CD10 IS A GOOD BIOMARKER TO PREDICT BACTERIAL INFECTION IN SEPSIS-SUSPECTED PATIENTS

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ABSTRACT

Objective: *This study first examined if CD10 expression can be used as a biomarker to predict bacterial infection in the suspected sepsis. Then, the correlation between CD10 expression and the severity of sepsis.*

Methods: *The blood samples from 168 suspected septic patients were collected for CRP, PCT and CD66b+CD10- expression. The results were statistically evaluated with the scores of SOFA, APACHE II, and MODS.*

Results: *The prediction accuracy of CD10 expression for bacterial infection was much higher than that of CRP and PCT. However, the AUC of CD66b+CD10-, CRP and PCT were too low to predict the 28-day mortality in septic patients. SOFA, APACHE II, and MODS showed no correlation with the expression of CD66b+CD10- in the peripheral blood of the suspect septic patients.*

Conclusion: *Peripheral blood CD66b+CD10- is an effective biomarker for the prediction of early bacterial infection, but invalid for death prediction and no correlation with the severity of sepsis.*

Keywords: *sepsis-CD10-infection-CRT-PCT.*

DOI: 10.19193/0393-6384_2019_5_448

Received *November 30, 2018;* ***Accepted*** *February 20, 2019*

Introduction

Sepsis is a worldwide life-threatening disease with high mortality and defined as the response of host to infection with dysfunction of organ[(1)]. Till now, no effect drug or management exists to treat sepsis. Thus, millions dollars and human power are spent every year on sepsis[(2)].

Previous studies showed that accurate and early diagnosis and administration of appropriate antibiotics would improve the outcomes of septic patients. While in the early stage of suspected sepsis, it is often difficult for clinicians to distinguish between bacterial and non-bacterial infection[(3)]. Tachycardia, leucocytosis, tachypnea, and pyrexia which are clinical signs of sepsis often overlap with other non-infectious conditions in critically ill patients. This difficulty is also a main cause of inappropriate or delayed use of antibiotics with various adverse events[(4)]. Scientists and clinicians have studied various biomarkers to differentiate between bacterial and non-bacterial infection of sepsis, but no single or biological indicator of sepsis is accepted universally[(4-5)]. Although C-reactive protein (CRP) and procalcitonin (PCT) are the most widely used biomarkers for patients with suspected bacterial sepsis, the ability to distinguish bacterial sepsis from other inflammatory conditions is limited[(5-6)].

The need for differentiating between bacterial and non-bacterial infection is unmet. CD10, also called common acute lymphoblastic leukemia antigen, neutral endopeptidase or enkephalinase, is a 100-kDa transmembrane glycoprotein. It is a major metalloproteinase to regulate levels of biologically active peptides which initiate inflammatory, cardiovascular, and neurogenic responses[(7-8)]. CD10 expresses on mature neutrophils at their latest stages of differentiation.

In Kaneko's study, a primate model of sepsis mediated by lipopolysaccharide (LPS) or Escherichia coli (E. coli) was founded by injecting LPS or E. coli to healthy volunteers. The results showed that the expression level of CD10 on surface of neutrophil reduced after injection[8]. This study is aimed to evaluate whether CD10 is a good biomarker to distinguish between bacterial and non-bacterial infection in the early stage of sepsis patients and the correlation between CD10 with severity of sepsis.

Methods

Patient characteristics

This study was approved by IRB of the Second Hospital of Dalian Medical University. From July, 2017 to Feb, 2018, totally 168 suspected sepsis patients were recruited from ICU, emergency ICU, emergency department, neurosurgery department, respiratory department and gastrointestinal department. The definition of sepsis is infection+SOFA≥2, organ dysfunction patient was defined as patient with SOFA≥2, suspected sepsis patient was defined as organ dysfunction patient with suspected bacterial infection. Bacterial infection was confirmed by bacterial culture with corresponding bacteria, or by classic symptoms of infection such as erysipelas. The sources of infection were from blood, sputum, specimen from surgery and urine tube. Non-bacterial infection was defined as negative results of blood culture or virus infection.

