

## Correlation of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, lymphocyte-to-monocyte ratio and carcinoembryonic antigen level in colorectal cancer

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Original Article

### ABSTRACT

#### ARTICLE INFO

##### Keywords:

neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, lymphocyte-monocyte ratio, carcinoembryonic antigen, colorectal cancer

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DOI : 10.20885/JKKI.Vol9.Iss2.art4

##### History:

Received: August 30, 2017

Accepted: May 5, 2018

Online: August 30, 2018

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**Background:** Colorectal cancer is one of cancer that has a high mortality rate. Cancer is associated with a systemic inflammatory response. Carcinoembryonic antigen (CEA) is a colorectal cancer prognostic marker, but this examination is quite expensive and not always ubiquitously available. Therefore, systemic inflammatory markers through routine blood examination are expected as a colorectal cancer marker.

**Objective:** The present study is to determine a correlation of neutrophil-lymphocyte ratio (NLR), the platelet-lymphocyte ratio (PLR), the lymphocyte-monocyte ratio (LMR) and CEA level in colorectal cancer patients.

**Methods:** This retrospective cross-sectional study was conducted in November 2016-January 2017 at Dr. Sardjito Hospital, Yogyakarta. As many as 209 patients who underwent surgery in January 2015-December 2016 with colorectal cancer histopathologic diagnosis were included. Demographic, clinical, histopathology and preoperative NLR, PLR, LMR and CEA level was obtained from medical records. Correlation of NLR, PLR, LMR and CEA level was analyzed with Somer's test.

**Result:** As many as 51.2% of subjects were male. Most subjects (n 42.6%) were diagnosed at 45-60 years old. The most common location of the tumor is in rectum (65.1%). Histopathology examination showed well differentiation in 51.7% subjects. The Somer's analysis showed there was correlation of NLR and CEA level ( $p=0.023$ ,  $r=0.164$ ), there was a correlation between PLR and CEA level ( $p=0.016$ ,  $r=0.146$ ). Higher NLR and PLR was proportional to higher CEA level. There was a correlation of LMR and CEA level ( $p=0.001$ ,  $r=-0.188$ ), lower LMR correlates to higher CEA level.

**Conclusion:** There is significant positive correlation of NLR and CEA level, PLR and CEA level, and negative correlation of LMR and CEA level in colorectal cancer patients.

**Latar Belakang:** Kanker kolorektal merupakan kanker dengan angka kematian yang tinggi. Kanker berhubungan dengan respon inflamasi sistemik. Carcinoembryonic antigen (CEA) merupakan penanda yang diperhitungkan dalam prognostik kanker kolorektal namun cukup mahal dan tidak selalu ada. Oleh karena itu, penanda inflamasi sistemik melalui pemeriksaan darah rutin diharapkan dapat dikembangkan sebagai penanda pada kanker kolorektal.

**Tujuan:** Mengetahui korelasi rasio neutrofil-limfosit (RNL), rasio platelet-limfosit (RPL), rasio limfosit-monosit (RLM) dan level CEA pada pasien kanker kolorektal.

**Metode:** Penelitian ini merupakan penelitian retrospektif cross sectional yang dilaksanakan pada November 2016-Februari 2017 di RSUP Dr.Sardjito, Yogyakarta pada 209 pasien yang menjalani operasi pada Januari 2015-Desember 2016 dengan histopatologi kanker kolorektal. Data demografik, klinis, pemeriksaan histopatologis dan laboratorium RNL, RPL, RLM dan CEA preoperatif diambil dari rekam medis pasien. Hubungan antara RNL, RPL, RLM dan level CEA dianalisis dengan uji Somer's.

**Hasil:** Sebanyak 51,2% subyek penelitian adalah laki-laki. Usia terdiagnosis tersering (42,6%) pada usia 45-60 tahun. Lokasi tersering tumor berada di rectum (65,1%). Gambaran histopatologis diferensiasi baik pada 51,7% subyek. Terdapat korelasi RNL dan level CEA ( $p=0,023$ ,  $r=0,164$ ), terdapat korelasi RPL dan level CEA ( $p=0,016$ ,  $r=0,146$ ). Tingginya RNL dan RPL sebanding dengan semakin tingginya level CEA. Terdapat korelasi RLM dan level CEA ( $p=0,001$ ,  $r=-0,188$ ), dimana semakin rendah RLM semakin tinggi level CEA.

