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Research Article

Biomarkers in Diagnosing late onset Neonatal Sepsis

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Abstract

Background

Late onset bacterial sepsis of neonates (LOS) is a highly mortal disease, although it is still a diagnostic challenge. Unfortunately a gold standard diagnostic test has yet to be described. In the current study it was aimed to investigate the diagnostic value of sTREM-1, PTX-3 and Pro-ADM in LOS and compare with currently used biomarkers.

Methods

In this multi-centric prospective study, patients were recruited from different NICUs among Turkey. Neonates, hospitalized with a suspicion of LOS were included. Patients were divided into three groups as; proven sepsis, clinical sepsis and control group regarding clinical and laboratory findings. The primary outcome was to evaluate any difference between groups regarding the diagnostic value of the biomarkers.

Results

The study consisted of 192 patients; proven sepsis (n=86), clinical sepsis (n=60) and control (n=46) groups. Mean CRP (P<0.001), mean WBC (P=0.015) and mean procalcitonin (PCT) (P=0.004) levels were significantly higher and mean platelet level was significantly lower (P<0.001) in clinical sepsis and proven sepsis groups. No statistical difference was present between groups regarding mean PTX-3 (P = 0.189), Pro-ADM (P = 0.536) and sTREM-1 (P = 0.091) levels.

Conclusion

Serum PTX-3, Pro-ADM and sTREM-1 levels did not indicate LOS diagnosis. Regarding previous studies

10

their relation to correlation with disease progression may make more sense. High CRP and PCT and low platelet levels were the most significant biomarkers for detecting LOS in our cohort.

Keywords
Biomarker, neonatal late onset sepsis, pentraxin-3, pro-ADM, sTREM-1

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

Introduction
Neonatal sepsis is still one of the leading causes of neonatal mortality. Late onset sepsis (LOS), is basically defined as sepsis occurring after 72 hours to 6 days of life. As it is a major health issue, frequently observed in premature neonates, up to 20 % very low birth weight hospitalized newborns experience at least one episode of culture positive sepsis [1]. In addition to high mortality rate, morbidities such as neuro-developmental disorders, broncho-pulmonary dysplasia and prolonged hospital stay may manifest as well [2, 3]. Because of non-specific and subtle symptoms, clinical diagnosis of this highly mortal disease is quite difficult [4]. Blood culture is the gold standard diagnostic test for the neonatal sepsis, although has some limitations such as the length of the time that the results released and false negative results. That is why many laboratory parameters have been proposed in addition to clinical findings in terms of supporting diagnosis. However, none of these tests have been proved to be the gold standard [5, 6].

Sepsis has complex patho-physiological steps. Therefore focusing on molecules related to these steps will be effective in finding novel biomarkers in terms of prompt diagnosis and decision making in disease management.
Human triggering receptor expressed on myeloid cells-1 (TREM-1) is a glycoprotein. As a member of the immunoglobulin family it mediates acute immune response and highly expressed in neutrophils and monocytes/macrophages in case of infections and inflammatory conditions [7, 8]. The soluble form of TREM-1 (sTREM-1) has been detected in higher amounts in the bloodstream of septic patients. Although some studies have been performed onthe diagnostic value of sTREM-1 and stated its beneficence in diagnosing sepsis, some reports the vice versa [9, 10].
Pentraxin 3 (PTX-3) is a member of pentraxin super family and is produced as a cytokine induced protein by fibroblasts and vascular endothelial cells in response to inflammatory stimulus [11]. In previous studies, PTX-3 was suggested as an indicator of tissue inflammation and damage [12]. Although there is scant information about PTX-3 in neonatal patients, recent studies, proposing its use in the diagnosis of neonatal sepsis have been reported [13, 14].

Pro Adrenomedullin (Pro-ADM) was found to have different predictive and prognostic values in infectious and ischemic conditions. Recent studies noted importance of Pro-ADM on the definition of prognosis and stratification of pediatric and adult sepsis patients [15, 16]. There is scant information in the literature, except for one study stating the pro-ADM to be a prognostic factor in neonatal sepsis [17].

In the current study it was aimed to determine the diagnostic importance of these molecules and compare their relation to biomarkers used in the diagnosis of sepsis in daily routine in LOS.

