Original Article

Can Pre-operative Neutrophil-lymphocyte Ratio Predict Microscopic Perforated Colon Cancer Outcome?

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Key Words
Colon cancer;
Perforation;
Neutrophil-lymphocyte ratio;
Survival

Purpose. Current studies showed inflammatory microenvironment associated with cancer progression. Neutrophil-to-lymphocyte ratio (NLR) is a known significant prognostic marker in resectable colorectal cancer patients; however, there is no research for perforated colon cancer patients.
Method. We presented a retrospective study from our database of colorectal cancer research from 1995 to 2015. Microscopic-perforation colon cancer patients reviewed by pathology report were included. Exclusion criteria were short-term mortality (< 30 days mortality), post-polypectomy perforation and confirmed distal metastasis patient. Clinicopathological factors, long-term overall survival, disease-free survival were analyzed and compared between high (NLR > 5) and low (NLR ≤ 5) groups.
Main outcome measures. The primary outcome was overall survival.
Result. Comparing higher and lower neutrophil-to-lymphocyte ratio (NLR) groups in microscopic perforated colon cancer patients, the leukocytosis (WBC > 12000/uL) (12.9% vs. 59.7%, $p < 0.001$), lower serum albumin level (albumin < 3.5 g/dL) (32.3% vs. 54.5%, $p = 0.003$), and emergent operation rate (5.4% vs. 26%, $p < 0.001$) were significantly higher in higher NLR groups.
However, long term overall survival rate ($p = 0.610$) and disease-free survival rate ($p = 0.139$) showed no significant difference between these two groups. The prognostic factors for overall survival and disease free survival in this study were patients aged > 65 years, Hb ≤ 10 g/dL, and N stage.
Limitations. This is a retrospective, non-randomized study, and also limited by selection bias and small case number in a single institute.
Conclusion. Neutrophil-to-lymphocyte ratio (NLR) has limited predictive value for outcome in microscopic perforation patients.
[*J Soc Colon Rectal Surgeon* (*Taiwan*) *2018;29:204-210*]

Colon and rectal cancer (CRC) is now the third most commonly diagnosed cancer in men, the second in women, and also the fourth leading cause of cancer-related death worldwide.[1] According to the latest report in Taiwan based on Health Promotion Administration for all cancers, colon and rectal cancer is the 2nd in incidence and 3rd in mortality. (government general budget of Health Promotion Administration, Taiwan. (https://www.hpa.gov.tw/Pages/List.aspx?nodeid=269)). Although there are some improvements

Received: June 29, 2018. Accepted: August 14, 2018.
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in current cancer treatments, the long-term survival outcomes are still unsatisfied. There were increasing reports showed that systemic chronic inflammatory reaction is related to the promotion of carcinogenesis in cancers.[2,3] There were more and more studies focused on inflammation-based prognostic factors, such as C-reactive protein (CRP), albumin, platelet to lymphocyte ratio (PLR), and the neutrophil-to-lymphocyte ratio (NLR), to identify high-risk patients for CRC recurrence after primary surgery.[4-7] Neutrophil-to-lymphocyte ratio (NLR) is now a significant prognostic marker in patients with resectable colorectal cancer (CRC). NLR is considered not only a tumor-related inflammatory environment marker but also a presentation of host immunity for anti-tumor ability. Pre-operative NLR > 5 significantly associated with poor overall survival.[8-10]

Based on Linkou Chang Gung Memorial Hospital's data, pre-operative NLR influenced the disease-free survival in stages I to III CRC patient, and elevated NLR (estimate cut-off value > 3) was associated with worse outcome (5-year disease-free survival 66.3% vs. 78.9% in colon cancer, $p < 0.001$; 60.5% vs. 66.2% in rectal cancer, $p = 0.008$).[11]

However, there's no report discussed between perforated colon cancer and NLR. Thus, we present data from our prospective database on the correlation of preoperative NLR with clinicopathologic features and the outcomes on survival between perforated colon cancer patients.