If a patient was diagnosed as organ dysfunction with suspected bacterial infection within 48 hours after his/her entrance of hospital, this patient was recruited into this study as a suspected sepsis patient after signing the consent. Patients under 18 years old, with tumor or leukemia, immunosurpessive or immunopromotive treatment, history of organ transplantation, refusal of consent were excluded. Number instead of name was used to identify patients. After recruiting, 2ml peripheral intravenous blood was taken within first 4 hours of suspected bacterial infection symptoms appeared and was stored in ethylenediaminetetraacetic acid (EDTA)-treated tubes and managed within 1 hour to detect CRP, PCT and CD66b+CD10- expression. MODS and APACH.

Were also recorded

Flow cytometry analysis

100 ul peripheral venous blood of each patient was drained into flow cytometry tube (from BD), then stained for 15 min in dark room using 5ul fluorocrome-conjugated mAbs or specific isotype controls: PE mouse anti-human CD66b (Biolegend), PE-Cy7 mouse anti-human CD10 (Biolegend), PE mouse IgG1 κ isotype control (Biolegend), and PE-Cy7 mouse IgG1 κ isotype control (Biolegend). BD FACSCalibur (Becton, Dickinson and Company, New Jersey, USA) was used for analyzing labeled cells and data were analyzed with Flow-Jo software (Tree Star, Ashland, OR).

CRP and PCT

Once peripheral venous blood samples were taken from sepsis-suspected patients, they were separated by centrifugation (at 1700×g for 5 min) within 1 hour, and aliquots were stored at -20 °C until further assayed.

PCT were detected by E-170 automatic analyzer (Roche), and CRP was measured by ELTA automatic analyzer (RADIM).

Statistics

The comparison of variables was performed using an unpaired 2-tailed Mann-Whitney U test (for comparison between 2 groups) or a 1-way analysis of variance (ANOVA) with the Dunnett posttest (when multiple comparisons to control group were made). P values of ,.05 were considered significant and asterisks indicate significant increases: *P, .05; **P $^{\#}$.01; ***P $^{\#}$.001. Graphs were elaborated using GraphPad Prism version 5 software (GraphPad Software, Inc).

Results

Demographic data

Totally 182 sepsis-suspected patients were recruited in this study. 3 patients' family refused to join the study, 2 patients quit during the study, 8 patients stopped treatment and left hospital without follow-up and 1 patient was diagnosed as HIV after microbial culture. Thus 168 patients' data were calculated in this study. The demographic data of 168 patients were showed in table 1. In these 168 sepsis-suspected patients, blood culture showed that 105 patients were bacterial infection and the rest of 63 patients were belong to non-bacterial infection.

	All suspect sepsis patients (n=168)	Non-bacterial infection (n=63)	Bacterial infection (n=105)	P
Age (years)	57[22-93]	59[20-89]	55[28-90]	0.87
Sex (male)	87(51.7%)	33(52.4%)	54(51.4%)	0.53
Main diagnosis				
Intestinal obstruction	37	17	20	0.062
Perforation of digestive tract	32	0	32	<0.001
Pancreatitis	12	2	10	<0.001
Bile duck shock	8	6	2	0.023
Pneumonia	29	10	19	0.036
Multiple trauma	14	5	9	0.037
Uremia	4	2	2	1
Myocardial infarction	8	6	2	0.037
Undiagnosed fever	24	5	19	<0.001
Severity				
SOFA	5 [2-14]	4 [2-8]	5[2-14]	0.55
APACHE II	14[9-20]	13[9-19]	15[10-20]	0.73
MODS	4[2-8]	4[2-8]	4[2-8]	1.0
Biomarkers				
$CD66b^+CD10^-$(%)	6.8[0.2-12.1]	1.3 [0.2-4.2]	10.1[5.6-12.1]	<0.001
CPR(mg/L)	103[31-190]	83[31-151]	116[52-190]	0.025
PCT(ng/L)	0.25[0.03-1.89]	0.15[0.03-1.88]	0.33[0.19-1.89]	0.033
Death within 28 days	48 (28.6%)	13 (20.6%)	35 (33.3%)	0.038
Site of infection				
Respiratory	26 (15.5%)	-	26 (24.8%)	-
Blood	11 (6.5%)	-	11 (10.5%)	-
Abdominal	32 (19.0%)	-	32 (30.5%)	-
Tissue/bone	7 (4.1%)	-	7 (6.7%)	-
Multiple	16 (9.5%)	-	16 (1.2%)	-
Others (fever, chills, etc.)	13 (7.7%)	-	13 (2.4%)	-
Source of infection				
Staphylococcus aureus	24 (14.3)	-	24 (22.9%)	-
Streptococcus	26 (15.5%)	-	26 (24.8%)	-
E.coli	42 (25.0%)	-	42 (40%)	-
Others	12 (7.0%)	-	12 (11.4%)	-
Gramstain G+	56 (33.3%)	-	56 (53.3%)	-
G-	49 (29.1%)	-	49 (46.7%)	-

