The Correlation of Antibacterial Peptides Concentration in Umbilical Cord Blood and Early-Onset Sepsis in Preterm Infants

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Research Article

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Abstract

Background: The role of serum LL37 in systemic inflammation has been confirmed, and the influence of it in umbilical cord blood to early-onset sepsis in preterm infants is currently being investigated.

Results: The level of LL37 of sepsis group was higher than those of in control group (362.13±46.71 vs 248.13±83.30 ng/ml), the levels of CRP, WBC and MPV in sepsis group were higher than those of in control group (6.25±4.19 vs 2.89±2.77 mg/L; 17.60±12.35 vs 8.24±3.55×10^9/L; 11.10±1.11 vs 8.93±0.68 fL), the level of PLT was lower than those of in control group (175.20±38.51 vs 245.75±49.85×10^9/L) (P < 0.05). The expression of LL37 was negatively correlated with PLT (r = -0.9347, P < 0.0001), and positively correlated with MPV ((r =0.9463 , P < 0.0001), the expression of PLT was negatively correlated with MPV (r = -0.9641, P < 0.0001). The area under curve of LL37 for diagnosis of early-onset sepsis was 0.875, the prediction probability was 0.7, the sensitivity was 90.0% and the specificity was 80.0%.

Conclusions The higher level of LL37 in umbilical cord blood was associated with the development of early-onset sepsis in preterm infants.

1. Background

Neonatal sepsis is a systemic inflammatory response syndrome (SIRS) and is also the major cause of morbidity and mortality, especially in preterm infants[1]. Early-onset sepsis (EOS) has been defined based on the age at onset, as occurring in the first 3 days of life and is caused by bacterial pathogens transmitted vertically before or during delivery[2]. Early recognition and diagnosis of early-onset neonatal sepsis is required to prevent the transition into septic shock, which is associated with a mortality rate of at least 40%[3]. Numerous studies have been conducted to assess the role of C-reactive protein (CRP), White blood cell (WBC), and other biomarkers for diagnosis of neonatal sepsis[4], but recently, there are some studies that report the important clinical value of antibacterial peptides for the diagnosis of sepsis[5].

The innate immune system is the first line of defense against microorganisms, of which cathelicidin family is one of the major antimicrobial peptide families in mammals, and LL37 is the only known human cathelicidin[6]. LL37 exhibits diverse biological activities, including antimicrobial activity, anti-apoptosis, regulating inflammatory response and angiogenesis[7]. We previously revealed that LL37 was one of the paracrine factors of umbilical cord blood mesenchymal stem cells and inhibited bacterial growth in ventilator -associated pneumonia (VAP) and sepsis[8–9]. Therefore, the level of LL37 in umbilical cord blood may be able to influence the occurrence of early-onset neonatal sepsis.

Thrombocytopenia is frequently observed following the onset of sepsis, and the decreased degree of platelet is closely related to the degree of SIRS. Mean platelet volume (MPV) is a parameter of the average volume of peripheral blood platelets, which directly reflects the morphology of platelets, and is

also a potential indicator for evaluating the pro-inflammatory state and thrombosis[10]. However, few studies explore the predictive value of platelets and MPV for sepsis.

The aim of this study was to quantify the levels of antimicrobial peptides LL37 in umbilical cord blood, explore the predictability of LL37 for preterm infants at risk of early-onset sepsis.

2. Results

2.1 General Characteristics

There were 55 single preterm infants (≤33 weeks gestation) born from January 2019 to June 2019 who had been collected umbilical cord blood. Among them, ten preterm infants diagnosed as early-onset sepsis, and twenty preterm infants were enrolled as control group matched by 1:2 according to gestational age and sex. There were no significant differences in maternal age, prenatal glucocorticoid, prenatal antibiotics, diabetes, eclampsia in two groups. In sepsis group, the occurrence of neonatal septic shock was significantly higher than that in control group, the difference was statistically significant (P = 0.030). But there were no significant difference in sex, gestational age, birth weight, delivery method and other infectious disease such as meningitis in two groups (P > 0.05). Patient characteristics are summarized in Table 1.

