy. The Laboratory confirmed sepsis (LCS) group included those diagnosed with evidence of growth of a recognized pathogen in pure culture.

The Control group comprised those children who did not have sepsis, or any inflammatory conditions, laboratory or radiographic results attributable to sepsis, or present with clinical findings described by another disease condition. The control group were mostly children, age and sex matched diagnosed with malaria, not hospitalized and gave consent that extra blood sample should be taken for our tests. The controls recruited were retrospectively harmonized with the septic group, according to weight and age. Classification of subjects according to disease descriptions was made by two paediatricians and subjects were included in the septic group (LCS and CS) or Controls only when both clinicians agreed. Only the septic group (LCS and CS), and Controls were factored in the analysis. In all 60 septic and 30 controls were recruited into the study.

2.5. Sample and data collection

Structured questionnaires were administered to both septic and controls subjects to obtain socio-demographic data. Information about

clinical care and other conditions were inferred from folders and their hospital bio data. One [1] to three [3] ml of venous blood samples were obtained by venipuncture at the time of admission, about 2 ml was transferred into gel separator tubes and the rest of blood sample was used for blood culture. The clotted sample was centrifuged and the serum obtained stored at -80 °C till all samples were ready for analysis.

2.6. Procedure for blood culture

Before blood collection, the site of venipuncture and the top of the culture bottle (rubber cap) were cleaned thoroughly with 70% alcohol. A minimum of 1ml blood sample was obtained into the BD Ped culture broth bottles (BD 7 Loveton Circle Sparks Maryland USA). The culture bottles were incubated in a BACTEC blood culture system at 35 °C for 5 days. Culture bottles that flagged positively were removed from the BACTEC system and a sterile syringe and needle were used to aseptically draw approximately 1ml of the broth for sub-culturing on solid media. Two [2] drops of the aseptically aspirated broth were incubated onto three different culture media plates (Blood agar BA, Chocolate Agar, CA, and MacConkey Agar). MacConkey agar was incubated aerobically while the BA and CA plates were incubated in Candle jars. All plates were incubated for 18–24 h at 37 °C. The blood culture bottles that were indicative of negative or no bacteria growth were discarded after five [5] days.

2.7. Gram staining, identification of bacteria and antimicrobial testing

Gram stain was carried out on bacteria colonies isolated from the solid media using the standard method. Bacteria identification involved the use of bacteria morphology, as well as biochemical and physiological properties such as their appearance on Culture media. Biochemical tests such as the Coagulase test and Catalase test were done for Gram-positive isolates while other biochemical tests such as the production of H2S gas, Urease, Indole, and Citrate utilization tests were carried out on isolated bacteria of the Enterobacteriaceae family [24]. Antimicrobial sensitivity testing pattern was performed for each isolated organism using the Kirby Bauer disc diffusion method [25].

2.8. Haematological and biochemical assays

Haematological parameters were determined using an automated five-part differential haematology analyzer (Sysmex XT 2000i). Biochemical parameters were measured spectrophotometrically.

2.9. Measurement of PCT, hs-CRP, and sCD14 by ELISA

Biochemical reagents for presepsin, procalcitonin, and highly-sensitive C-reactive protein were purchased from Greenstone Swiss Technologies, China. These reagents were used to measure samples of both the septic and the uninfected subjects according to the manufacturer's instructions by sand-wish ELISA method. Subsequently, their respective absorbance was measured spectrophotometrically using Rayto RT-2000 (Rayto Life and Analytical Sciences Co., Ltd, China) microplate reader.

2.10. Statistical analysis

The data obtained were entered into Microsoft Excel software. The Statistical Package for Social Sciences (SPSS release 20.0, Copyright ©SPSS Inc.) was used for the analysis of the data. Continuous variables with normal distribution were expressed as mean ± standard deviation (SD) and the median and interquartile range (IQR) used for variables that were not normally distributed. The receiver operating characteristic (ROC) curve was used in evaluating the diagnostic efficiencies of PCT, hs-CRP, and sCD14-ST by determining the area under the curve and selection of independent predictors of sepsis was done using multiple logistic regression analysis. A combination of the biomarkers into a single Bioscore was done using cut off values determined from the ROC curves. Individual values were either scored as 0 or 1 depending on whether the value was above or below the cut off value determined by the ROC curves. The Bioscore ranged from 0 (where PCT, hs-CRP, and sCD14-ST all have values below the cut off) to 3 (where all three parameters have values greater than the cut off). The confidence interval of 95% was used and a *p-value* < 0.05 was considered to be statistically significant.

