

The predictive value of the inflammatory markers P-selectin and MCP1 in determining the length of stay and 30-day survival in the differentiation of sepsis patients

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Abstract

Objectives: Sepsis is the leading disease that is diagnosed late and still has a mortal course in emergency departments. The primary factors that will reduce both morbidity and mortality are early diagnosis and an early treatment approach. Therefore, in this study, P-selectin and MCP1 levels, which are known to be markers of inflammation, were examined in patients being followed up in intensive care.

Methods: Patients evaluated with a preliminary diagnosis of sepsis in the emergency intensive care unit between September 2015 and August 2016 were classified as having sepsis or infection according to the Q-SOFA criteria, and the P-selectin values were compared.

Results: In the sepsis group, GCS was determined as 13 (12-13), SBP 90 (80-110), tachypnea 24 (22-26), lactate 3.8 (0.6-16.0), MAP 70 (60-77), and LOS 16 days (9.5-20.3). In the ROC analysis, the sensitivity of P-selectin and MCP1 in the differentiation of patients with and without sepsis was 95.7%, and 73.8%, and the specificity was 97.8% and 73.8%, respectively. According to the cutoff values, the sensitivity and specificity in the prediction of patient mortality were 71.4% and 65.6% in P-selectin and 78.6% and 65.6% in MCP1.

Conclusion: The P-selectin and MCP1 values in the emergency department can differentiate sepsis patients according to the Q-SOFA criteria and showed 30-day mortality at a significant level. Therefore, in patients with suspected sepsis in an emergency department, MCP1 can be of benefit to physicians in their decisions regarding LOS and transfer to intensive care.

Keywords: Q-SOFA, Critical care, MCP1, P-selectin, Biomarkers, Sepsis, Mortality. (JPMA 68: 1321; 2018)

Introduction

Sepsis is a common cause of hospital admission and is a disease with significant levels of morbidity and mortality worldwide. In the USA, the annual incidence of sepsis is approximately 300-1031 per 100,000 of the population and the rate is increasing.¹ However, in-hospital mortality from sepsis fell from 35% in 2000 to 20% in 2013. Many studies have been conducted in this area, and research is ongoing.² This mortality rate could be further reduced with early suspicion and early diagnosis.

Sepsis with acute organ failure is widespread, fatal, and constitutes a significant national healthcare economic burden.³ As abnormalities in clotting and fibrinolysis are often seen in patients with sepsis, the properties of the vascular endothelium can be changed from anticoagulant to procoagulant.⁴ Fibrin accumulation and complementary activation may cause extensive vascular

wall damage and may be related to multiple organ failure.⁵

Endothelial activation is one of the differentiating characteristics of sepsis. The activated endothelium initiates inflammation by triggering leukocytes and thrombocytes, which will then express cell adhesion molecules on the surface. One such molecules is P-selectin, which is of vital importance for the natural immune system. P-selectin is found on the surface of the endothelium and thrombocytes and cuts the rate of leukocytes there and enables dissemination from the blood vessel to the infection or inflammation region.⁶ A series of reports have stated that the over-synthesis of P-selectin could form a procoagulant state that transmits TF to the growing thrombocyte thrombus of microparticles in the circulation which carry PSGL-1, which is a receptor against P-selectin.⁷

Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, is known to contribute to the pathogenesis of atherosclerosis by promoting the uptake of inflammatory cells to the vessel wall.⁸ It is responsible for the entry of monocytes to the vascular inflammation

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area.⁹⁻¹¹ CCL2 is an important molecule for the regulation of leukocyte function, endothelial activation, and monocyte chemotaxis, which mediate various inflammation-supporting biological activities.¹²

The aim of this study was to evaluate the power of P-selectin and MCP1 levels in determining the length of stay (LOS) and mortality in patients with suspected sepsis who were being followed up in the intensive care unit (ICU), as it was hypothesised that these markers would be elevated in patients with infection compared to patients without sepsis.

Materials and Methods

Adnan Menderes University Emergency Department patients were enrolled to the prospective study who were suspected to have sepsis and followed up in intensive care unit. Patient information was noted in the study form. According to the Q-SOFA criteria, patients were divided into two groups as sepsis and infection group. Samples for P-selectin and MCP1 were separated from the blood samples at the time of application and stored at -80°C.