Table 1
Maternal and Neonatal Characteristics

	Sepsis Group	Control group	*P*
Maternal	32.30 ± 6.25	31.10 ± 5.86	0.609
Age(y)	7(70.0%)	18(90.0%)	0.300
Prenatal glucocorticoids,n,(%)	7(70.0%)	8(40.0%)	0.245
Prenatal antibiotics,n,(%)	5(50%)	3(15%)	0.078
Diabetes,n,(%)	1(10%)	3(15%)	1.000
Eclampsia,n,(%)	8(80%)	16(80%)	1.000
Neonatal			
Male,n,(%)			
Gestational age(w)	29.73 ± 1.94	30.39 ± 1.61	0.336
Birth weight(kg)	1.27 ± 0.32	1.46 ± 0.23	0.071
Cesarean section,n,(%)	4(40.0%)	10(50.0%)	0.709
Septic shock,n,(%)	3(30%)	0(0%)	0.030
Meningitis,n,(%)	2(20%)	0(0%)	0.103

Data are expressed as n (%) or mean ± standard deviation. $P < 0.05$, the difference was statistically significant.

2.2 The levels of LL37 and other inflammatory mediators

The levels of LL37 in umbilical cord blood of sepsis group were significantly higher compared with controls (362.13 ± 46.71 vs 248.13 ± 83.30 ng/ml). The levels of CRP, WBC, and MPV in sepsis group were also higher than those in control group (CRP:6.25 ± 4.19 vs 2.89 ± 2.77 mg/L; WBC: 17.60 ± 12.35 vs 8.24 ± 3.55×109/L; MPV: 11.10 ± 1.11 vs 8.93 ± 0.68 fL), the levels of PLT in sepsis group were significantly lower (175.20 ± 38.51 vs 245.75 ± 49.85×109/L), all of the difference above was statistically significant($P < 0.05$) (Fig. 1 and Fig. 2).

2.3 Correlation between levels of LL37 and mediators of inflammation

In sepsis group, the LL37 levels correlated with the levels of PLT (P 0.0001, r = -0.9347), MPV (P 0.0001, r = 0.9463). The levels of PLT correlated with the levels of MPV (P 0.0001, r= -0.9641). No significant correlation was found in control group (Fig. 3).

2.4 The analysis of receiver operating characteristic curve (ROC)

Antimicrobial peptides LL37, CRP and WBC all have early diagnostic value for early-onset neonatal sepsis. However, the area under the ROC curve (AUC) diagnosed with LL37 is the largest (LL37:0.875, CRP: 0.775 and WBC: 0.825), and when the prediction probability of LL37 was 0.7, the sensitivity was 90.0%, and the specificity was 80.0% (Fig. 4 and Table 2).

Table 2
The diagnostic value of LL37, CRP and WBC for early-onset neonatal sepsis

Index	AUC(95%*CI*)	Cutoff	Sensitivity (%)	Specificity (%)
LL37	0.875(0.747–1.003)	0.70	90.0	80.0
CRP	0.775(0.581–0.969)	0.60	80.0	80.0
WBC	0.825(0.674–0.976)	0.55	80.0	75.0

CRP: C-reactive protein, WBC: white blood cells, AUC: the area under the receiver operating characteristic curve, 95% CI: 95% confidence interval.

3. Discussion

3.1 Principal findings

Neonatal sepsis is the major cause of prematurity and infant mortality. Early diagnosis and effective treatment is the key to save the lives of neonates. In this prospective study, we determined the concentrations of LL37 in umbilical cord blood and other inflammatory factors in peripheral blood in preterm infants. The key findings of this study are the following: (1) The preterm infants at risk of early-onset sepsis had higher levels of antimicrobial peptide LL37, CRP, WBC and MPV, and a lower level of PLT. (2) The expression of antimicrobial peptide LL37 was negatively correlated with PLT level and positively correlated with MPV level, the expression of PLT was negatively correlated with MPV level in preterm infants at risk of early-onset sepsis. (3) The early individually value of antimicrobial peptide LL37 for early-onset sepsis is higher than CRP and WBC.