3. Results

Table 1 shows the comparison of parameters between septic and control group. Mean values of hs-CRP, sCD14, PCT and WBC were significantly higher in the sepsis group than the controls *(P < 0.05)* except mean Hb which was higher in the controls than the sepsis group.

Figure 1 shows the frequency distribution of cultured isolates in blood samples of study participants, the overall prevalence of the blood pathogens was 14 (23.3%) out the 60 sepsis group. The most common bacterial isolate was Coagulase-negative Staphylococcus (CNS) (28.7%), followed by Coagulase positive Staphylococcus (CPS) *(21.4%),* Klebsiella spp (21.4%). E. *coli* (14.3%), Methicillin-resistant Staphylococcus aureus (MRSA) (7.1%), and *Pseudo. Aeruginosa* (7.1%). Procalcitonin, and highly-sensitive C-reactive protein significantly correlated with Klebsiella spp. whilst Pseudo. Aeruginosa correlated significantly with presepsin levels (Figure 2).

Table 2 shows the comparison of laboratory and clinical parameters between patients with clinical sepsis (CS) and Laboratory confirmed sepsis (LCS). The levels of hs-CRP, sCD14 and PCT were higher in the LCS patients than those with CS except platelet count which was significantly higher in the patients with CS (165.0 (136.0–211.0)) compared to those with LCS (125.0 (79.75–144.0)) *(P = 005)*.

Table 1. Comparison of laboratory parameters between the septic and control group.

Variable	Sepsis group (n = 60)	Controls (n = 30)	P-value
Age (mean ± SD) (years)	2.30 ± 0.70	2.45 ± 0.40	0.199
Weight (kg) (median IQR)	3.65 (2.81–9.80)	3.40 (2.78–13.33)	0.981
Gender (Male/Female)	29/31	16/14	0.652
WBC ($\times 10^3$ uL) (median (IQR))	14.32 (11.16–18.34)	10.41 (9.76–12.66)	**<0.0001**
Hb (g/dL) (Mean ± SD)	12.4 ± 4.20	14.66 ± 1.88	**0.0062**
hs-CRP (μg/L)	22.18 (14.35–31.30)	13.08 (3.59–18.51)	**<0.0001**
sCD14 (μg/L)	25.46 (19.20–66.23)	18.09 (13.82–20.98)	**<0.0001**
PCT (ng/L)	632.8 (465.70–1468.0)	434.20 (345.0–523.3)	**<0.0001**
Fever (>38 °C)	23/37	27/3	**0.007**
Leukocyte counts (<5×10^3/uL)	59/60	0/30	0.999
Platelets (<100×10^3/uL)	50/60	0/30	**0.027**

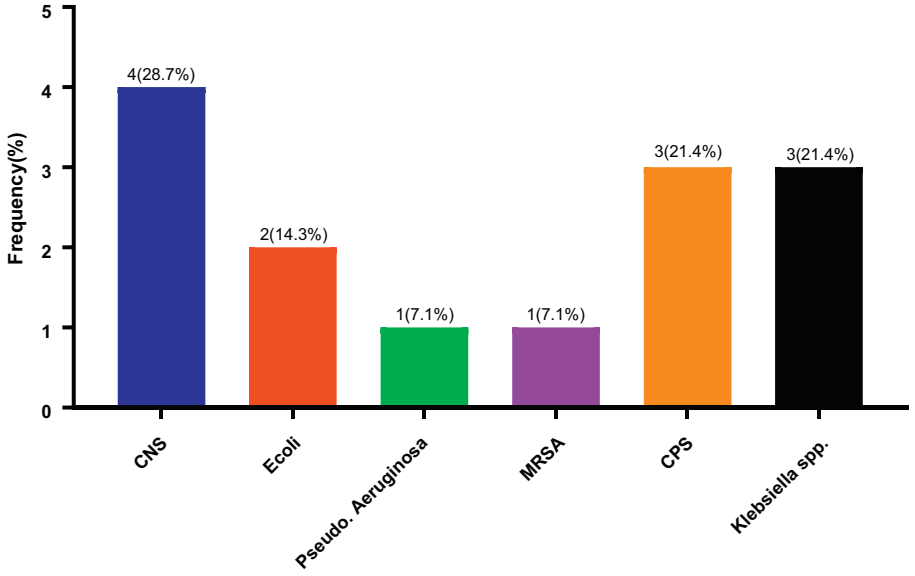

Figure 1. Frequency distribution of bacterial isolates among study participants with Laboratory confirmed sepsis (LCS).