Table. 1: The demographic data of 168 patients recruited. Data were shown as mean [min-max] or number [percent].

Specificity and sensitivity of CD66b+CD10-, CRP and PCT to diagnose bacterial infection in sepsis-suspected patient

The sensitivity and specificity of CD66b+CD10-(%), CRP, and PCT in predicting bacterial infections in sepsis-suspected patients were shown in Figures 1 and table 2. The AUC of CD66b+CD10- in peripheral blood of patients with sepsis was 0.9056, which was much higher than that of CRP (AUC=0.8021) and PCT (AUC=0.7210) and statistical significance was found by MedCalc (P was 0.04 and <0.001, respectively). Data showed that CD66b+CD10- in peripheral blood was a good biomarker to predict bacterial infection, and the prediction accuracy was much higher than that of CRP and PCT. The prediction accuracy of CRP was also statistically significant when compared with PCT (P=0.03).

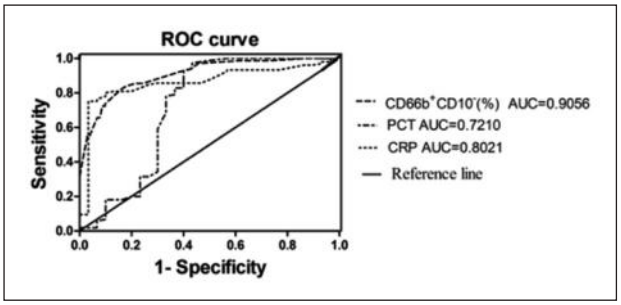

Figure 1: Specificity and sensitivity of CD66b+CD10-(%), CRP and PCT to diagnose bacterial infection in sepsis-suspected patient. Data shown were the receiver operating characteristic (ROC) curve of CD66b+CD10-(%), CRP and PCT.

Biomarkers	AUC	95% CI	Cut-off	Sensitivity	95% CI	Specificity	95% CI
$CD66b^+CD10^-$	0.906	0.862-0.943	3.35	0.865	0.800-0.926	0.903	0.779-0.922
PCT (ng/L)	0.721	0.584-0.857	0.31	0.781	0.690-0.856	0.667	0.472-0.827
CRP (mg/L)	0.802	0.779-0.925	102	0.7524	0.659-0.831	0.800	0.828-0.901

Table. 2: Specificity and sensitivity of CD66b+CD10-(%), CRP and PCT to diagnose bacterial infection in sepsis-suspected patient. Data were shown as mean [min-max].

Sensitivity and specificity of CD66b+CD10-(%), CRP, and PCT to predict 28-day mortality in septic patients.