3.2 Strengths and weaknesses

This study has strengths as follows: First, we measured the concentrations of LL37 in umbilical cord blood in preterm infants, analyzed the relationship between LL37 and neonatal early-onset sepsis, which was not reported previously. LL37 is released as an 18-kDa pro-peptide, produced by hCAP-18, which is significantly increased in inflammation and infection, has the function of amplifying Toll-like receptor signals[11–12]. Bucki et al confirmed that LL37 rose immediately after infection, and reached a peak within 2 hours, which provided a theoretical basis for early diagnosis of sepsis[13]. Second, in our study, the

expression of antimicrobial peptide LL37 was negatively correlated with PLT level and positively correlated with MPV level in preterm infants at risk of early-onset sepsis. LL37 was demonstrated that be able to inhibit P selectin expression, which is a major platelet alpha-granule protein that is highly expressed on the platelet surface during activation[14]. However, P-selectin can lead activated platelets adhere to neutrophils and monocytes[15], with innate immune response of LL37. There may be a link between the anti-inflammation activity of LL37 and its antiplatelet aggregation activity. However, this study also has some limitations. First, the number of early-onset sepsis cases was limited and the efficacy needed to expand the sample size for further evaluation. Second, LPS activates inflammatory cells to secrete TNF-a, IL-1β, IL-6 and other cytokines, which mediate the development of sepsis[16]. LL37 was proved to be able to bind to LPS and suppress the interaction between LPS and LPS binding protein as well as bind to macrophage CD14 receptors, thus inhibiting LPS-induced TNF-a expression[17–18]. We may conduct a larger study to assess the mechanism of LL37 and sepsis. Third, the study found the correlation of LL37 with PLT and MPV. However, We didn't know specific signals or pathways that LL37 influenced them. Wen et al. reported that LL37 also could reduce phosphorylation of Src kinase and AktSer[473], indicating that the modulation of Src/PI3k/Akt signaling pathway involved in the antiplatelet activity of LL37[14]. Src kinase plays an important role in activating platelet[19], and phosphatidylinositol 3-kinase (PI3K) is the most crucial one among the downstream effectors of Src kinase, Akt is the most well-known activation marker of

PI3K. Therefore, we may conduct an animal study to explore how the LL37 influence platelet in early-onset neonatal sepsis.

3.3 Comparison with previous studies

In the present studies, it is not surprisingly to find significant elevation in the serum level of CRP, PCT, and WBC in the newborn with sepsis. CRP is the most common laboratory tests in the diagnosis of neonatal sepsis[20], which will take a long time for level to increase, so making it low sensitive for early diagnosis of neonatal sepsis[21]. As well as this study provides low sensitivity (80.0 %) with limited specificity (80.0%) for CRP. However, conditions where there is spurious increase in level of CRP such as meconium aspiration syndrome, premature infant exposure to glucocorticoids, maternal fever during labor, fetal distress, prolonged labor, perinatal asphyxia and intraventricular hemorrhage (IVH), thus making it a nonspecific biomarker for diagnosis of neonatal sepsis[21–22].

Leucopenia has been proved to have low sensitivity (29%) but high specificity (91%) for diagnosis of neonatal sepsis[23], which defined as WBC count less than 5000 to 7500/mm3. But in our study, there is a different sensitivity (80.0%) and specificity (75.0%). Besides WBC, absolute neutrophil count (ANC), and immature to total leukocyte ratio (I:T) also have been used to diagnosis of neonatal sepsis. Hornik et al. found that low WBC counts, low ANC and high I: T neutrophil ratios were associated with increased odds of infection[24]. But all have significant limitations in the diagnosis of neonatal sepsis.

Procalcitonin (PCT) is an acute phase reactant protein and has been reported to be associated with immunomodulation associated with systemic inflammatory response syndrome (SIRS). The levels of PCT increase significantly during systemic

bacterial infection such as early-onset or late-onset sepsis[25–26], and Chiesa et al. also showed in diagnosis of EOS, PCT had sensitivity of 92%, specificity of 97%[26]. However, false increase of PCT is seen in many conditions such as premature newborn, intracranial hemorrhage, birth asphyxia, neonatal hypoxemia, and healthy infants born to mothers with chorioamnionitis[27]. Therefore, PCT must to be studied further in larger groups of infants so as to improve its diagnostic accuracy. As there are limited cases in our study, PCT showed no statistical difference in two groups, and without increasing significantly in early-onset sepsis.