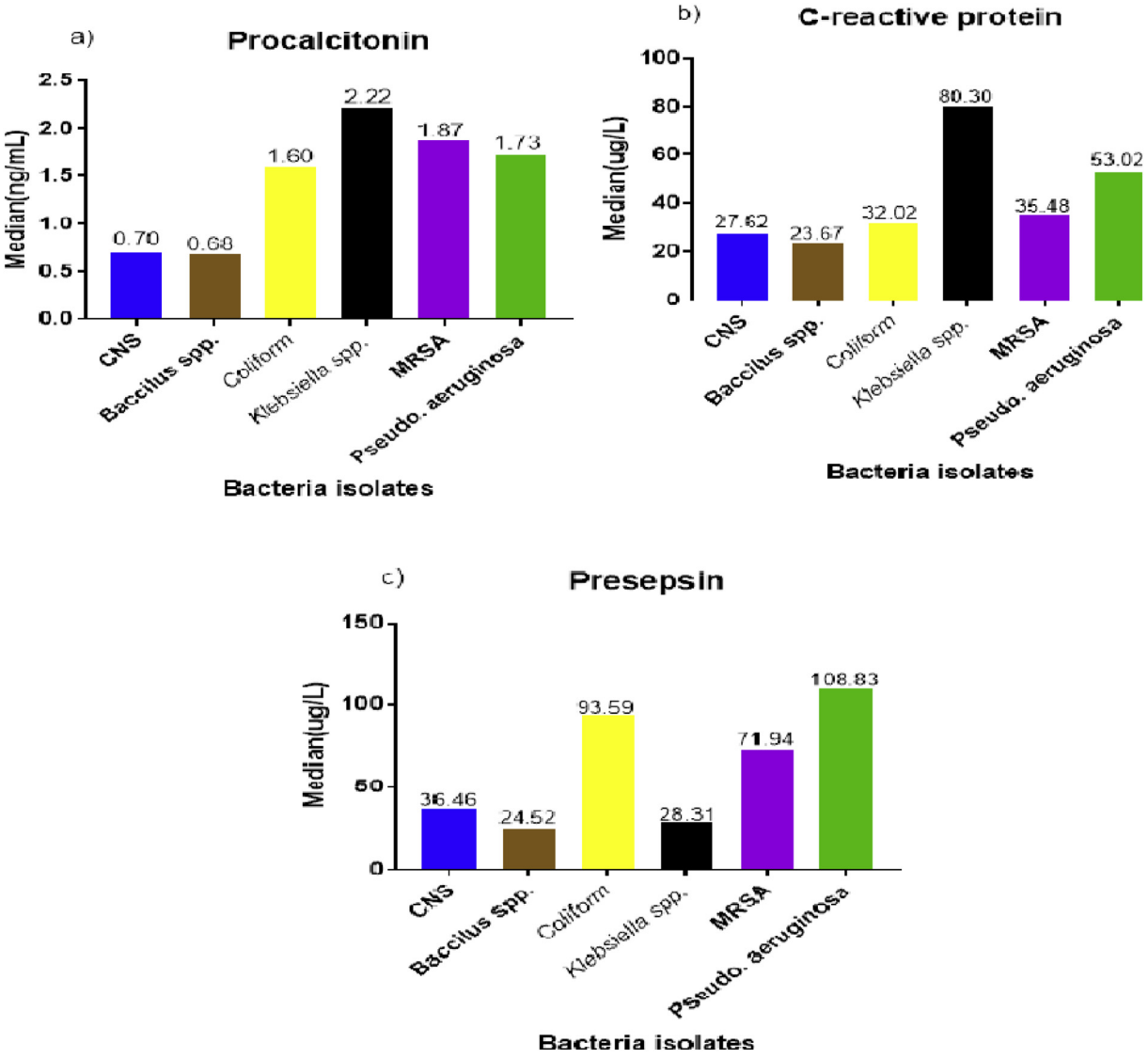

Figure 2. Comparing types of bacteria isolated from patients with the median levels of; a) procalcitonin, and b) highly-sensitive C-reactive protein c) pre-sepsin. Procalcitonin, and highly-sensitive C-reactive protein significantly correlated with *Klebsiella spp*. whilst *Pseudo. Aeruginosa* correlated significantly with presepsin levels.