Material And Methods
Study design
In this prospective study, neonates hospitalized in level 3 NICUs among Turkey during a two-year period (2014-2015) were recruited. The study was approved by the Ethical Committee of Gulhane Military School of Medicine and Teaching Hospital and written informed consent was obtained from the legal guardian of the newborn.

Patients and Data Collection
Both preterm and term newborns hospitalized in NICUs with the suspicion of LOS were enrolled. The defined inclusion criteria were: being at the postnatal age of ≥ 72 hours and presenting with non-specific findings of sepsis such as apnea, needing supplemental oxygen or mechanical ventilation, temperature instability, hypotension, bradycardia or tachicardia, feeding intolerance, abdominal distension, and necrotizing enterocolitis. Patients with early onset sepsis, congenital abnormalities and inborn error of metabolism were excluded from the study.
A web based patient clinical form was used for data collection. Clinical and demographic findings were recorded and a serum sample was obtained from each patient. A physician from each attending center was responsible for the enrollment end registration of the patient to the system. Patients were followed up for clinical manifestations, laboratory findings and positive blood culture and were classified into three different groups as either, proven sepsis group, clinical sepsis group and control group.

Diagnosis of infection

The classification of the patients was made according to the criteria reported by Goldstein et al [18]. Patients with the clinical manifestations of sepsis in addition to positive laboratory tests without blood culture positivity were classified in the group of clinical sepsis. Proven sepsis was defined as clinical sepsis plus any positive blood culture. Patients accepted to NICU with a clinical suspicion of sepsis but without any further clinical and laboratory findings were accepted as the control group. Antibiotic treatment was initiated to all enrolled newborns according to standard protocols.

After skin disinfection, blood cultures were obtained from neonates on admission via peripheral vein or central catheter when first placed. At least 1 ml of blood sample was withdrawn.

Laboratory analysis

Blood samples were withdrawn from a peripheral vein of the newborns at the admission before the initiation of any treatment. After being isolated, serum samples were kept in -80 oC in each center until they were collected for biochemical analysis. Each patient was given a number which was written on the tube and the text box in the web registry system thus, any ambiguities about sample collection were overcome.

The mean values of white blood cell count (WBC), hemoglobin, platelet, C-reactive protein (CRP), procalcitonin (PCT) and Interleukin-6 levels (IL-6) and immature and total neutrophil ratios (manual cell count) were compared between groups. The levels of the molecules, mean PTX-3, Pro-ADM and sTREM-1 were measured and compared between groups.

Statistical analysis

SPSS for Windows 15.0 (SPSS Inc., Chicago, IL, USA) wasused for statistical analysis. Descriptive statistics were presented as mean, median [inter-quartilerange (IQR)], standard deviation, frequency, and percent. The distribution characteristics of continuous variables were evaluated with Kolmogorov Smirnov test. As a parametric test Student t test was used and as a nonparametric test Mann-Whitney U test was used. Pearson Chi-square test or Fisher's exact test were used to compare categorical variables as appropriate. Spearman correlation test was used to determine the linear association between variables. Receiver operating characteristic (ROC) curve analysis was used to determine cut-off points for the markers. Then sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were calculated with respect to determined cut-off values. Univariate logistic regression analysis was used to calculate odds ratio (OR) values. P value < 0.05 was accepted as statistically significant.