With this study, we investigated the levels of LL37 in umbilical cord blood and found that it was significantly higher in preterm with early-onset sepsis than that in healthy control group. The sensitivity and specificity of LL37 were 90.0% and 80.0%, respectively, values that were higher than the most obtained for the other markers. The inflammatory mediators (interleukin IL-6IL-1, tumor necrosis factor TNF) released after severe infection, activating the IF-IL-6 site on the antimicrobial peptide LL37 by Kinase/signal and transcription activator (JAK/STAT) pathway or Ras-dependent mitogen-activated protein kinase (MAPK) pathway, so significantly increasing the expression of LL37[28]. This may be the main mechanism.

4. Conclusions

In summary, the expression of antimicrobial peptide LL37 increased significantly in umbilical cord blood in preterm at a risk of early-onset sepsis, which has certain clinical application value for early diagnosis and evaluation of sepsis, and is expected to become a new index for clinical prediction of severe infection screening.

5. Methods

5.1 Study population

This study was approved by the Ethics Committee of Guangdong Women and Children's Hospital (201801065). Written informed consent from mothers giving birth 33 weeks gestation (singleton) from January 2019 to June 2019 was obtained before collection of umbilical cord blood samples. The excluded criteria as follows: (1) Mothers with a history of infection (virus infection, chorioamnionitis) before delivery and premature rupture of membranes; (2) Preterm infants with congenital abnormalities, meconium pollution of amniotic fluid. Umbilical cord blood was sampled during delivery using a standardized procedure. Placentae were examined for evidence of histological chorioamnionitis. All clinical data of maternal and preterm infants (33 weeks gestation, singleton)) were collected.

5.2 Measurement of Concentrations of Antimicrobial Peptides LL37

The level of LL37 in umbilical cord blood was measured by sandwich ELISA as follows: The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human LL37. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human LL37 and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human LL37, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of Human LL37[29].

5.3 Measurement of Inflammatory Mediators

The average value of inflammatory mediators for three days consecutively after birth were compared. The concentration of C-reactive protein (CRP) in venous blood was measured by routine latex-enhanced immunoturbidimetry (Roche Diagnostics). Routine white blood cell count (WBC) was determined by flow cytometry (Sysmex). The platelet (PLT) and mean platelet volume (MPV) were measured by whole blood cell automatic analyzer (Japanese Sysmex KX221).

5.4 Statistical Analysis

All data were tested for normal distribution with the Kolmogorov–Smirnov test. The normal distribution data was represented as the mean ± standard deviation. Dichotomous data was expressed by the frequency and relevant percentage. The correlation between the measurement data was analyzed by Pearson correlation. Receiver operating characteristic curve (ROC) was applied to evaluate the ability of antibacterial peptide LL37 and related inflammation indicators to diagnose early-onset sepsis, and analyze its sensitivity and specificity. P 0.05 was considered statistically significant. All analyses were performed by using SPSS version 19.0 software.

Abbreviations

SIRS Systemic inflammatory response syndrome

EOS Early-onset sepsis

CRP C-reactive protein

WBC White blood cell

VAP Ventilator -associated pneumonia

MPV Mean platelet volume

ROC Receiver operating characteristic curve

IVH Intraventricular hemorrhage

ANC Absolute neutrophil count

PCT Procalcitonin

Declarations

Ethics approval and Consent to participate

This study was approved by the Ethics Committee of Guangdong Women and Children's Hospital (201801065). Besides, it was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Written informed consent from mothers giving birth ≥33 weeks gestation (singleton) from January 2019 to June 2019 was obtained before collection of umbilical cord blood samples.

Consent for publication

Not applicable.

Availability of data and material

Data are available on various websites and have been made publicly available (more information can be found in the Results section). If asked, Jiayu Miao could be contracted.

Competing interests

The authors declare that they have no competing interests.

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Ethics approval: This study was approved by the Ethics Committee of Guangdong Women and Children's Hospital (201801065).

Author contribution

Jiayu Miao collected all data and wrote the manuscript, Ying Liu and Zengqing Li revised the manuscript, Zhuxiao Ren and Zhicheng Zhong collected the samples, Longli Yan, Fang Xu, Xin Xia and Jianlan Wang collected the references, Jie Yang revised and reported the manuscript. All authors read and approved the final manuscript.

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Not applicable.