**Kesimpulan:** Terdapat korelasi positif RNL dan level CEA, RPL dan level CEA, serta terdapat korelasi negatif RLM dan level CEA pada pasien kanker kolorektal.

## INTRODUCTION

The incidence of colorectal cancer is the third most common in men (746,000 cases; 10.0% of total cancer) and the second in women (614,000 cases; 9.2% of total cancer) worldwide. Colorectal cancer becomes the third leading cause of worldwide cancer mortality.<sup>1</sup> In Indonesia, the incidence of colorectal cancer is about 15,985 in men and 11,787 in women; with mortality is about 10.2% in men and 8.5% in women.<sup>2</sup> Despite the progress in therapy, the overall prognosis of colorectal cancer is still weak because most patients are diagnosed at an advanced stage, which leads to a poorer prognosis.<sup>3</sup>

Cancer as a chronic process is associated with an inflammatory response. Systemic inflammatory marker studies are expected to develop biomarkers for colorectal cancer

through a simple, ubiquitously available, and inexpensive examination. One of them is through a routine blood test. Neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the lymphocyte-to-monocyte ratio (LMR) has been reported can be used to predict the prognosis of colorectal cancer patients.<sup>4-9</sup> However, the clinical utility of the NLR, PLR, and LMR remains poorly defined and inconsistent. On the other hand, CEA is a well-known biomarker in colorectal cancer and has value in the follow-up of colorectal cancer patients. Patients with preoperative serum CEA > 5 ng/mL have a worse prognosis than patients with lower CEA level.<sup>3,10,11</sup> On the other hand, CEA testing is not always available and quite expensive. Therefore, this study aims to know the correlation of NLR, PLR, LMR and CEA level in colorectal cancer patients.

## METHODS

### Study design

This present cross-sectional study is a retrospective which was conducted at Dr. Sardjito Hospital, Yogyakarta in November 2016-February 2017. Data were taken from the patient's medical records.

### Population and Subjects

The study population was colorectal cancer patients. The inclusion criteria of the subjects were colorectal cancer patients who underwent surgery at RSUP Dr. Sardjito in January 2015-December 2016, with histopathological examination confirmed colorectal cancer. The exclusion criteria of the subjects were the presence of infections, diseases of the blood system, other intestinal diseases, i.e., colitis ulcerative and Crohn disease; with other cancers, with a history of cancer in other organs; incomplete clinical parameters and pre-operative laboratory results.

### Ethical consideration

This study was approved by Educational and Research Section of Dr. Sardjito Hospital, Yogyakarta.

## Measurement

Demographic data (sex and age), clinical data on tumor location, and the histopathologic form and differentiation of colorectal cancer were retrieved. The laboratory examination of platelet values, neutrophils, lymphocytes, monocytes and serum CEA were taken at the earliest within 30 days before surgery.

The platelets, neutrophils, lymphocytes, monocytes were obtained from routine blood tests with automatic hematology analyzer. NLR is the ratio between the numbers of neutrophil and lymphocyte, with NLR cut-off was 2.56.<sup>12</sup> PLR is the ratio between the numbers of platelet and lymphocyte, with PLR cut-off was 258. LMR is the ratio between the numbers of lymphocytes and monocytes, with LMR cut-off was 2.38.<sup>13</sup>

CEA is a tumor marker for colorectal cancer. A total of 10  $\mu$ L blood serum was taken to check the CEA level. The CEA level was measured with electrochemiluminescence immunoassay (ECLIA) method on the Cobas immunoassay analyzer. The CEA level is in the range of 0.200-

1,000 ng/mL. The lower limit detection is reported with <0.200 ng/mL while the upper limit is reported with >1000 ng/mL. The CEA level cut-off is 5.00 ng/mL.<sup>3,10,11</sup>

## Data Analysis

The data were analyzed with SPSS 22. The data were analyzed descriptively and then continued with Somer's test to know the correlation of NLR, PLR, LMR and CEA level in colorectal cancer patients. The p-value <0.05 was considered significant.

## RESULT

From 329 subjects with histopathology of colorectal cancer, 209 subjects were included in this study. The mean  $\pm$  SD age of the subjects was 57.30  $\pm$  12.33 years. The youngest age diagnosed with colorectal cancer was 25.34 years. The characteristics and the laboratory examination results of subjects were shown in Table 1 and Table 2.