In the 105 patients who were diagnosed as bacterial infections (who were also confirmed as sepsis according to the sepsis-3 criteria: sepsis= infection + SOFA≥2), 70 survived and 35 died within 28 days. The proportion of CD66b+CD10- in surviving sepsis patients was 9.8% (9.0%-11.8%), and 9.7% (9.1%-12.1%) in dead group, with no statistical difference (P=0.35); The concentration of CRP in surviving sepsis patients was 111 (52-178) mg/L and 119 (66-190) mg/L in dead group, without statistical difference (P=0.44). The concentration of PCT in alive septic patients was 0.27 (0.19-1.67) ng/L and 0.35 (0.20-1.89) ng/L in dead group, and no statistical difference was found (P=0.09). (Table 3). In predicting the 28-day mortality in patients with sepsis, data from figure 2 and table 4 showed that the AUC of CD66b+CD10-(%) was 0.6722 (95% CI 0.5650-0.7795) , the AUC of CRP was 0.5971 (95% CI 0.5761-0.7994) and PCT's AUC was 0.6878 (95% CI 0.4905-0.7038). These three indicators have poor prediction accuracy for 28-day mortality in patients with sepsis, and there were no statistical differences among the groups.

Biomarkers	Septic patients (n=105)	Survival (n=70)	Death (n=35)	P	AUC(95% CI)	Cut-Off
$CD66b^{+}CD10^{-}$	10.1[9.0-12.1]	9.8[9.0-11.8]	9.7[9.1-12.1]	0.35	0.565-0.779	7.65
CRP (mg/L)	116[52-190]	111[52-178]	119[66-190]	0.44	0.576-0.799	107
PCT(ng/L)	0.33[0.19-1.89]	0.27[0.19-1.67]	0.55[0.20-1.89]	0.06	0.490-0.704	0.46

Table. 3: Sensitivity and specificity of CD66b+CD10-(%), CRP, and PCT to predict 28-day mortality in septic patients. Data were shown as mean [min-max].

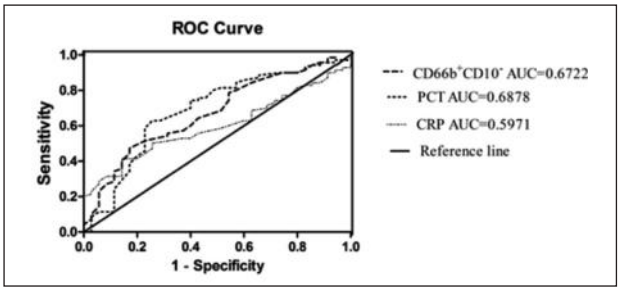

Figure 2: Sensitivity and specificity of CD66b+CD10-, CRP, and PCT to predict 28-day mortality in septic patients. Data shown were the receiver operating characteristic (ROC) curve of CD66b+CD10-, CRP and PCT.

The correlation between CD66b+CD10-(%) and disease severity in peripheral blood of patients with sepsis

We examined the correlation between CD66b+CD10- in peripheral blood of septic patients and SOFA, APACHE II, and MODS, and data were shown as figure 3A, 3B and 3C respectively. The results showed that the correlation between CD66b+CD10- ratio and SOFA, APACHE II, and MODS in peripheral blood of septic patients was poor, with R2 was 0.07606, 0.08463, and 0.01150 respectively, and no statistical significance was found (P=0.067, 0.0525 and 0.4832, respectively).

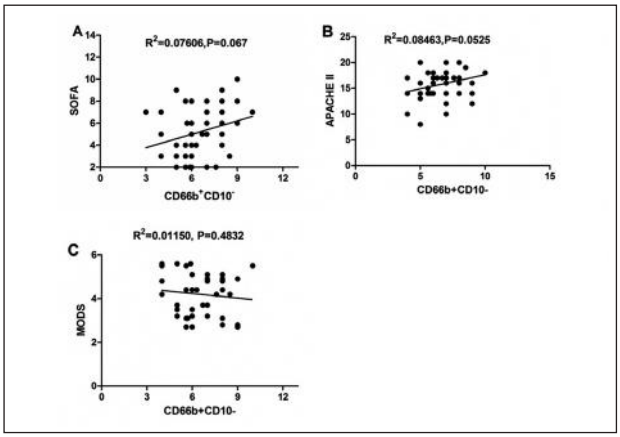

Figure 3: The correlation between CD66b+CD10- and disease severity in peripheral blood of patients with sepsis. 3A: the correlation between CD66b+CD10- and SOFA, 3B: the correlation between CD66b+CD10- and APACHE II, 3C: the correlation between CD66b+CD10- and MODS.