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Figures

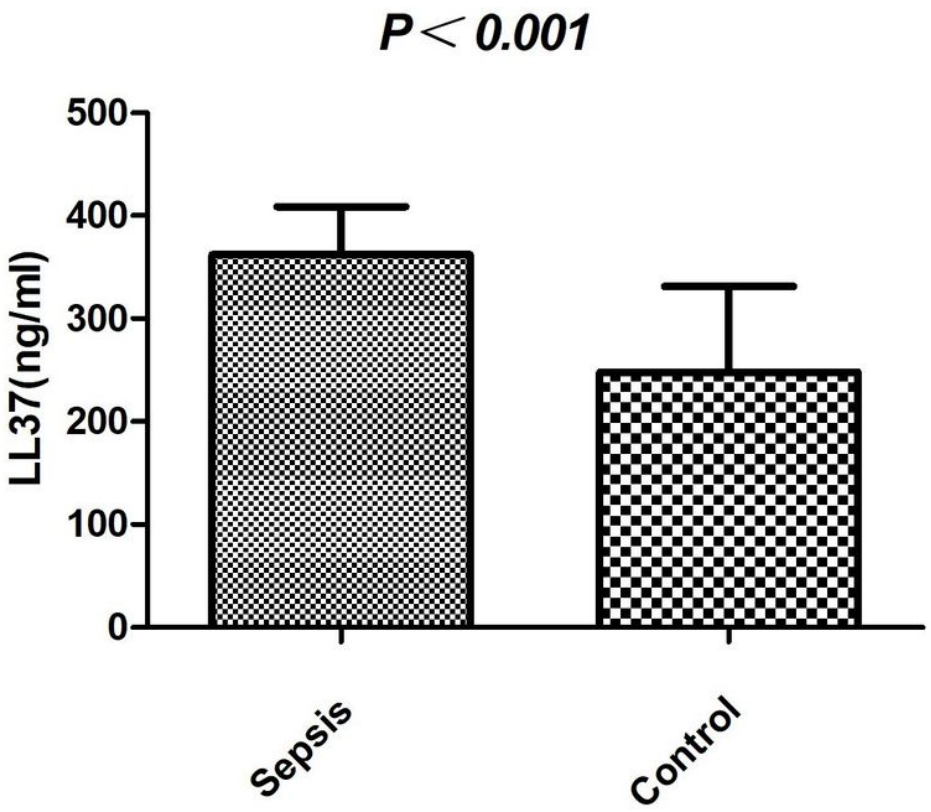

Figure 1

The level of LL37 in umbilical cord blood of two groups;