Materials and Methods

Study population and inclusion/exclusion criteria

From January 1, 1995 to December 31, 2015, 240 pathologically proven perforated colon cancer patients who underwent surgical resection at Chang Gung Memorial Hospital, Linkou were reviewed retrospectively in this study. The definition of colon perforated was based on pathology reports, whether gross or microscopic perforation was mentioned. We had excluded patients who were post-polypectomy perforation (n = 5); who were short-term mortality (less then 30 days post-operation mortality, n = 4), and who had impressed distal metastasis before operation (n = 61). Pre-operation evaluations and post operation follow-up data were collected for analysis. Recurrence or second primary cancer was recorded with date, stage, location, treatments, and cause of death.

Data collection and presentation

Patient and demographic characteristics before operation included age, gender, hepatitis status, diabetes status, liver cirrhosis, serum albumin, CEA level, WBC count, Hb, and NLR. Perioperative factors included tumor location, and emergency operation or not. Tumor TNM stage, histology, and pathology features were included.

Definition and cuff-off value of NLR

The NLR was defined as calculated by dividing the number of neutrophils by number of lymphocytes from serum differential count.

The preoperative NLR were recorded on the latest data before operation, ether at ER or during admission. The cut-off value of NLR in this study was defined as 5.0.

Study end point

The end point in this study was survival time. The cause of death was recorded as an event. Patients were recorded alive at last follow up, with disease or not. Survival duration was measured from the operation date to the date of death or last known follow-up.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Data Editor 24.0 (SAS institute Inc., Cary, NC, USA), and values of $p < 0.05$ was considered statistically significant. Pearson's chi-squared test was used for clinicopathologic descriptive data to determine the correlation between higher and lower NLR groups. Kaplan-Meier methods were used to assess

survival outcome by different subgroups. The Cox proportional hazards model was used for univariate and multivariate analyses, and variables with $p < 0.05$ in univariate analysis were entered into further multivariate analyses to determined independent survival risks.

Result

After reviewing pathology report, 240 patients were confirmed perforated colon cancer (gross or microscopic perforation). All patients underwent surgical treatment, including emergent explore laparotomy. We excluded 5 patients due to post-polypectomy and had operation within 24 hours, 4 patients due to early mortality (less then 30 post-operation days) and 61 patients due to confirmed distal metastasis (Fig. 1). 170 patients were finally enrolled in this study. The lower neutrophil-to-lymphocyte ratio (NLR) was defined as less and equal to 5 was 54.7 % (n = 93); the higher NLR was defined as greater then 5, and was about 45.3% (n = 77). The mean follow-up time in overall patients was 73.5 months, ranging from 1.7 to 135.7 months.

Clinicopathologic factors in patients according to low versus high preoperative neutrophil-to-lymphocyte ratio

Clinicopathological factors were compared between higher (greater than 5.0) and lower NLR groups (5.0 or less) and showed in Table 1. In higher NLR group, there significant correlation in higher possibility of leukocytosis (WBC > 12000/uL, (12.9% vs. 59.7%, $p < 0.001$), lower serum albumin level (albumin < 3.5 g/dL, 32.3% vs. 54.5%, $p = 0.003$), and more likelihood of having emergent operation (5.4% vs. 26%, $p < 0.001$).

Kaplan-Meier analysis of overall survival (OS) and NLR

The 5-year OS rate was 57% in higher NLR group, and 65% in lower NLR group. In 3-year OS, higher NLR group was 73% and lower NLR group was 76%. Both overall survival rates showed no statistical difference between higher and lower NLR groups ($p = 0.610$) (Fig. 2).

Kaplan-Meier analysis of disease free survival (DFS) and NLR

Disease free survival also showed no statistically significant difference between higher and lower NLR groups. 5-year disease-free survival rate was 55% in higher NLR group, 69% in lower NLR group. In 3-year disease-free survival rate, higher NLR group was 63% and the lower group was 72%($p = 0.139$) (Fig. 3).