Table 1. Characteristics of subjects (n=209)

Characteristics	n	%
Sex		
Male	107	51.2
Female	102	48.8
Age (year)		
<30	5	2.4
30-45	29	13.9
45-60	89	42.6
$\geq$ 60	86	41.1
Tumor location		
Rectum	136	65.1
Colon	73	34.9
Differentiation		
Poor	21	10.0
Moderate	77	36.8
Well	108	51.7
Mucinous	3	1.4

Tabel 2. Laboratory results of subjects (n=209)

Examinantions	Median (minimum-maximum)	
	n	%
CEA (ng/mL)	13.08 (0.20-1,000.00)	
≥ 5	151	72.2
< 5	58	27.8
Leukocyte (103/μL)	8.26 (3.24-33.69)	
Neutrophile (103/μL)	5.80 (1.74-31.78)	
Lymphocyte (103/μL)	1.59 (0.19-11.60)	
Monocyte (103/μL)	0.51 (0.02-1.65)	
Platelet (103/μL)	348.00 (75.00-1,229.00)	
NLR	3.51 (0.72-74.90)	
≥2.56	148	70.8
<2.56	61	29.2
PLR	229.52 (54.97-2,194.74)	
≥258	84	40.2
<258	125	59.8
LMR	3.06 (0.32-34.12)	
>2.38	133	63.6
≤2.38	76	36.4

CEA=carcinoembrionic antigen; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; LMR=lymphocyte-to-monocyte ratio

The Somer's analysis showed a positive correlation between NLR and CEA ( $p < 0.05$ ,  $r = 0.164$ ) and positive correlation of PLR and CEA level ( $p < 0.05$ ,  $r = 0.146$ ). Higher NLR and PLR

are proportional to higher CEA level. There was a negative correlation between LMR and CEA level ( $p < 0.001$ ,  $r = -0.188$ ), lower LMR correlate with higher CEA level (Table 3).

Tabel 3. Correlation of NLR, PLR, LMR and CEA level (n=209)

		CEA ≥5 ng/mL		CEA <5 ng/mL		r	p
		n	%	n	%		
NLR	≥2.56	114	54.5%	34	16.3%	0.164	0.023*
	<2.56	37	17.7%	24	11.5%		
PLR	≥258	68	32.5%	16	7.7%	0.146	0.016*
	<258	83	39.7%	42	20.1%		
LMR	>2.38	87	41.6%	46	22.0%	-0.188	0.001**
	≤2.38	64	30.6%	12	5.7%		

\* $p < 0.05$ ; \*\* $p < 0.01$

CEA=carcinoembrionic antigen; NLR=neutrophil-to-lymphocyte ratio; PLR=platelet-to-lymphocyte ratio; LMR=lymphocyte-to-monocyte ratio

## DISCUSSION

The incidence of colorectal cancer in males was greater as reported before. Colorectal cancer

incidence in men and women under 50 years continued to increase from 1992 to 2012 by 2.1% per year. More than 86% of people diagnosed

when less than 50 years old have symptoms, with more advanced stage and have a worse prognosis. The location of the tumor in general concerns the left side, especially in the rectum (3.9% per year).<sup>1,3</sup>

Carcinoembryonic antigen level examination is not recommended in the screening or diagnostics of colorectal cancer due to the low sensitivity and specificity. Also, a high level of CEA can occur in other diseases, such as gastritis, peptic ulcer, diverticulitis, liver disease, chronic obstructive pulmonary disease, diabetes mellitus, and acute or chronic inflammatory state. The CEA level is also found to be higher in smokers. However, CEA level is a valuable biomarker in the follow-up of colorectal cancer patients. The CEA level >5.00 ng/mL before surgery has a poorer prognosis than the lower level. A high CEA level after surgery implies the need for evaluation or further action.<sup>3,10,11</sup>

Our study showed that higher NLR is proportional to the CEA level. Systemic inflammatory response plays a role in the development of multiple cancers through genetic mutations, genomic instability, epigenetic modification, cancer cell proliferation in various stages, and tumor metastasis.<sup>14-16</sup> Macrophages are abundant in intestinal lamina propria, and tumor-associated macrophages (TAMs) are associated with tumor development. Type I macrophages (M1) produce proinflammatory cytokines involved in the pathogens and tumor cells killing mechanism, such as Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), interleukin (IL)-12, and creating oxidative environments by producing inducible Nitric Oxide Synthase (iNOS) and reactive oxygen species (ROS).<sup>15</sup>