Results

A total of 203 patients were recruited. Among the cohort; 7 patients were excluded because of a birth history of chorioamnionitis; 2 patients were excluded due to inadequate serum and 2 were excluded because of inappropriate blood sampling (hemolysis) during the follow up. Remaining 192 patients were included in the study and were classified into three main groups as either proven sepsis (n=86), clinical sepsis (n=46) and control group (n=40) (Figure-1). The groups were similar regarding number of patients (P = 0.596). In all groups the ages of inclusion were similar (P=0.934).
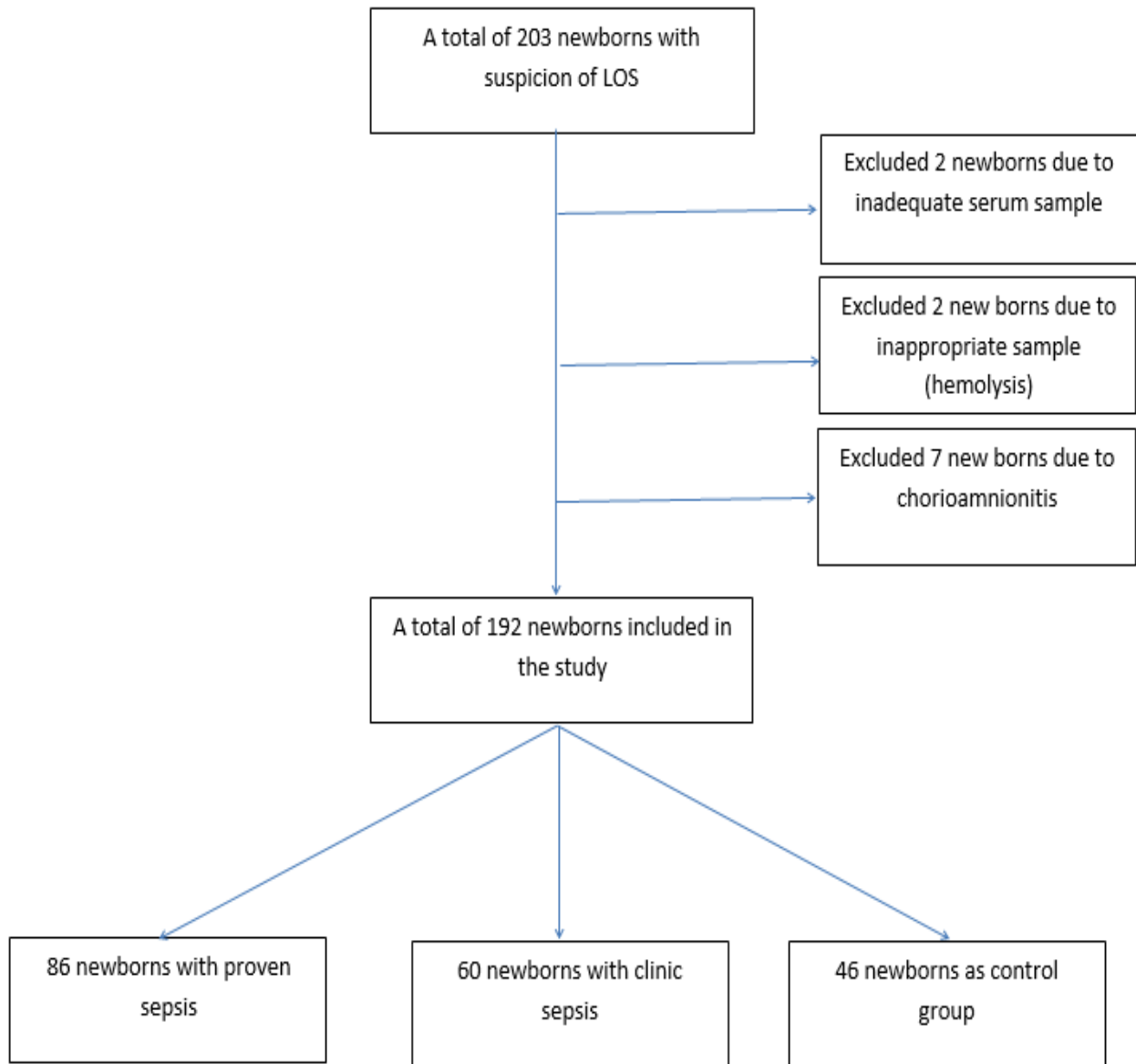
Figure 1 Flow-chart of thestudy

The ratio of cesarean delivery was similar in all groups (P=0.234). Mean gestational age of the neonates was the lowest in proven sepsis group (P=0.026). The groups were also significantly different according to birth weights of the patients (P=0.044). Proven sepsis group had the lowest birth weight while control group had the highest. Groups were similar according to mean maternal age, premature ruptures of membranes ratio, maternal urinary tract infection and fever during pregnancy. However, antenatal antibiotic application (P=0.001) was significantly higher in control group as compared to other groups. The results are presented in Table-1.