Discussion

Chronic inflammation-induced carcinogenesis has been a commonly acceptable concept in several malignancies.[3] In colon cancer, the systemically inflammatory not only affects cytokines, promotion of angiogenesis, but also metastasis.[12] Therefore, several systemic inflammatory associated markers such as C-reactive protein (CRP), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), NLR and signed inflammation-based prognostic models were used as outcome predictors.[4-7] As we know, tu-

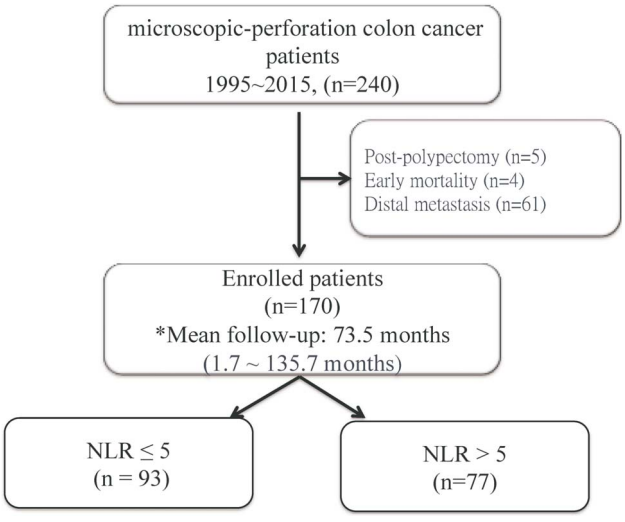
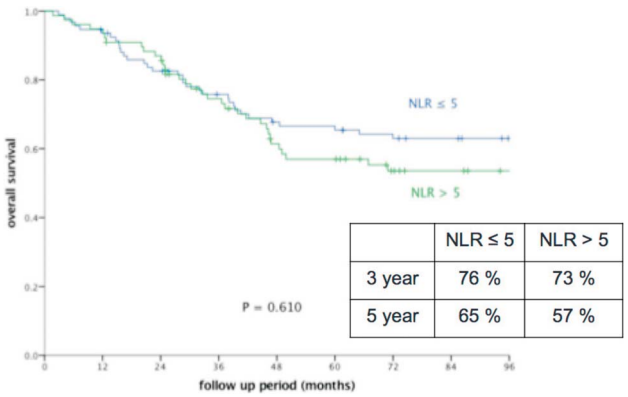


Fig. 1. Flow chart – study population.