Discussion

Sepsis is a time-dependent disease, and the first 12 hours, some clinicians even suggested the first 4 hours of diagnosis are critical to the patient's prognosis. If the diagnosis is too late and effective antibiotic treatment is not given in time,sepsis may rapidly deteriorate and even progress to septic shock with more mortality and morbidity[9-10]. Therefore, rapid diagnosis and appropriate antibiotic treatment are the keys to the treatment of sepsis.

Good clinical markers should detect infections in a timely manner with high sensitivity and specificity, and can have a certain guiding significance for the prognosis of sepsis and the severity of the disease[4]. There is an unmet need for a diagnostic tool that distinguishes bacterial and non-bacterial causes of sepsis. Although many previous scholars have analyzed and studied various biomarkers in many studies, no single or multiple biological indicators have been universally recognized. The current gold standard for microbiological diagnosis is still blood culture, followed by routine identification and use of antimicrobial spectrum. The disadvantage is that it takes time to obtain the results, it may take 2-3 days to obtain the microbiological results, and the sensitivity is low, especially for those who have previously received antibiotic treatment[4].

The peripheral blood samples in this study were obtained from patients with suspected signs of sepsis within 48 hours after admission and were treated within 1 hour after obtaining. Combined with the final blood culture results, the results showed that CD66b+CD10- in peripheral blood of sepsis-suspected patients had a high prediction accuracy for bacterial infection with the AUC was 0.9056 (P<0.0001). Which means CD66b+CD10- is a good early biomarker to determine the bacteria infection in sepsis-suspected patients. The AUC of CRP and PCT were 0.8021 and 0.7210 respectively, which were much lower than that of CD66b+CD10-, so they could not be used as a good indicator for early diagnosis of bacterial infection. This result is consistent with previous research results[11].

In previous studies, the most widely studied biomarkers for suspected bacterial sepsis in global medical work and research scholars were CRP and PCT. CRP is the most widely used marker of inflammation due to its kinetics and availability in clinical laboratories[2]. However, even after the infection disappears, the plasma CRP concentration is still elevated, which leads to an increase in other inflam-

mation-related conditions, so the main disadvantage of CRP is its low specificity[2]. However, in this experiment, the predictive accuracy of CRP in the first 4 hours of suspected bacterial infection reached AUC=0.8021 (P=0.002), so it also had certain prediction accuracy. PCT may be the most specific indicator of bacterial sepsis, and its concentration is associated with severity, mortality, and organ failure. However, this marker has a fatal shortcoming, that is, it is not an early marker, but usually peaks at 6-12 hours after onset and may also increase in non-infectious conditions[11]. In this experiment, the prediction accuracy of PCT in the first 4 hours of suspected bacterial infection was not high, which was consistent with previous conclusions. Moreover, CRP and PCT have limited ability to separate bacterial sepsis from other inflammatory diseases.

This study examined not only the value of peripheral blood CD66b+CD10-, CRP and PCT in the survival and death group of patients with sepsis, but the accuracy of CD66b+CD10-, CRP and PCT in predicting the death of sepsis patients. The results showed that the proportion of CD66b+CD10- in the peripheral blood of patients with sepsis was 9.8% (9.0%-11.8%) in the surviving patients, and 9.7% (9.1%-12.1%) in the death group and the difference was not significant (P = 0.35); the concentration of CRP in surviving sepsis was 111 (52-178) (mg/L), and in the death group was 119 (66-190) mg/L without statistical difference (P = 0.44); PCT concentration in viable sepsis patients was 0.27 (0.19-1.67) ng/L, and 0.35 (0.20-1.89) in the death group with no significant the difference (P=0.09). In the analysis of 28-day mortality in patients with sepsis, we can see from Figure 2 that the AUC of CD66b+CD10- is 0.6722, the AUC of CRP is 0.5971, and the AUC of PCT is 0.6878. The accuracy of these 3 biomarkers in predicting 28-day mortality in patients with sepsis was very low, and there was no statistical difference among the groups. Therefore, we could conclude that the CD66b+CD10- in peripheral blood of patients with sepsis is not a good indicator for predicting death in patients with sepsis. In a previous study by Kaneko et al., after administration of LPS intravenously to healthy individuals, CD10 decreased within the first 4 hours and no longer decreased after 4 hours. This may explain that CD10 is a good indicator of early diagnosis of bacterial infections, but not a good predictor of death. The predictive accuracy of CRP and PCT for death in patients with sepsis is also consistent with previous results [2-11].