Table 2. Comparison of laboratory parameters in patients with LCS and CS.

Variables	LCS (n = 14)	CS (n = 46)	P-value
Age (mean ± SD) (years)	2.20 ± 0.90	2.40 ± 0.54	0.309
Weight (kg) (median (IQR))	4.50 (2.76–16.18)	3.50 (2.77–9.10)	0.342
Gender (Male/Female)	6/8	24/22	0.761
WBC ($\times 10^3/\mu L$) (median (IQR))	12.88 (9.66–144.00)	15.44 (11.30–20.01)	0.104
Hb (g/dL) (Mean ± SD)	13.88 ± 3.80	11.99 ± 4.29	0.147
hs-CRP (μg/L)	35.29 (22.80–81.10)	20.70 (12.30–25.52)	**0.001**
sCD14 (μg/L)	69.54 (24.00–132.7)	22.76 (18.46–28.27)	**0.004**
PCT (ng/L)	1653 (655.1–2033)	595.1 (459.2–930.3)	**0.001**
Platelets ($\times 10^3/\mu L$)	125.0 (79.75–144.0)	165.0 (136.0–211.0)	**0.005**
Temperature (°C)	37.38 ± 0.962	37.40 ± 1.164	0.272
Fever (>38 °C)	6/8	16/30	0.524
Heart Rate (>160 beats/mins)	3/11	3/43	0.139
Respiratory Rate (>60/mins)	4/10	8/38	0.453
Capillary Refill (<3/<2 s)	12/2	21/25	**0.013**
Feeding Difficulties (Yes/No)	4/10	22/24	0.227
Leukocytes counts ($<5\times 10^3$/uL)	1/13	0/46	0.237
Platelets ($<100\times 10^3$/uL)	5/9	5/41	**0.047**
Blood glucose (<2.5 mmol/L)	0/14	3/43	0.999
RR (cpm)	49.0 (42.0–66.50)	46.0 (38.0–56.0)	0.254

LCS-Laboratory confirmed sepsis; CS-Clinically confirmed sepsis; P-value<0.05 = statistically significant, Hb = Hemoglobin, WBC = White blood cell count, hs-CRP = High-sensitive C-reactive protein, sCD14-ST = presepsin, PCT = Procalicitonin, RR = Respiratory rate. P-value <0.05 was considered statistically significant (P-value of significant variable are in bold print).

