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CD11b as a Biomarker for Canine Systemic Inflammatory Response Syndrome and Sepsis

Do-Hyeon Yu, Dong-Ho Noh, Ru-Hui Song, Jun-Hwan Kim, Da-Mi Lee, Sue-Hee Kim, Chul Park and Jinho Park[1]

College of Veterinary Medicine & Bio-Safety Research Institute, Chonbuk National University, Jeonju, Jeonbuk 561-756, Korea

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Abstract : The aim of this study is to investigate neutrophil activation markers among canine ICU (intensive care unit) control, systemic inflammatory response syndrome (SIRS) and sepsis. These markers include WBC (white blood cells), platelets counts, blood film examination (neutrophilic band to segmentation ratio and neutrophilic degenerative changes), and flow cytometric analysis (CD11b expression of neutrophils). As a result, the mean CD11b fluorescence intensity of neutrophils and the neutrophilic degenerative change scores were both significantly higher in sepsis group ($P < 0.05$). In addition, mortality was also found to be correlated with the up-regulation of CD11b expression in circulating neutrophils. This study demonstrates that CD11b expression of neutrophils could be more a reliable biomarker to predict prognosis in ICU patients than traditional blood film examination according to this study.

Key words **:** neutrophil marker, CD11b, sepsis, systemic inflammatory response syndrome.

Introduction

Sepsis is a syndrome that is increasing in incidence, high mortality in companion animals (7). The estimated incidence of sepsis is 1-5% of feline cases and 6-10% of canine cases and the median estimates of survival are 10-25% and 25-50% in these animals, respectively (8). A better understanding of the pathophysiology and cellular mechanisms of sepsis is indispensable, and the identification of biomarkers that could quantify disease severity and predict patient prognosis would be beneficial in both research studies and clinical applications.

Since the activation of blood leukocytes is a commonly observed phenomenon in sepsis, an increase in the expression of the integrins of these neutrophils, in particular CD11b, is considered to be a good marker of cell activation (9). High CD11b expression of peripheral blood neutrophils in critically ill patients is believed to indicate a high risk of organ failure (3,6).

In the present study, we investigated CD11b expression by flow cytometry in non-septic systemic inflammatory response syndrome (SIRS) and septic dogs, and we also evaluated this parameter according to outcome. In addition, CBC (complete blood counts) and manual observations of the morphological changes of the neutrophils were also compared.

Materials and Methods

Patients

This was a prospective, observational study. Fourteen ICU (intensive care unit) cases recruited between November 2009 and January 2010 were categorized into one of three groups, which were the ICU control group without inflammation or infection (n = 4, congestive heart failure, intestinal intussusceptions, gastric foreign body, parasite infestation), SIRS without sepsis group (pancreatitis [n = 3], fever of unknown origin [n = 1]) and the sepsis group (n = 6, urinary tract infection, pyometra, bacterial pneumonia, canine distemper virus infection, canine parvoviral enteritis). Septic status was determined by bacterial culture or suspected bacterial infection. Pancreatitis was diagnosed by using IDEXX® cPLI (canine pancreatic lipase immunoreactivity) assay kit and pancreatic complications were excluded by diagnostic imaging system. Infectious diseases were also ruled out by bacterial culture in the fever of unknown origin case and this case was suspected as autoimmune disease which was responsive to steroid.

Diagnostic criteria for SIRS were based on the study by de Laforcade *et al*. (2), and mortality was defined as death or euthanasia due to poor prognosis. Survival was defined as discharge from the hospital.

Hematological tests

CBC including WBC and platelet counts was performed with an automated impedance cell counter (Vet ABC blood counter ABX Diagnostics, Montpellier, France) from EDTA anti-coagulated blood. The ratio of neutrophilic bands to segmented nuclei, and a semi-quantitative evaluation of the degenerative changes of the neutrophils (5) were blindly identified with manual differential counts. Neutrophilic degenerative changes were scored as grade 1 for the Doehle bodies and basophilia of the cytoplasm, grade 2 for foamy cytoplasm, grade 3 for dark blue-

[1]Corresponding author.
E-mail : jpark@jbnu.ac.kr

gray foamy cytoplasm and toxic granules, and grade 4 for having an indistinct nuclear membrane and karyolysis for grade.