The sepsis group was formed of patients with a score of ≥ 2 according to the Q-SOFA criteria (systolic blood pressure < 100 mmHg, GCS < 15 , tachypnea > 22), and the second group was formed of patients without sepsis.¹³

Patient inclusion criteria: The study included patients admitted to the emergency ICU with a preliminary diagnosis of sepsis and who were followed up for at least 30 days between September 2015 and August 2016.

Patient exclusion criteria: Patients with malignancy, chronic ischaemic disease, or stroke treatment were excluded from the study.

Blood collection and measurement of sP-selectin: Blood samples were obtained from the patients with suspected sepsis for routine testing. The samples were centrifuged, then stored at -80°C until assay. The P-selectin levels in the samples were determined using a commercial SUNLONG (Sun Long Biotech Co., LTD) human P-selectin ELISA kit (catalogue number SL1687Hu). Sensitivity was stated as 6.5 pg/ml. The intra-assay and inter-assay values were reported as 10% and 12%, respectively. The assay range was indicated as 16pg/ml-1000 pg/ml. The test results were calculated with the BIOELISA reader Elx800 using a standard curve.

Blood collection and measurement of MCP1: Blood samples were obtained from the patients with suspected sepsis for routine testing. The samples were centrifuged, then stored at -80°C until assay. The MCP1 levels in the samples were determined using a commercial SUNLONG

(Sun Long Biotech Co., LTD) human MCP1 ELISA kit (catalogue number SL1198Hu). Sensitivity was stated as 3.2 pg/ml. The intra-assay and inter-assay values were reported as 10% and 12%, respectively. The assay range was indicated as 10pg/ml-500 pg/ml. The test results were calculated with the BIOELISA reader Elx800 using a standard curve.

Ethical approval: The study methods were submitted to and approved by the Institutional Review Board of the Local Ethics Committee, and the need for informed consent was waived (Institutional Review Board research protocol 2015-660). The study was conducted in accordance with the Declaration of Helsinki.

Statistical analyses: Descriptive statistics for categorical variables were stated as number (n) and percentage (%). The Chi-square test was used to compare data between groups. The fit of continuous variables to normal distribution was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were stated as median values (25-75%), as none of the variables conformed to normal distribution. The Mann Whitney U-test was used for group comparisons. Spearman correlation coefficients were calculated to evaluate the relationships between variables. Receiver operator characteristic (ROC) curve analysis was applied to identify the optimal cutoff point of P-selectin and MCP1 (at which the sensitivity and specificity would be maximal) for the prediction of sepsis. The areas under the curve (AUCs) were calculated as measures of the accuracy of the tests and were compared using the Z test. Cox regression analysis and Kaplan-Meier analysis were used to determine the survival rates. Power analysis was performed for MCP1 and P selectin last of the study. A value of $p < 0.05$ was accepted as statistically significant (2-tailed test).

Results

Eighty-eight patients were evaluated, comprising 46 in the sepsis group and 42 in the infection group. The study group consisted of 46 patients with sepsis (18 female, 28 male, median age 67.0 (55.8-78.3)). There were 42 patients (30 female, 12 male, median age 47.0 (33.0-59.0)) in infection group.

In the sepsis group, GCS was determined as 13 (12-13), SBP 90 (80-110), tachypnea 24 (22-26), lactate 3.8 (0.6-16.0), MAP 70 (60-77), and LOS days 16 (9.5-20.3). Mortality was seen in 15 (32.6%) patients of the sepsis group.

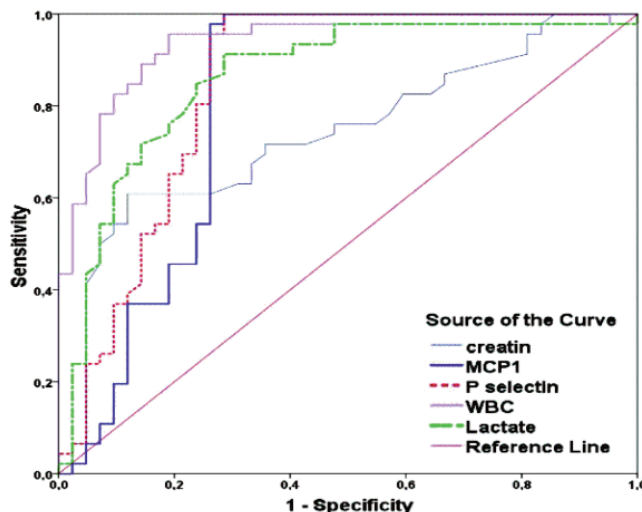
The demographic and laboratory values of the sepsis and infection groups are shown in Table-1. A statistically significant difference was determined between the

Table-1: Baseline characteristics and laboratory findings of the study groups.