Type I macrophages produce IL-23, which triggers IL-17. IL-17 subsequently induces IL-1, IL-6, IL-8, Chemokine ligand 1 and TNF- $\alpha$  production in stromal, epithelial and endothelial cells, and monocyte subsets. Together, these proinflammatory cytokines recruit neutrophils to peripheral tissues to do phagocytosis and apoptosis. Neutrophil apoptosis decreases IL-23 secretion and reduces production of IL-17, granulocyte-Colony Stimulating Factor (GCSF)

and granulopoiesis. In chronic inflammatory conditions such as colorectal cancer, these processes are disrupted. IL-23 continues to be produced, promoting IL-17 expression, as well as increasing neutrophils and monocytes in peripheral tissues.<sup>15</sup> These changes cause the neutrophils to be abundant in chronic inflammation, in which tumor develops. Neutrophil apoptosis that is not eliminated by macrophages release intracellular granules that increase tissue damage. RNL associated with CEA levels has been reported in several studies.<sup>12,17-20</sup>

In contrast, type II macrophages (M2) moderate the inflammatory response, eliminate the residual apoptotic neutrophil, increase angiogenesis by producing proangiogenic cytokines, such as Vascular Endothelial Growth Factor (VEGF)-A, VEGF-C, TNF- $\alpha$ , IL-8; and stimulate the production of arginase for cell replication, collagen deposition, and tissue repair. TAMs often express as M2 phenotype when the tumor is vascular, developing, and invading.<sup>15,18</sup>

Lymphocytes are the fundamental component of the innate and adaptive immune system and play a role in cellular immunosurveillance and immunoediting, and tumor suppression. Lymphocytes infiltrate the microtumor environment and trigger of the immune-antitumor response. The interaction of cluster of differentiation 8 (CD8+) and CD4+ T-lymphocytes induces cytotoxic cells and tumor cell apoptosis. Also, lymphocytes produce cytokines that inhibit the proliferation and metastasis of cancer cells.<sup>12,19-21</sup>

Our study showed that higher PLR is proportional to the CEA level. The meta-analysis study reported an increase in PLR associated with poor prognosis. The interactions between platelets and tumors are not fully understood. Platelets play a role in VEGF formation through the release of platelet-proangiogenic mediators in the tumor blood vessels. Proinflammatory mediators also stimulate megakaryocyte proliferation through Platelet-Derived Growth Factor (PDGF) that supports tumor development. Also, platelets release microparticles that help

tumor cells escape from natural killer cells.<sup>7,9,21</sup>

Our study showed lower LMR correlate with higher CEA level. The low LMR associated with a poor prognosis in colorectal cancer is reported in previous studies.<sup>8,13,21</sup> Cancer cells recruit myeloid-derived suppressor cells that possess some of the innate immune cell characteristics, such as monocytes, macrophages, neutrophils, and dendritic cells that are known to have immunosuppressive activity and assist angiogenesis tumor. Increased myeloid-derived suppressor numbers may be reflected in an increase in the number of monocytes.<sup>13,16</sup> Monocytes secrete cytokines, such as TNF- $\alpha$  and IL-1. Also TAMs, circulating monocytes, have a role in suppressing adaptive immunity and increasing angiogenesis, invasion, and migration. Therefore, elevated levels of circulating serum monocytes may reflect increased levels of TAMs and a worse prognosis.<sup>13,15</sup>

## CONCLUSION

This study concludes that there was a significant proportional correlation of NLR and CEA. There was a significant proportional correlation of PLR and CEA level. There was a negative correlation between LMR and CEA level.

The limitation in this study include potential confounding factors such as ischemia, acute coronary syndrome, metabolic syndrome, diabetes mellitus and renal or liver dysfunction and there were not set the optimal cut-off of NLR, PLR, and LMR. Therefore, longitudinal research with prognostic design, a more significant number of subjects, multicenter with more variables, at a more specific stage of cancer would be useful for further investigation.

## CONFLICT OF INTEREST

We declare that there is no conflict of interest.

## Acknowledgement

Maynard Andrew Maramis (Department of Surgery, Universitas Gadjah Mada/Dr. Sardjito Hospital, Yogyakarta) for the cooperation.

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