Measurement of cell-surface antigen expression

Detecting expression of the cell surface molecule CD11b (marker of phagocytic cells, integrin αM, complement receptor CR3, ICAM's receptor) of granulocytes was performed using EDTA anti-coagulated blood. Blood was diluted in FACS buffer (PBS solution containing 1% bovine serum albumin) to 1×10^6 WBC in a volume of 200 μL. The mouse anti-dog CD11b monoclonal antibody (AbD Serotec, Oxford) was incubated for 30 min at 4°C. The RPE-conjugated rabbit F $(ab')_2$ anti-mouse IgG secondary antibody (AbD Serotec, Oxford) was then incubated for 20 min at 4°C. FACS lysing solution (BD Bioscience) was used to lyse red blood cells and fixate the cells by incubating the cells for 10 min in the dark at room temperature. The flow cytometric analysis was performed using a FACS Calibur (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA) flow cytometer. Granulocyte populations were gated using forward and sideward scatter parameters. The geometric mean fluorescence intensity (GMFI) of RPE-CD11b was measured from the strictly gated neutrophils. Data was acquired using a minimum of 10,000 events, and data analysis, including spectral compensation, was performed using FCS Express (FCS Express Version 3, De Novo Software).

Statistical analysis

Data are presented as the mean ± standard deviation (SD). A Shapiro-Wilk test was used to verify normality of the data. One-way ANOVA tests with the Scheffe *post hoc* test were used in order to compare significant differences in the variables among the three groups. When the null hypothesis was rejected, the Kruskal-Wallis non-parametric test was used. The chi-square test was used for the categorical variables. To evaluate the relationship between variables and survival, comparisons were made using either the Student-*t* test (parametric) or the Mann-Whitney U test (non-parametric). A two-tailed *P* value < 0.05 was considered to be statistically significant. The SPSS statistical software packages (SPSS 17.0, SPSS Inc, Chicago, IL) were used to perform the statistical evaluations.

Table 1. WBC, platelet counts and neutrophilic band/segmentation ratio in relation to the categorized groups and survival.

	WBC ($\times 10^9$/L)	Platelets ($\times 10^9$/L)	Band/Seg
ICU control	9.15 ± 4.01	332.25 ± 179.86	0.09 ± 0.04
SIRS	9.90 ± 4.55	181.25 ± 196.75	0.37 ± 0.36
sepsis	22.58 ± 14.52	247.60 ± 112.88	0.28 ± 0.30
P value	0.067[a]	0.252[a]	0.434[b]
survivors	16.14 ± 12.92	291.22 ± 173.21	0.19 ± 0.24
non-survivors	10.95 ± 4.85	167.75 ± 96.22	0.35 ± 0.34
P value	0.463[c]	0.315[d]	0.215[c]

[a]Kruskal-Wallis non-parametric test; [b]One-way ANOVA test; [c]Mann-Whitney U test; [d]Student *t*-test.

Results

The average age of the 14 dogs was 6.72 years, and the ranged from 6 months to 11 years. There were nine males (1 castrated) and five females (1 spayed), and the most frequent breeds were Maltese (21%), mixed (10.5%), and poodle (10.5%). There were nine survivors and five non-survivors (26.3% mortality rate).

CD11b expression (GMFI, 81.68 ± 30.11 for ICU control, 182.25 ± 10.40 for SIRS group and 221.72 ± 127.47 for sepsis group) and the degenerative change scores of the neutrophils are significantly different among the groups ($P = 0.049$ and 0.046, respectively) (Fig 1a). In addition, the CD11b expression in the sepsis group was significantly higher than that in the ICU control group when the Scheffe *post hoc* test ($P = 0.049$) was used. However, the number of WBC and platelets, as well as the ratio of, neutrophilic bands to segmentation, did not show statistical differences (Table 1).

For the comparison of outcome, the CD11b expression levels in non-survivors were significantly higher than in survivors ($P = 0.020$) (Fig 1b). There was no statistically significant difference between survivors and non-survivors with regard to the number of WBCs or platelets, the neutrophilic band/segmentation ratio, or the neutrophilic degenerative change scores (Table 2).