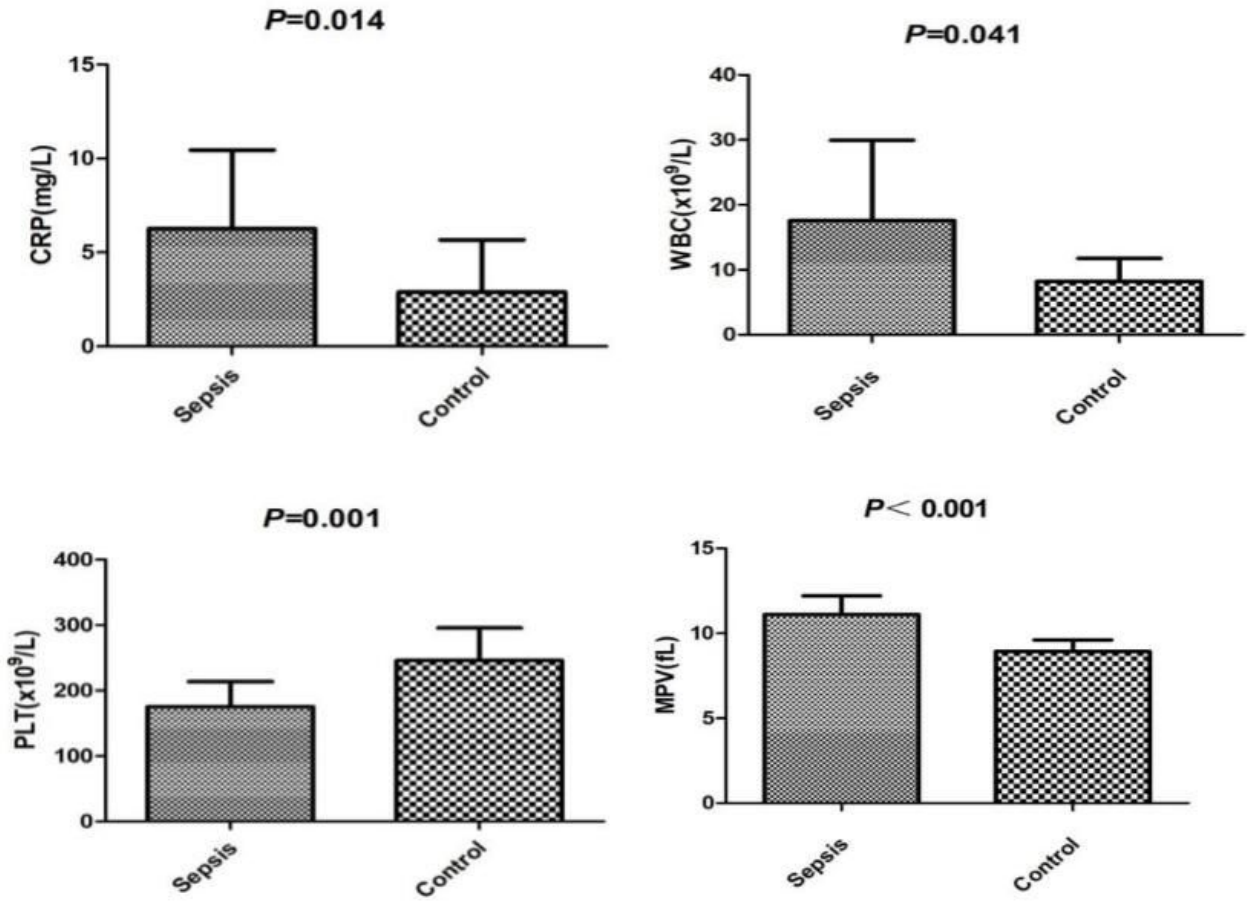

Figure 2

The levels of CRP, WBC, PLT and MPV in two groups;