	Infection n= 42	Sepsis n= 46	p Value
Age	47.0 (33.0-59.0)	67.0 (55.8-78.3)	0.104
Gender (female)	30 (50.8%)	18 (60%)	0.419
3-month survival/ex	0	15(32.6%)	<0.001
Length of stay	12.0(9.0-17.5)	16.0(9.5-20.3)	0.304
GKS	15(14-15)	13(12-13)	<0.001
Tachypnea	18(14-23)	24(22-26)	<0.001
MAP	73.3(70.0-80.0)	70.0(60.0-76.7)	0.122
BUN	45.0(31.8-52.8)	45.0(32.9-58)	0.699
Creatinine	1.0(0.8-1.2)	1.6(1.0-2.0)	<0.001
ALT	26.0(24.0-29.3)	25.0(15.8-36.0)	0.582
AST	37.0(34.5-42.0)	36.0(22.8-48.0)	0.848
LDH	235.0(184.0-275.8)	303(236-350)	0.560
Fibronectin	37.2(17.7-131.4)	21.2(10.7-29.3)	0.343
MCP-1	97.0(67.3-333.2)	348.3(137.5-773.8)	<0.001
P-Selectin	1478(68.7-546.8)	839.1(565.5-2138.5)	<0.001
CRP	112.3(41.0-137.0)	123.1(74.5-167.5)	0.354
Procalcitonin	1.3(0.4-3.8)	6.8(2.5-19.3)	0.073
WBC	8.5(6.4-9.6)	18.7(13.6-23.5)	<0.001
Lymphocyte	0.8(0.6-1.3)	1.4(0.8-2.0)	0.172
Lactate	1.86(0.78-14.60)	3.80(0.60-16.00)	<0.001
Thrombocyte	269.0(235.0-359.8)	256.0(185.8-356.5)	0.946

GKS: Glaskow Coma Scoring;
 MAP: Mean Arterial Pressure;
 BUN: Blood Urea Nitrogen;
 ALT: Alanine Aminotransferase;
 AST: Aspartate Aminotransferase;
 LDH: Lactate dehydrogenase;
 MCP1: Monocyte chemoattractant protein-1;
 CRP: C-reaktif protein ;
 WBC: white blood cell.

groups in respect to 30-day survival, creatinine, P-selectin, white blood cell (WBC), and MCP1 values (all p<0.001). ROC analysis was applied to the values with a significant



Graphic-1: The results of the ROC analysis applied to the markers determined as significant between the sepsis and infection groups.

difference between the groups. The results of the ROC analysis are shown in Table-2 and Graphic-1.

ROC analysis was then applied to evaluate these markers in the non-surviving patients. WBC (AUC 0.395, 95% CI 0.220-0.571, p=0.262) and creatinine (AUC 0.549, 95% CI 0.357-0.742, p=0.599) were not found to be significant. The MCP1, P-selectin, and lactate values were determined to be significant (Graphic 2, Table 3). No significant difference was found between the significant MCP1, P-selectin, and lactate values in the Z-test; all 3 determined mortality to the

same degree. Values were determined between MCP1 and P-selectin of p=0.794, between lactate and P-selectin

Table-2: ROC analysis results of the markers with significance between the sepsis and infection groups.

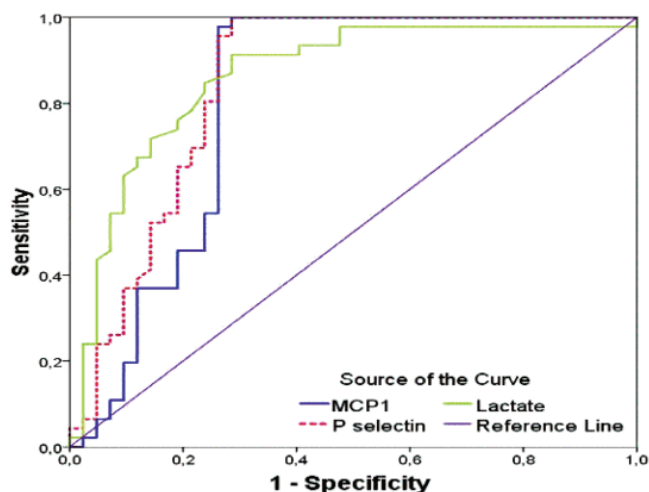
	AUC	Cut off	(95% CI)	P Value	Sensitivity	Specificity
Creatinine	0.739	1.025	(0.634-0.844)	0.000	76.10%	52.4%
MCP1	0.808	125.19	(0.704-0.912)	0.000	97.8%	73.8%
P Selectin	0.847	354.08	(0.757-0.936)	0.000	95.7%	73.8%
WBC	0.931	11.190	(0.876-0.987)	0.000	91.3%	83.3%
Lactate	0.862	1.82	(0.781-0.943)	0.000	91.3%	72.4%

MCP1: Monocyte chemoattractant protein-1; WBC: white blood cell.