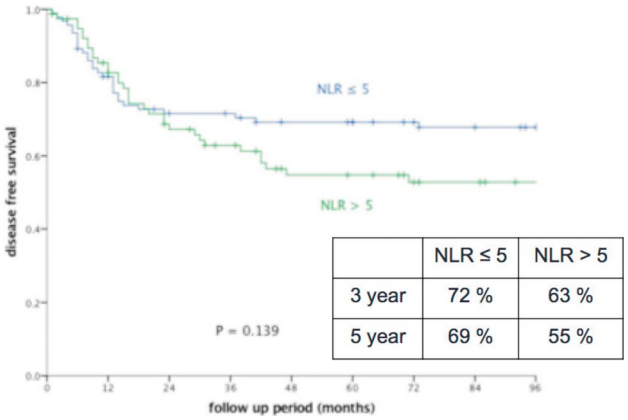
Table 1. Demographic data

	Total (n = 170)	NLR ≤ 5 (n = 93)	NLR > 5 (n = 77)	*p*-value
Age				0.565
≤ 65 y/o	99 (58.2%)	56 (60.2%)	43 (55.8%)	
> 65 y/o	71 (41.8%)	37 (39.8%)	34 (44.2%)	
Sex				0.131
Female	77 (45.3%)	47 (50.5%)	30 (39.0%)	
Male	93(54.7%)	46 (49.5%)	47 (61.0%)	
HB				0.347
≤ 10 g/dL	75 (44.1%)	38 (40.9%)	37 (48.1%)	
> 10 g/dL	95 (55.9%)	55 (59.1%)	40 (51.9%)	
WBC				< 0.001
≤ 12000 /uL	112 (65.9%)	81 (87.1%)	31 (40.3%)	
> 12000 /uL	58 (34.1%)	12 (12.9%)	46 (59.7%)	
CEA				0.397
≤ 5 ng/mL	70 (41.2%)	41 (44.1%)	29 (37.7%)	
> 5 ng/mL	100 (58.8%)	52 (55.9%)	48 (62.3%)	
Albumin				0.003
≤ 3.5 g/dL	72 (42.4%)	30 (32.3%)	42 (54.5%)	
> 3.5 g/dL	98 (57.6%)	63 (67.7%)	35 (45.5%)	
DM				0.346
No	146 (58.9%)	82 (88.2%)	64 (83.1%)	
Yes	24 (14.1%)	11 (11.8%)	13 (16.9%)	
Hepatitis				0.809
No	165 (97.1%)	90 (96.8%)	75 (97.4%)	
Yes	5 (2.9%)	3 (3.2%)	2 (2.6%)	
Liver cirrhosis				0.893
No	168 (98.8%)	92 (98.9%)	76 (98.7%)	
Yes	2 (1.2%)	1 (1.13%)	1 (1.3%)	
Location				0.432
Right	63 (37.1%)	32 (34.4%)	31 (40.3%)	
Left	107 (62.9%)	61 (65.6%)	46 (59.7%)	
Emergency OP				< 0.001
No	142 (85.3%)	88 (94.6%)	57 (74.0%)	
Yes	25 (14.7%)	5 (5.4%)	20 (26.0%)	
Histology type				0.408
Adenocarcinoma	146 (85.9%)	78 (83.9%)	68 (88.3%)	
Mucinous	24 (14.1%)	15 (16.1%)	9 (11.7%)	
Histology grade				0.166
Well diff.	11 (6.5%)	9 (9.7%)	2 (2.6%)	
Moderate	133 (78.2%)	71 (76.3%)	62 (80.5%)	
Poor	26 (15.3%)	13 (14.0%)	13 (16.9%)	
T stage				0.338
2	1 (0.6%)	0 (0%)	1 (1.3%)	
3	24 (14.1%)	11 (11.8%)	13 (16.9%)	
4	145 (85.3%)	82 (88.2%)	63 (81.8%)	
N stage				0.268
0	89 (52.4%)	49 (52.7%)	40 (51.9%)	
1	54 (31.8%)	33 (35.5%)	21 (27.3%)	
2	27 (15.9%)	11 (11.9%)	16 (20.8%)	
TNM stage				0.545
1	1 (0.6%)	0 (0%)	1 (1.3%)	
2	89 (52.4%)	49 (52.7%)	40 (51.9%)	
3	80 (47.1%)	44 (47.3%)	36 (46.8%)	



	NLR ≤ 5	NLR > 5
3 year	76 %	73 %
5 year	65 %	57 %

Fig. 2. Overall survival rate between high and low NLR patients.



	NLR ≤ 5	NLR > 5
3 year	72 %	63 %
5 year	69 %	55 %

Fig. 3. Disease-free survival rate between high and low NLR patients.

mor associated cytokines and growth factors enter the circulation, causing systemic response. Neutrophilia, angiogenesis and lymphopenia could be observed. In short, the elevated cytokine concentrations and increased tumor macrophage infiltration may suggest that the NLR reflects the regulation of the innate immune response.

The higher NLR also associated with higher T and N stage in pathological review and a higher incidence of extramural venous invasion,[13] but in our study, T and N status were not associated with higher NLR in microscopic perforated colon cancer patients.

There were also many papers focusing on the preoperative NLR to the outcome of colon cancer. Normal NLR was identified between 0.78 and 3.53.[14] The higher NLR (> 5) was known as a prognostic factor with poor overall survival and early recurrence;[9,10,15] however, in perforated colon cancer patients, in our study, the overall survival and disease free survival were both not statistically significant in predicting patient outcome by higher NLR (OS *p* value = 0.610 and DFS *p* value = 0.139). In our study, the independent risk factors for overall survival and disease free survival were age > 65 years, anemia (Hb ≤ 10 g/dL), and N stage (Table 2, 3).

In microscopic perforated colon cancer patients, no matter they have peritonitis in clinical or not, enhanced inflammation reactions were undoubted. The rapid changed and elevated WBC count, neutrophil percentage in acute phase reaction can also affected with bacterial infections. Pre-operative NLR then has less meaning in present the real interaction between cancer and our immune system.

Limitations

This was a retrospectively study, the data base was from a single center, and the patient number was small. Second, the pre-op data were collected in ER, or during admission if patient had planned elective surgery.