	Control group (n=46)	Proven sepsis group (n=86)	Clinical sepsis group (n=60)	P value
Female/male ratio	22/24	49/37	33/27	0.596
Cesarean section (%)	84,8	80,2	71,7	0,234
Mean gestational age (wk) (mean±sd)	34,04 ± 4,29	32,54 ± 4,99	34,51 ± 3,98	0,026
Mean gestational weight (g) (mean±sd)	2449,45 ± 992,48	1815,06 ± 964,60	2263,58 ± 999,53	0,001
Mean hour of inclusion (mean mean±sd)	322,80 ± 296,82	322,87 ± 248,76	338,81 ± 300,54	0,934
Mean maternal age (years ±sd)	29,60±6,78	29,63 ± 7,01	28,63 ± 5,97	0,635
Premature rupture of membranes (%)	4,3	7,0	8,3	0,487
Maternal UTI (%)	26,1	20,9	8,3	0,108
Antenatal maternal fever (%)	0,0	2,3	3,3	0,481
Antenatal maternal anibiotics (%)	19,6	10,5	1,7	0,001

Table 1 Demographic and clinical findings of the patients

The ratio of blood culture positive sepsis was 44.7 % and the urinary culture positivity was 3.6 % among the study population. The most common pathogen isolated from blood was Coagulase-negative staphylococcus (CoNS) (43.0 %) followed by Klebsiella spp (23.2 %), Staphylococci other than CoNS (12.7), Group B Streptococcus (8.1 %), Escherichia Coli (3.5 %), and others (e.g. Acinetobacter, Stenotrophomonas, Enterobacter, Enterococcus, Serratia, Pseudomonas, Candida) (12.7 %). The most common pathogen isolated from urine was Klebsiella spp (5.8 %) followed by, Escherichia Coli (2.3 %), Enterobacter (2.3 %).

Mean hemoglobin (P=0.263) level, I/T ratios (P=0.646) and IL-6 (P=0.149) levels were similar in all groups. Mean WBC was different between groups and was highest in proven sepsis group (P=0.015) and mean platelet level was lowest in proven sepsis group when compared to other groups (P=0.001). Both the mean CRP (P=0.001) and mean PCT (P=0.004) levels were significantly different between groups and were both higher in clinical sepsis and proven sepsis groups compared to control group (P=0.001) and also higher in clinical sepsis group as compared to proven sepsis group (Table-2).
Mean PTX-3 (P = 0.189), Pro-ADM (P = 0.536) and sTREM-1 (P = 0.091) levels were comparable between the three groups (Table-3).

	Control group (n=46)	Proven sepsis group (n=86)	Clinical sepsis group (n=60)	P value
WBC (/mcL) (mean ± sd)	5,04 ± 10,11	9,86 ± 9,93	7,02 ± 7,72	0,015
Hemoglobin (g/dL) (mean ± sd)	13,78 ± 3,10	12,93 ± 3,05	12,91 ± 3,16	0,263
Platellet (/mcL) (mean ± sd)	336,03 ± 167,98	145,82 ± 130,52	180,71 ± 163,39	0,000
I/T ratio (%)	0,33 ± 0,19	1,35 ± 5,69	0,29 ± 0,19	0,646
CRP (mg/dL) (mean ± sd)	1,49 ± 1,27	37,37 ± 51,32	45,97 ± 41,27	0,000
Procalcitonin (ng/dL) (mean ± sd)	0,94 ± 2,98	7,83 ± 14,76	10,37 ± 18,72	0,004
IL-6 (pg/mL) (mean ± sd)	130,60 ± 174,23	1358,98 ± 1550,42	346,26 ± 391,99	0,149

Table 2 The laboratory results of the patients

	Control group (n=46)	Proven sepsis group (n=86)	Clinical sepsis group (n=60)	P value
Pentraxin-3 (ng/mL) (mean±sd)	1,09 ± 1,90	2,59 ± 7,52	1,35 ± 4,12	0.189
Pro-Adrenomedullin (pmol/ml) (mean±sd)	26,57 ± 19,93	21,75 ± 16,27	25,66 ± 18,00	0.536
STREM-1 (pg/mL) (mean±sd)	5,39 ± 6,95	13,41 ± 26,84	11,09 ± 20,04	0.091

Table 3 The comparison of PTX-3, Pro-ADM and sTREM-1 levels between groups

In the proven sepsis group CRP was highly significant for the diagnosis and the AUC was 0.870 with a sensitivity of 80.0 % and the specificity of 71.1 % when the cut point was accepted as 2 mg/dL. For the definition of clinical sepsis CRP had high significance as well with the sensitivity and specificity of 100.0 % and 71.1 % respectively and the AUC was 0.994. In the diagnosis of both proven and clinical sepsis PCT was moderately significant with sensitivity values of 58.7 % and 70.7 % and specificity values of 88.9 % and 88.9 % respectively. The AUC values for PCT were 0.792 and 0.837 for proven and clinical sepsis respectively. The AUC for PTX-3, Pro-ADM and sTREM-1 was not calculated as they did not present significant difference.