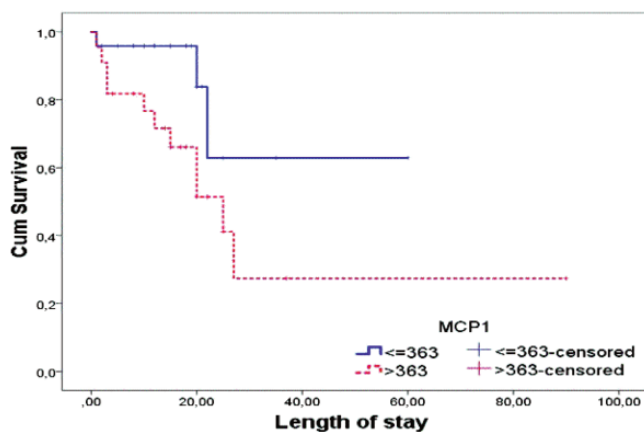
Table-3: ROC analysis of the MCP1, P selectin and lactate values for the evaluation of mortality in the sepsis patients.

	AUC	95% CI	P Value	Cut-off	Sensitivity (95%CI)	Specificity (95%CI)	+LR	-LR
P selectin	0.717	0.564-0.839	0.021	847.8	71.43% (41.9-91.6)	65.62%(46.8-81.4)	2.08	0.44
MCP1	0.746	0.596-0.862	0.002	363.0	78.57% (49.2-95.3)	65.62%(46.8-81.4)	2.29	0.33
Lactate	0.775	0.647-0.873	<0.001	4.12	57.14% (28.9-82.3)	91.11%(78.8-97.5)	4.02	0.71

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Graphic-2: The ROC analysis of the MCP1, P-selectin, and lactate values to evaluate the mortality course in sepsis patients.



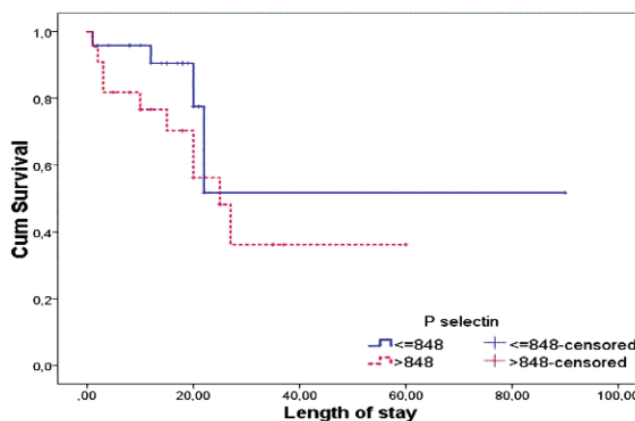
Graphic-3: The effect of MCP1 on LOS.

$p=0.722$, and between lactate and MCP1 $p=0.884$.

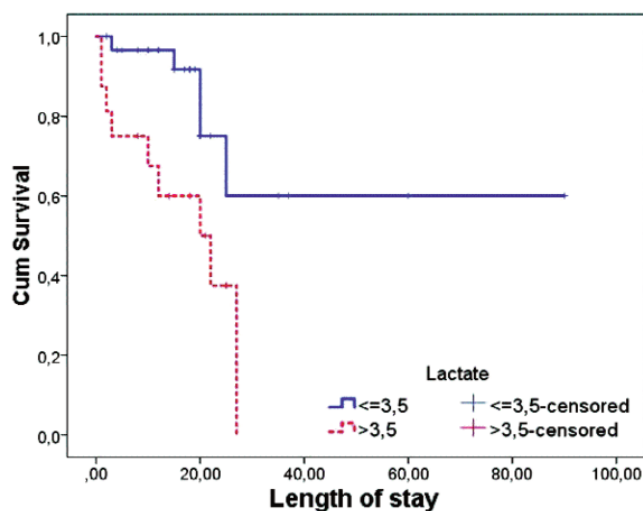
The Kaplan-Meier analysis of MCP1 is shown in Graphic 3. The mean LOS was 36.2 ± 10.1 days for those with an MCP1 cutoff value >363 and 44.8 ± 7.9 days for those below. The difference was determined to be statistically significant ($p=0.039$).