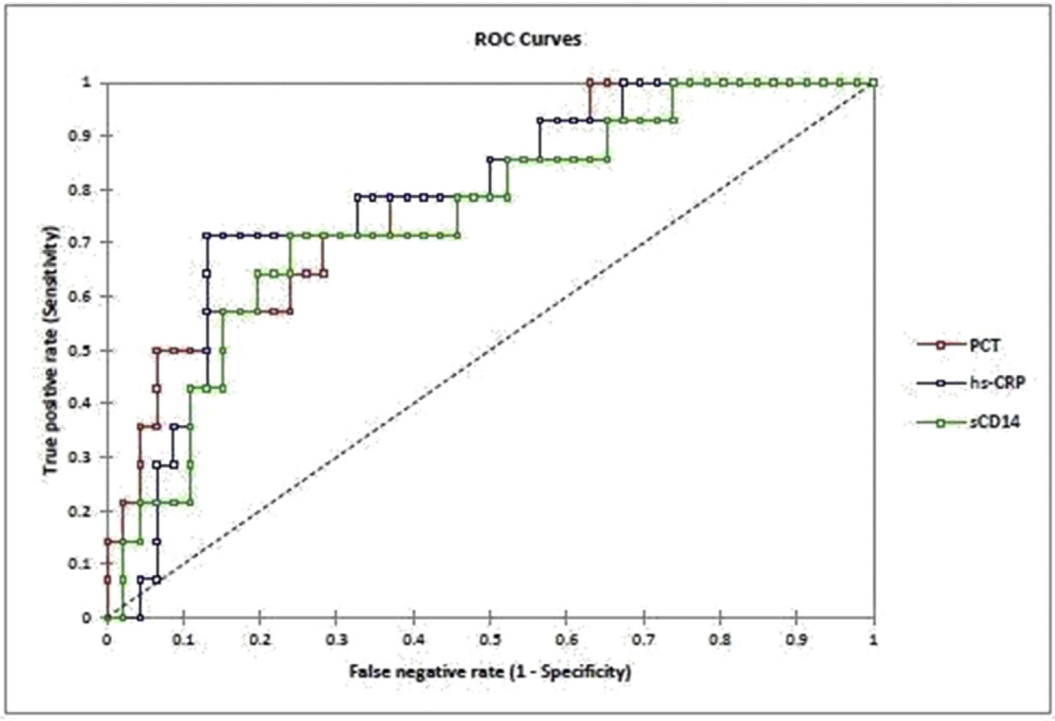

Figure 3. The receiver operating characteristics (ROC) curves of biomarkers using blood culture as the gold standard.

Table 3. Diagnostic Performance of biomarkers in the diagnosis of sepsis.

Biomarker	Cut off	Sensitivity (95% CI)	Specificity (95% CI)	NPV (%)	PPV (%)	TP	TN	FP	FN	J_{max}
Procalcitonin	1.57 ng/ml	57.14 (32.60–8.51)	86.96 (73.84–94.16)	57.14	86.96	8	40	6	6	0.44
hs-C-Reactive Protein	26.92 μg/L	71.43 (44.89–88.44)	86.96 (73.84–94.16)	62.5	90.91	10	40	6	4	0.58
sCD14	28.05 μg/L,m	71.43 (44.89–88.44)	76.09 (61.84–86.16)	47.62	89.74	10	35	11	4	0.48

P-value<0.05 = statistically significant, sCD14-ST = presepsin, Jmax = Youden index, CI = Confidence Interval, NPV = Negative Predicted Value, PPV = Positive Predicted Value, TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative.

Table 4. The diagnostic performance of Bioscore models using multiple logistic regression analysis in the diagnosis of sepsis.

Bioscore	Sensitivity (95% CI)	Specificity (95% CI)	NPV (%)	PPV (%)	TP	TN	FP	FN
CRP + PCT								
0	71.43 (44.89–88.44)	86.96 (73.84–94.16)	90.91	62.5	10	40	6	4
1	57.14 (32.60–78.51)	86.96 (73.84–94.16)	86.96	57.14	8	40	6	6
2	0.0 (0.0–25.63)	100 (90.57–100.0)	76.37			46		14
CRP + sCD14								
0	71.43 (44.89–88.44)	78.26 (64.16–87.84)	90	50	10	36	10	4
1	64.29 (38.59–83.63)	86.96 (73.84–94.16)	88.89	60	9	40	6	5
2	0.0 (0.0–25.63)	100 (90.57–100.0)	76.67			46		14
PCT + sCD14								
0	83.33 (41.60–98.40)	70.37 (57.06–80.87)	97.44	23.81	5	3	16	1
1	83.33 (41.60–98.40)	77.78 (64.85–86.87)	91.3	14.29	2	42	12	4
2	0.0 (0.0–44.79)	100 (92.86–100)	90			54		6
CRP + sCD14+PCT								
0	71.43 (44.89–88.44)	76.09 (61.84–86.16)	89.4	47.62	10	35	11	4
1	71.43 (44.89–88.44)	86.96 (73.84–94.16)	90.91	62.5	10	40	6	4
2	57.14 (32.60–78.51)	86.96 (73.84–94.16)	86.96	57.14	8	40	6	6
3	0.0 (0.0–25.63)	100 (90.57–100)	76.67				46	14

P-value<0.05 = statistically significant, sCD14-ST = presepsin, CRP = C-reactive Protein, PCT = Procalcltonin, CI = Confidence Interval, NPV = Negative Predicted Value, PPV = Positive Predicted Value, TP = True Positive, TN = True Negative, FP = False Positive, FN = False Negative.

Table 5. The diagnostic performance of Bioscore models using multiple logistic regression analysis in the diagnosis of sepsis.