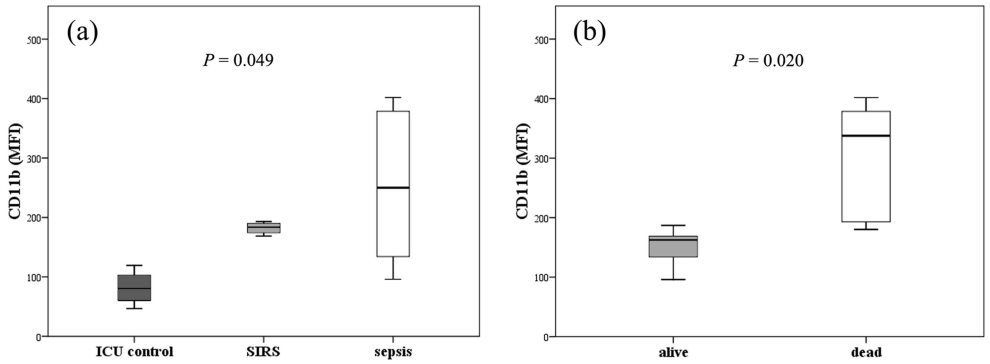

Fig 1. The geometric mean fluorescence intensity (GMFI) of CD11b expression of strictly gated neutrophils by flow cytometry according to the categorized group (ICU control/SIRS/sepsis) (a) and survival (b). CD11b expression in the sepsis group and the non-survivors was significantly higher than the ICU control group and the survivors, respectively ($P = 0.049$ and $P = 0.020$, respectively).

Table 2. Counts in neutrophilic degenerative change scores in relation to the categorized groups and survival.

Grade	0	1	2	3	4	*P* value
ICU control	3	1	0	0	0	
SIRS	0	0	1	0	3	0.029*
sepsis	0	0	1	0	5	0.010*
survivors	3	1	1	0	4	
non-survivors	0	0	1	0	4	0.190

*versus control group

Discussion

Neutrophil changes are commonly associated with infections and inflammations. Cytoplasmic immaturity and cellular degeneration were involved in the morphogenesis of neutrophils in the dog (4), and the more severe the toxic or degenerative changes indicates the severe the inflammatory state in the animal (10). In addition, activated blood neutrophils anchor via specialized adhesion molecules on the surface of endothelial cells. These inflammatory molecules increase the surface expression and avidity of the β2 integrins, CD11a and CD11b, which promotes firm adhesion to endothelium (1). Our study also revealed that neutrophilic degenerative change scores were higher in patients with canine SIRS and sepsis. We also observed higher CD11b expression of neutrophils in the dogs with sepsis, when compared with the ICU controls. Both of the neutrophilic markers seemed to correlate with disease severity, but only the CD11b expression was found to be significantly different between survivors and non-survivors. CD11b expression of neutrophils could be more reliable marker to predict prognosis in ICU patients than traditional blood film examination according to this study. This study is the first report that CD11b, as a prognostic marker. Since CD11b is involved in the adhesion of neutrophils, it can be speculated that leukocytes with high CD11b expression are rapidly sequestered from the circulation and cannot be detected in blood samples when the inflammatory response is induced (9). Thus, evaluation of CD11b expression in patients with inflammation would yield more reliable data. Nevertheless, the limitation of this study is the lack of a large study population. A broader spectrum of this research area should be performed in future studies. CD11b expression in patients with sepsis or septic shock and MODS (multiple organ failure syndrome) should be also evaluated.

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The authors declare no conflict of interest.

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개 전신성염증반응증후군 및 패혈증의 진단적 표지자로서 CD11b의 활용

유도현 · 노동호 · 송루희 · 김준환 · 이다미 · 김수희 · 박철 · 박진호[1]

전북대학교 수의과대학

요 약 : 본 연구는 중환자의 개에서 전신성염증반응증후군 및 패혈증에 의하여 유발되는 백혈구 활성화 지표를 분석하기 위하여 실행되었다. 분석된 지표로는 백혈구 수치, 혈소판 수치, 혈액 도말 표본의 백혈구 변화상 및 백혈구 표면의 CD11b발현 정도 등을 검사하였다. 그 결과, 패혈증군에서 백혈구의 CD11b 발현 값 (유세포 분석)이 현저하게 높게 관찰되었으며($P < 0.05$), 퇴행성 변화 정도도 같은 군에서 현저하게 높게 관찰 되었다($P < 0.05$). 또한 비 생존군의 CD11b 발현 값도 생존군에서의 값보다 현저하게 높았다. 이러한 연구 결과로 볼 때, 백혈구의 CD11b 발현은 중환자의 개에서 그 예후를 평가하는데 유용하다고 여겨진다.

주요어 : 백혈구 표지자, CD11b, 패혈증, 전신성염증반응증후군