Discussion

This study primarily focused on the diagnostic value of novel biomarkers in newborns with the suspicion of LOS. As far as we are concerned this study is the first one to assess PTX-3, sTREM-1 and Pro-ADM concurrently in LOS. Our results indicated that the markers did not have any significant value to diagnose LOS in neonatal patients.

Despite recent advances, neonatal infections are still important health problems. Diagnosing at an earlier stage is of importance in disease management. In addition to anamnesis and clinical findings, laboratory markers are the mainstay of a rapid and accurate diagnosis. Although blood culture is accepted as the gold standard test, it is mostly time consuming and has low sensitivity. Besides, empirical administration practice of broad-spectrum antibiotics caused the microorganisms to retaliate with drug resistance [19]. In this conjunction, the diagnosis of neonatal sepsis keeps on being a challenge.

As the microorganism enters the blood stream a complex immune response occurs. During the course of the sepsis many molecules are produced and released in order to combat with the agent and inform

adjacent and distal tissues of the body. Some of these molecules have been discovered and are used in daily practice in terms of supporting clinical diagnosis and help in the management of the therapy. However, a gold standard biomarker to diagnose and to guide the treatment of neonatal sepsis has yet to be described [20].

STREM-1 has been proposed as a promising biomarker in detecting neonatal sepsis with high sensitivity and specificity. Adly et al investigated neonates presenting with either early or late sepsis (63 culture-positive and 49 culture-negative and 40 healthy controls) and reported that elevated sTREM-1 could be considered as an early marker for the indication of sepsis severity and poor prognosis in neonatal sepsis [21]. In another study Arízaga-Ballesteros V et al demonstrated the effectiveness of sTREM-1 in the prediction of septic shock and death in LOS [22]. On the other hand Routsi et al reported that sTREM-1 was not significantly higher in patients with sepsis which was in conjunction with our previous study on EOS patients [23]. Furthermore they noted that it tended to increase in patients presenting progression to septic shock and an indication of a poor outcome [24]. Regarding these previous studies, investigators tend to assume sTREM-1 as a pro-inflammatory cytokine, but it can be an anti-inflammatory protein as well which was proposed by Giamarellos-Bourboulis et al. They stated that sTREM-1 levels correlated with IL-10 levels thus it could be anti-inflammatory like IL-10 and besides that, the less the sTREM-1/TNF-alpha ratio, the more severe the patient was, like transiting from sepsis to severe sepsis. This means higher TNF-alpha and lower sTREM-1 may represent severe sepsis [25].

In our results, sTREM-1 levels were similar between neonates with and without sepsis. This could be due to the time of the blood sample collection which was made at the admission with the clinical findings only. And at that time no patient had any sign of progression to a severe clinical course. Moreover, if sTREM-1 is an anti-inflammatory protein like stated in the aforementioned study, we did not have enough time to observe its elevation or decline in the course of the disease.

Pentraxins are endogenous proteins, synthesized in response to inflammatory signals. It shares similar structures with CRP although CRP is synthesized in liver and PTX is locally produced by the inflamed tissue. It has been suggestedas a predictor for poor outcome, shock and even organ failure [11, 26]. However, there is scarce information in pediatric infections and PTX-3 in the literature. Kim et al reported in their study that PTX-3 was able to indicate disease severity in lower respiratory tract infections in children [27]. In a recent study El Gendy et al compared 80 children in pediatric intensive care unit with 80 healthy controls and stated higher PTX-3 in study group as compared to healthy counterparts. However, they observed no difference between septic and non-septic critically ill children in the study group [28].