Finally, we analyzed the correlation between the proportion of CD66b+CD10- in peripheral blood of patients with sepsis and the severity of sepsis. We compared the proportion of CD66b+CD10- in peripheral blood of patients with sepsis with SOFA, APACHEII and MODS. The results showed that CD66b+CD10- in peripheral blood of patients with sepsis was poorly correlated with SOFA, APACHEII and MODS, and R2 was 0.07606, 0.08463 and 0.01150, respectively with no statistical significance (P values were 0.067, 0.0525, and 0.4832 respectively). Therefore, we conclude that CD66b+CD10- in peripheral blood of patients with sepsis is not associated with the severity of sepsis.

Conclusion

- Peripheral blood $CD66b^{+}CD10^{-}$ in patients with suspected sepsis can predict early bacterial infection in patients with suspected sepsis.
- Peripheral blood $CD66b^{+}CD10^{-}$ in patients with sepsis is not a good predictor of death in patients with sepsis.
- There is no correlation between $CD66b^{+}CD10^{-}$ in peripheral blood of patients with sepsis and SOFA, APACHEII and MODS in the severity of sepsis.

References

1) Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA, 2016, 315(8): 801-810.
2) Esposito S, De Simone G, Boccia G, et al. Sepsis and septic shock: New definitions, new diagnostic and therapeutic approaches. J Glob Antimicrob Resist, 2017, 10204-212.
3) Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA, 2016, 315(8): 762-774.
4) van Engelen TSR, Wiersinga WJ, Scicluna BP, et al. Biomarkers in Sepsis. Crit Care Clin, 2018, 34(1): 139-152.
5) Sharma D, Farahbakhsh N, Shastri S, et al. Biomarkers for diagnosis of neonatal sepsis: a literature review. J Matern Fetal Neonatal Med, 2017, 1-14.
6) Yang AP, Liu J, Yue LH, et al. Neutrophil CD64 combined with PCT, CRP and WBC improves the sensitivity for the early diagnosis of neonatal sepsis. Clin Chem Lab Med, 2016, 54(2): 345-351.
7) Marini O, Costa S, Bevilacqua D, et al. Mature CD10(+) and immature CD10(-) neutrophils present in G-CSF-treated donors display opposite effects on T cells. Blood, 2017, 129(10): 1343-1356.

8) Shipp MA, Stefano GB, Switzer SN, et al. CD10 (CALLA) /Neutral Endopeptidase 24.11 Modulates Inflammatory Peptide-Induced Changes in Neutrophil Morphology, Migration, and Adhesion Proteins and Is Itself Regulated by Neutrophil Activation. Blood, 1991, 787.
9) Urrechaga E, Boveda O, Aguirre U. Role of leucocytes cell population data in the early detection of sepsis. J Clin Pathol, 2017.
10) Seymour CW, Gesten F, Prescott HC, et al. Time to Treatment and Mortality during Mandated Emergency Care for Sepsis. N Engl J Med, 2017, 376(23): 2235-2244.
11) Karon BS, Tolan NV, Wockenfus AM, et al. Evaluation of lactate, white blood cell count, neutrophil count, procalcitonin and immature granulocyte count as biomarkers for sepsis in emergency department patients. Clin Biochem, 2017, 50(16-17): 956-958.

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