Table 2. Univariate and multivariate analysis by the Cox proportional hazard model to demonstrate the adjusted hazard ratios of potential factors on overall survival

	Univariate *p* value	Multivariate *p* value	HR
Age > 65 y/o	< 0.001	< 0.001	2.824
Sex	0.974		
HB ≤ 10 g/dL	0.046	0.021	0.574
WBC >12000/uL	0.581		
CEA > 5 ng/mL	0.198		
Albumin ≤ 3.5 g/dL	0.399		
NLR > 5	0.610		
DM	0.079		
Hepatitis	0.258		
Liver cirrhosis	0.476		
Location	0.755		
Emergency OP	0.056		
Histology type	0.198		
Histology grade	0.906		
T stage	0.429		
N stage	< 0.001	< 0.001	
0			
1			1.569
2			5.978

Table 3. Univariate and multivariate analysis by the Cox proportional hazard model to demonstrate the adjusted hazard ratios of potential factors on disease free survival

	Univariate *p* value	Multivariate *p* value	HR
Age > 65 y/o	0.045	0.010	1.950
Sex	0.668		
HB ≤ 10 g/dL	0.047	0.029	0.578
WBC > 12000/uL	0.128		
CEA > 5 ng/mL	0.158		
Albumin ≤ 3.5 g/dL	0.377		
NLR > 5	0.144		
DM	0.108		
Hepatitis	0.426		
Liver cirrhosis	0.504		
Location	0.258		
Emergency OP	0.097		
Histology type	0.519		
Histology grade	0.714		
T stage	0.715		
N stage	< 0.001	< 0.001	
0			
1			1.984
2			5.513

Therefore, the result may not reflect the same clinical conditions. Further larger studies may be required for validation of this finding.

Conclusion

Higher pre-operation NLR ratio (NLR > 5) is known associated with poorer outcome; however, in microscopic perforated patient, the higher neutrophil count not only affected by inflammatory associated with cancer, but also with infection due to perforation. The NLR ratio thus cannot be represented as a single marker for inflammatory microenvironment. In stage I~III colon cancer, based on our study, higher NLR ratio does not correlate with poor outcomes (OS $p = 0.610$; DFS $p = 0.139$), and has limited benefit in predict patient's outcome.

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原　著

手術前嗜中性球與淋巴球的比率是否可以預測大腸癌破裂病患的預後

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目的　目前研究顯示微觀的發炎環境與癌症的進程相關。嗜中性球與淋巴球的比率 (NLR) 在可切除的大腸直腸癌是一個已知重要的預測因子，但目前並沒有相關文章討論到大腸癌破裂的病人預後。

方法　以回溯性研究的方法，根據病理報告挑選本院 1995 年至 2015 年顯微鏡下大腸癌破裂並接受手術之病患。排除條件為短期死亡 (手術後 30 天內)，瘜肉切除破裂，以及確診遠端轉移的病人。依照嗜中性球與淋巴球的比率 (NLR) 分為高低兩組 (> 5 或 ≤ 5) 分別比較病患之臨床病理表現、長期整體存活率 (OS) 及無病存活率 (DFS)。

主要結果分析　存活分析。

結果　在大腸破裂癌病患中，嗜中性球與淋巴球的比率較高的病患在統計上有顯著的傾象有：白血球增多 (WBC > 12000/uL) (12.9% vs. 59.7%, $p < 0.001$)、較低的血液中白蛋白 (albumin < 3.5 g/dL) (32.3% vs. 54.5%, $p = 0.003$)、緊急手術 (5.4% vs. 26%, $p < 0.001$)。嗜中性球與淋巴球比率高低兩組間長期整體存活率 (OS, $p = 0.610$) 及無病存活率 (DFS, $p = 0.139$) 並無顯著差異。在本次研究中，對整體存活率及無病存活率的預測因子皆為病人年紀病人年紀大於 65 歲，血紅素小於 10，以及淋巴結是否轉移 (N stage)。

限制　這是一個回朔性，非隨機的臨床研究。因為是單一中心的小樣本分析，也可能存在選樣偏差因為選樣偏差。

結論　手術前嗜中性球與淋巴球比率對預測微觀下大腸癌破裂病人的存活率幫助有限。

關鍵詞　大腸癌、破裂、嗜中性球與淋巴球比率、存活率。