Bioscore	Coefficient	Standard Error	OR (95% CI)	P-value
Model 1 (CRP + PCT)				
0	1 (referent)	1 (referent)	1 (referent)	1 (referent)
1	10.81	943.3	4.0e7 (5.32–6.0e13)	**0.003**
2	-4.11	471.67	13.33 (3.24–64.99)	**0.003**
Model 2 (CRP + sCD14)				
0	1 (referent)	1 (referent)	1 (referent)	1 (referent)
1	-0.33	0.79	2.25 (0.102–20.96)	0.535
2	1.47	7.32	13.50 (3.35–65.13)	**0.002**
Model 3 (PCT + sCD14)				
0	1 (referent)	1 (referent)	1 (referent)	1 (referent)
2	1.22	0.49	11.67 (2.82–57.08)	**0.006**
Bioscore (PCT + CRP + sCD14)				
0	1 (referent)	1 (referent)	1 (referent)	1 (referent)
1	0.588	1.216	1.80 (0.17–19.50)	0.629
2	2.89	1.333	18.0 (1.32–245.59)	**0.030**
3	2.757	0.819	15.75 (3.16–78.41)	**0.001**

P-value<0.05 = statistically significant, hs-CRP = High-sensitive C-reactive protein, sCD14-ST = presepsin, PCT = Procalicitonin. P-value <0.05 was considered statistically significant (P-value of significant variable are in bold print).

Figure 3 shows the receiver operating characteristics (ROC) curves for the diagnostic performance of biomarkers of sepsis (LCS). Using blood culture as the gold standard, the area under the curve (AUC) was 0.79, 0.78 and 0.75 for PCT, hs-CRP, and sCD14-ST respectively. On an individual biomarker basis, PCT proved superior to hs-CRP and sCD14.

Table 3 denotes the diagnostic performance of PCT, hs-CRP, and sCD14. Procalcitonin and c-reactive proteins exhibited similar better specificity 86.96 (73.84–94.16) than prespesin 76.09 (61.84–86.16), however, presepsin showed better sensitivity, this further necessitates the combination of these biomarkers to complement each to enhance accurate diagnosis.

Tables 4 and 5 shows the diagnostic performance of Bioscore models using multiple logistic regression analysis in the diagnosis of sepsis. With Model 1 (CRP + PCT), a score of either 1 or 2 is statistically significant in the diagnosis of sepsis *(P < 0.05)*. The odds of diagnosing sepsis are very high with a score of 2 (13.33 (3.24–64.99)) compared to a score of 1 (4.0e7 (5.32–6.0e13)). In Model 2 CRP + sCD14, a score of 2 has the highest odds (13.50 (3.35–65.13)) in the diagnosis of sepsis and its statistically significant *(P= 0002)*. In Model 3 PCT + sCD14, a score of 2 is highly significant (p = 0.0006) and with increased odds (11.67 (2.82–57.08)) in the diagnosis of sepsis compared to a score of 1. A Bioscore of 2 and 3 were statistically significant *(P < 0.05)* in predicting blood culture-proven sepsis with a score of 2 having the highest odds (18.0 (1.32–245.59)). In all, the odds of predicting sepsis increased proportionally from a score of 1–3 hence the Bioscore was highly effective in predicting microbiologically proven sepsis.

4. Discussion

This study evaluated the individual and combined diagnostic accuracies of PCT, hs-CRP and Presepsin using Bioscore model for early and accurate diagnosis of sepsis. The findings indicate that individually, PCT, hs-CRP, and sCD14 were significantly increased in septic subjects as compared to the controls. The combination of biomarkers to predict the outcome of the disease has been used in various disorders such as liver fibrosis, breast cancer, and cardiovascular diseases [26]. In the current study, a combination of PCT, hs-CRP, and sCD14 into a Bioscore seemed to be a more efficient way of differentiating between paediatric patients with or without blood culture proven sepsis beside the performance of each biomarker.

A single Bioscore was constructed for the three biomarkers to ascertain if the diagnostic performance of the individual marker would improve the diagnosis. The current study showed blood culture positivity rate of 23.3%. The most common bacterial isolates were Coagulase-negative Staphylococcus (CNS) (28.7%), followed by Coagulase positive Staphylococcus (CPS) *(21.4%),* Klebsiella spp. (21.4%). E. *coli* (14.3%), Methicillin-resistant Staphylococcus aureus (MRSA) (7.1%), and *Pseudo. Aeruginosa* (7.1%). Moreover, we observed that Procalcitonin, and highly-sensitive C-reactive protein significantly correlated with Klebsiella spp. whilst Pseudo. Aeruginosa correlated significantly with presepsin levels (Figure 2). The 23.3%. blood culture positivity rate observed in the current study is similar to a study conducted by Acquah et al., [25] on pediatric sepsis, who reported a 25.9% blood culture positivity rate. Other studies in Port Harcourt and Calabar both in Nigeria reported a relatively higher blood culture positivity rate of 34.2% and 48.9% respectively [27, 28, 29, 30]. Blood culture for the diagnosis of paediatric sepsis remains the gold standard although in many cases, the results for the culture remains negative even in the case of strong clinical evidence of septicemia, this low sensitivity can be attributed to lower concentration of bacteria as a result of inadequate quantity of blood sample that is taken for paediatric blood cultures compared to adults. Moreover, the initiation of empirical antibiotics on suspicion of sepsis further reduces the chances of isolating a viable organism.