According to the Kaplan-Meier analysis, the mean LOS was 31.6 ± 6.0 days for those with a P-selectin cutoff value >848 and 55.5 ± 15.8 days for those below. The difference was not statistically significant ($p=0.220$) (Graphic-4).

The mean LOS was 16.9 ± 2.8 days for those with a lactate cutoff value >3.5 and 61.9 ± 11.1 days for those below. The difference was determined to be statistically significant ($p=0.008$) (Graphic-5).



Graphic-4: The effect of P-selectin on LOS.



Graphic-5: The effect of lactate on LOS.

The effects on LOS of age, heart rate, lactate dehydrogenase (LDH), systolic blood pressure, tachypnea, Q-SOFA score, MCP1, P-selectin, and lactate were analysed with Cox regression analysis. As a result of the analysis, lactate was found to be statistically significant ($p<0.001$).

Discussion

This study can be considered unique in respect to being the first to compare P-selectin and MCP1 in sepsis patients, not with a control group but with a group of patients with infection other than sepsis, and showing that these markers could strongly differentiate the patients and were correlated with 30-day mortality.

P-selectin was first described in 1992 as a vascular adhesion molecule that is critical in the inflammatory

response. Palabrica et al. showed that P-selectin played an important role in blood clotting and thrombosis.¹⁴ In a study by Mosad et al., P-selectin was reported to be elevated in patients with evident disseminated intravascular coagulation (DIC) compared to those without.¹⁵ Furie stated that there was a significant increase in the P-selectin expression in thrombocytes of patients with severe Systemic Inflammatory Response Syndrome (SIRS) compared to the values in normal healthy volunteers.¹⁶ In the current study, there was a statistically significant difference between the groups according to those who met the sepsis criteria compared to those with infection.

In a study by Pusch et al., P-selectin levels were examined in patients with acute ischaemic stroke with carotid stenosis and in chronic diseases such as Parkinson, and P-selectin was found to be significantly high.¹⁷ P-selectin has also been determined to be significantly high in patients with diabetes mellitus who developed several other diseases, such as ischaemic optic neuropathy,¹⁸ obesity,¹⁹ and SIRS.²⁰ In Schrijver et al's study, cases were grouped as acute and chronic, and the control group was comprised of those who did not meet the latest sepsis criteria, which supports the current study.²¹ In another study by Chiu et al., P-selectin was found to be higher in patients at high risk of acute myocardial infarctus compared to a control group.²¹

Accumulated evidence has shown that MCP1 has an important role in the pathogenic mechanisms leading to sepsis.²²⁻²⁵ In various studies, levels of MCP1 expression have been shown to be significantly increased in various murine models of sepsis, reflecting mortality and impaired organ function in sepsis patients.^{22,26} In the current study, MCP1 in sepsis patients was seen to be significant both in the diagnosis and the prediction of LOS and mortality.

When comparing patients with sepsis from those with infection in an emergency department, WBC, lactate, MCP1, and P-selectin were found to be significant in the differentiation of patients; lactate, MCP1, and P-selectin were significant in the prediction of mortality. Lactate and MCP1 were determined to be significant in the prediction of LOS. As lactate can be elevated in several diseases, MCP1 should be considered for use in the determination of these three states in the differentiation of patients in emergency departments.

A limitation of our study, the patients in our sample size is not receiving with a power analysis because there are no studies on this subject in literature. So that patients were enrolled in the study over a one year period.

However, the power analysis was 0.91 for the MCP1 and 0.88 for the P-selectin.

Conclusions

As sepsis is one of the primary diseases for which it is difficult to make a definitive diagnosis in emergency departments, physicians are left with the problem of determining where these patients should be followed up. MCP1, which is a marker of endothelial activation, can statistically differentiate sepsis patients diagnosed according to the Q-SOFA criteria and can show 30-day mortality at a statistically significant level. Therefore, MCP1 levels can be considered of benefit to physicians on the subject of LOS, survival, and transfer to ICU for patients with suspected sepsis in an emergency department.

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Conflicts of Interests: None.

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