Knowledge on PTX-3 in neonatal age is even less than that. PTX-3 was reported as a diagnostic tool in pulmonary hypertension in newborns [29] and was also found to be in correlation with cardiovascular impairment in hypoxic respiratory failure in late preterm neonates [30]. Higher PTX-3 levels were associated with worsened neonatal outcome in a previous study on 28 newborns, born to mothers with preterm premature rupture of membranes [31]. To the best of our knowledge this is the only study on LOS in the literature. Supporting the study performed by Gendy et al, our results did not indicate any difference between the presence and absence of sepsis. This may be attributed to the nature of PTX-3 as it is produced by the damaged tissue itself, rather than systematically by liver. In our study group, none of the patients in admission were in advanced sepsis or septic shock to cause a severe tissue disruption to boost the expression of PTX-3.

ADM is secreted from the endothelial cells when the blood flow of a tissue is deteriorated in order to provide required circulation. Due to the short half-life of ADM, its more stable form, pro-ADM was discovered [32].

In neonatal patients, Pro-ADM was elaborated in terms of being a biomarker for sepsis and has been reported to be successful [33]. Sameh et al compared 60 proven sepsis and 30 healthy newborns and noted high sensitivity and specificity for pro-ADM in detecting neonatal sepsis [34]. High levels of ADM have not only been related to infection but also possible obstacles in its elimination from kidneys and lungs [35, 36].

In the current study, our sepsis groups did not demonstrate significant levels of pro-ADM as compared to control group. This could be due to none of our patients were at an advanced stage of sepsis like septic shock where tissue blood flow is deteriorated. Thus, Pro-ADM levels were similar between groups. Besides, upto our knowledge there is scant knowledge on the elimination kinetics of Pro-ADM in newborns. Preterm neonates, who are prone to LOS more than others, have lower renal functions and more frequent lung problems which might affect the elimination kinetics of Pro-ADM as well. In addition to that, Pro-ADM is detected in high levels in cardiac diseases due to disrupted blood flow to body tissues [36]. And especially preterm newborns might emerge with cardiac dysfunction, causing

insufficient circulation which may cause raised Pro-ADM levels as ADM is thought to increase in order to provide circulation to tissues per se. As a consequence, Pro-ADM should be cautiously evaluated in newborns as a biomarker in sepsis.

In our previous study published in 2019, we also evaluated the diagnostic accuracy of these three biomarkers in newborn early onset sepsis in a large cohort [23]. Our results compare well with the previous study which means we could not demonstrate any significant difference between neonates with and without sepsis. And as far as we are concerned this is the largest cohort of neonates with LOS, among whom these biomarkers were investigated

All groups were similar in terms of demographic findings and mean hour of inclusion. All parameters of antenatal maternal follow-up were comparable except for maternal antenatal antibiotic administration which was significantly higher in control group. This may be due to antenatal urinary tract infection rate being also higher in control group; although it did not reach statistical significance. Mean birth weight and mean gestational age parameters were the lowest in proven sepsis group which were expected as they are clinical findings to be associated with LOS [37].

In our study group we observed that the comparison of mean IL-6 levels and I/T ratios between the three groups did not reveal any significant difference. This might have sourced from two reasons as; not all the attending centers were convenient for measuring IL-6 levels in their laboratories, and not all of our patients' records consisted of I/T ratios. Significantly highest WBC levels and lowest PLT counts were noted in the proven sepsis group which is observed in LOS in general. Mean CRP levels and PCT levels were low in control group than other groups as expected.

The ratio of culture positive sepsis was 44.7 % among our study population. This was higher than the rates frequently reported in the literature [38]. In alliance with the literature, the most common pathogen obtained from blood culture was coagulase-negative staphylococcus (CoNS) [39]. This could be due to overrated gram positive bacterial isolation which might have occurred due to contamination.

To conclude

Despite advances in treatment alternatives and strategies, LOS is still a highly mortal condition. Early diagnosis and appropriate guidance of treatment is life saving and protective against antibiotic resistance. Although an effort has been put to make an early and accurate diagnosis, a reliable biomarker has yet to be described. As a biomarker, sTREM-1, pro-ADM and PTX-3 have been propounded as promising. However, they seem to have an increased expression with the progression of the disease and deterioration of the bodily tissues. This may suggest these markers to aid in follow up of the disease outcome rather than an early diagnosis. Furthermore, in terms of observing any correlation between the disease progression and these markers, repeated measurements may be of help.

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Conflicts of interest

The authors hereby declare no conflict of interest.

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