Furthermore, this study observed satisfactorily discriminating power (AUC = 78.7%) with a specificity of 86.96% and sensitivity of 57.14% for procalcitonin using blood culture as the gold standard. In a prospective study by Koksal, [13] among the Turkish population, a sensitivity of 48%, a specificity of 100% and an AUC of 77.0% were reported among neonates with sepsis before the administration of antibiotics. Again, using a cut off value of 0.5 ng/mL, [14] Adib, in their study showed that PCT best-predicted sepsis with 72.6% sensitivity and 65.5% specificity [15]. The variations observed between these studies may be due to the differences in the cut-off values used. Our study used a narrow cut off 284 value of 1.57 ng/ml, Koksal and colleagues used 2 ng/ml whilst Adib used a narrower cut off value of 0.5 ng/Ml [15].

The hs-CRP results were significantly elevated in the septic group than the control group *($P < 0.0001$)*. Moreover, hs-CRP levels were significantly higher in patients with LCS than CS *($P < 0.0013$)*. These reports corroborate well with findings from other cross-sectional studies conducted among paediatric with sepsis in Turkey, and Iran respectively [31, 32, 33]. In this study, hs-CRP recorded a specificity of 86.96%, the sensitivity of 71.43% (Table 3) and AUC of 78.4% (Figure 1). Few previous studies have reported a diagnostic pattern for hs-CRP among children with sepsis. A study conducted by Mussap et al [11] observed a lower sensitivity (48%), and AUC (64.0%) but a similar specificity of 87% for CRP. The high AUC reported in this study buttresses the clinical importance of using hs-CRP assay kits in diagnosing paediatric sepsis rather than the conventional CRP kits.

This study found a significantly higher level of presepsin (sCD14-ST) among the participants with sepsis than the controls *($P < 0.0001$)* and also in patients with LCS than those with CS. These findings are consistent with a similar study by Shozushima et al, [19] who evaluated serum presepsin levels and found significantly increased levels in septic patients in comparison with patients with systemic inflammatory response syndrome and healthy controls. For the first time, this study reported a sensitivity of 71.43%, a specificity of 76.09% and an AUC of 74.8% for presepsin in diagnosing paediatric sepsis in Ghana.

The combination of many biomarkers to improve diagnosis or predict the outcome of the disease has been used in various disorders such as liver fibrosis, breast cancer, and cardiovascular diseases [27, 28, 29]. A combination of these biomarkers into a Bioscore seemed to be a more efficient way of differentiating between paediatric patients with LCS and CS besides the performance of each biomarker. With at least two of the three markers above their respective threshold (Bioscore 2 or 3), a greater proportion (>75%) of the cases were shown to have sepsis. The "Bioscore," turned out to be associated with improved diagnostic accuracy in the diagnosis of sepsis in paediatric populace. Even though a small improvement in the diagnostic accuracy of sepsis by the combination of two or three biomarkers (PCT, hs-CRP, and presepsin) were observed against the increased cost of using multiple biomarkers, it overall economic advantages outweigh the cost of misdiagnosis, antibiotic misuse and even death, consequently, the usage of biomarker or combination of biomarkers is imperative.

The study is limited by the relatively small sample size, inability to take samples at various time points to ascertain their level of increment as well as inability to validate the Bioscore in different cohort. Thus, a larger sample size will be considered in further studies, though inferential analysis can be deduced from the current study.

5. Conclusion

The hs-CRP, PCT, and sCD14-ST are independent indicators of paediatric sepsis due to their high prognostic values. The incorporation of the combination of these three biomarkers into routine diagnostic tests for paediatric sepsis will aid in the early diagnosis of paediatric sepsis. Moreover, this study has provided robust thresholds for all three biomarkers as a basis for future research.

Declarations

Author contribution statement

S. Sakyi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Enimil: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

D. Adu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Ephraim and K. Danquah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

L. Fondjo and E. Toboh: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

D. Baidoe-Ansah: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

P. Adoba and B. Afranie: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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