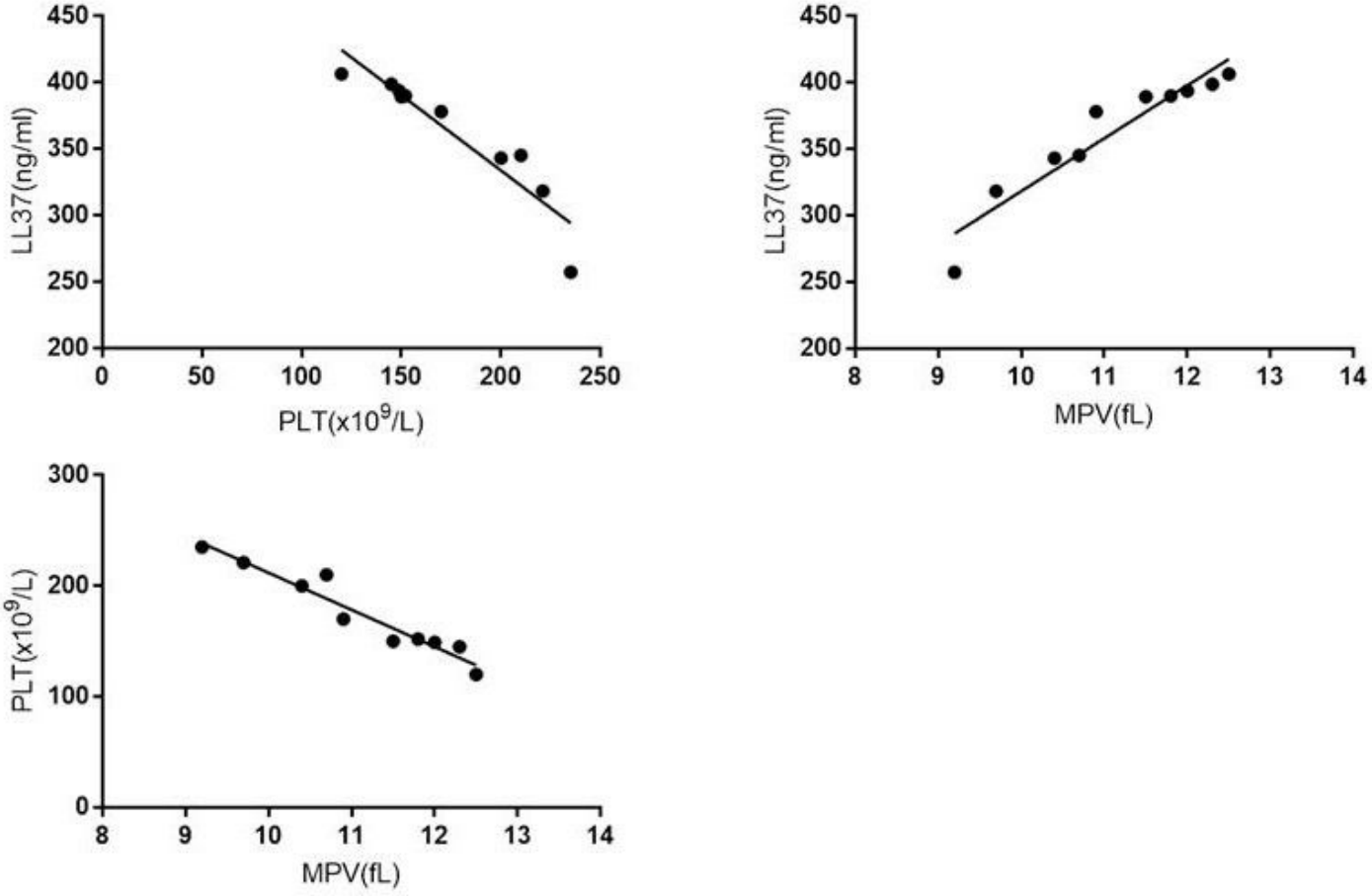

Figure 3

The correlation of LL37, PLT and MPV in sepsis group;

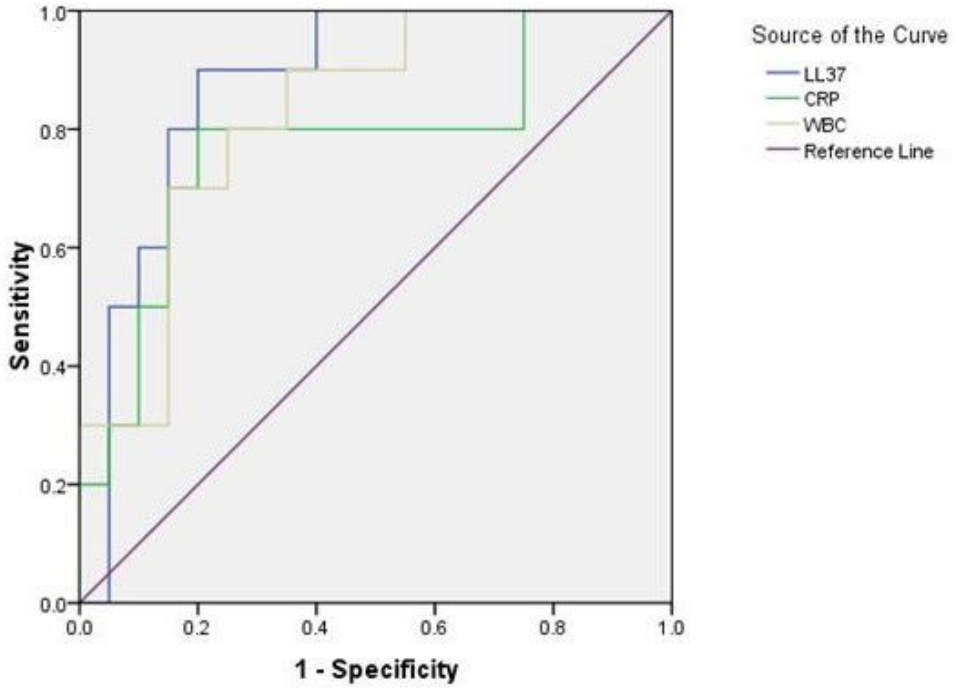

Figure 4

ROC curve of LL37, CRP and WBC for early diagnosis of early-onset sepsis.