



Neutrophilic Lymphocytic Ratio as Predictive Biomarker in Advanced Rectal Cancer Patients

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Abstract

Background: Preoperative Neutrophil-Lymphocytic ratio has been suggested in many studies in resected colorectal cancer as predictive marker for recurrence and survival. This study investigated impact of pretreatment Neutrophil-Lymphocytic ratio on treatment outcome in rectal cancer patients.

Methods: Retrospective analysis of pretreatment Neutrophil-Lymphocytic ratio for one hundred and forty two patients with locally advanced rectal carcinoma (LARC) treated by neoadjuvant concomitant chemo-radiotherapy followed by surgical resection, between June 2006 and June 2011 in Minia University Hospital and Al-Hussein University Hospital. In order to study neutrophil lymphocytic ratio as a predictive factor for disease free survival and overall survival, other factors like age, sex, tumor length, tumor distance from anal verge, number of resected lymph nodes, positive lymph nodes, excision margin, tumor differentiation and circumferential resection margin with their impact on disease free survival and overall survival were also studied.

Results: One hundred forty two patients with mean age of 65.1 ± 10.8 . 64% were females; more than one third (34.5%) underwent colostomy and $61.3\% \leq 5$ cm from anal verge. Pretreatment CEA was above 3 ng/ml in 57.7% of patients. Mean NLR was 4.1 ± 2.87 . NLR of 58.4% patients were >3 . Regarding disease free survival, In a univariate analysis, only involved excisional margin seen in 8 patients and positive CRM seen in 15 patients show significant impact on DFS (p-value 0.01, 0.001), respectively. On other hand, NLR didn't show significance on DFS. None of the studied factors showed impact on overall survival in a univariate analysis. However, Neutrophil lymphocytic ratio at cut-off 3 showed significance. Population group with NLR ratio ≥ 3 showed less survival with a mean of 52.5 month compared to those with NLR ratio <3 that showed mean survival of 60.7 (p-value 0.05).

Conclusion: Pretreatment NLR is a simple, easily accessible laboratory finding for identifying LARC patients who had poorer prognosis for the standard line of treatment.

Keywords: Colorectal cancer; Neutrophil-Lymphocytic; Cancer

Introduction

Colorectal carcinoma (CRC) is the fourth most frequent cause for death from cancer worldwide. Disparate factors increase a person's risk of developing the tumor, such as age, inflammatory bowel disease, personal and/or family (such as hereditary non-polyposis colorectal cancer; HNPCC) history of colorectal tumors (adenoma or adenocarcinoma) and environmental factors [1].

There have been major advances in the treatment of CRC in the last 10–15 years, involving the introduction of new cytotoxic and molecular targeted therapies. However, the use of these new strategies resulted in increased toxicities and is prohibitively expensive. Hence, there is a need for accurate predictors of outcomes from treatment, in particular, identifying those patients who are more likely to benefit. This may also be used to rationalize the increasingly expensive treatments, especially in under-resourced communities [2].

Tumour development and growth occurs as a result of interactions among the tumour, host-derived stromal tissues including blood vessels and host immune/inflammatory cells, with chronic inflammation having an important role in cancer development and progression [3].

The ratio of circulating neutrophils to lymphocytes (NLR) is considered indicator of systemic inflammatory response and has been proposed as a routinely available preoperative indicator of prognosis in patients undergoing resection of primary colorectal cancer in many trials [4].

The origin of this suggestion was a study of serial postoperative observations of neutrophils and lymphocytes which showed that the ratio of these two factors was an effective indicator of the intensity of physiological stress in ICU patients after CRC resection or surgery for abdominal sepsis or medical treatment of severe sepsis or septic shock [5].

Many studies for patients with primary CRC have reported a statistically significant association between preoperative NLR and Disease free survival as well as overall survival [6]. In spite of this association was not found in other studies [7].

In the present study we analyzed the potential association between the preoperative NLR and the colorectal cancer treatment outcomes. Many Laboratory and radiological investigations can be used NLR was chosen as it is cheap simple, and non-invasive test.

Patients and Methods

This study is a retrospective analysis for patients with locally

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advanced rectal carcinoma treated by neoadjuvant concomitant chemoradiotherapy followed by surgical resection, between June 2006 and June 2011 in Minia University Hospital and Al-Hussein University Hospital.

All patients were subjected to the following minimum pre-operative assessment; Clinical examination including DRE (digital rectal examination), CBC (complete blood count) including differential (neutrophil and lymphocyte), serum creatinine, blood urea, liver function tests, CT scan chest, abdomen and pelvis with IV contrast and MRI pelvis. The prognostic analysis included all patients for whom NLR data were available. The NLR was defined as the absolute count of neutrophils divided by the absolute count of lymphocytes determined from the full blood count routinely taken within 10 days before treatment.

Patients recruited to this study had locally advanced rectal carcinoma. Staged by MRI. Patients' stages varied from $cT_3N_0M_0$ to $cT_4N_3M_0$. A patient was considered to have a locally advanced disease if MRI pelvis showed involvement of the CRM (circumferential resection margin) by the tumor.

We excluded from analysis; patients with PS (performance Status) 3-4 ECOG, patients with documented infections before starting treatment, patients with coronary artery or cerebral vascular disease, patients with other concomitant malignancy and those with acute bowel complications like obstruction or perforation.

All patients were treated using 3-4 fields of photon beam radiation therapy using linear accelerator. The total radiotherapy dose 45 Gy, 25 fractions, 1.8 Gy/fraction over 5 weeks. Intravenous 5-fluorouracil was concomitantly given during first and fifth weeks of radiation therapy course.

We calculated overall survival time from the date of surgery to the date of death due to any cause and for patients remained alive at end of study date of last follow up or last contact was taken with times censored at last contact for patients who were lost to follow-up or who remained alive at the close of study.

Recurrence free survival was measured from the date of surgery until the date of recurrence. Times were censored at last contact for patients who were lost to follow-up or who remained alive and recurrence-free at the close of study in June 2012.

All previous data was collected from patients data base at the 2 centers mentioned above. Using Statistical package for social sciences (SPSS) version 12. Quantitative variables were summarized using mean and SD, median minimum and maximum values. Qualitative data were summarized using frequencies and percentage [8]. Survival analysis was done using Kaplan-Meier, comparisons was done using Log-rank test. Differences were considered significant when $p \leq 0.05$ and highly significant when $p \leq 0.01$ [9].

Results

In this study we analyzed, 142 patients with rectal cancer treated by concomitant chemoradiotherapy and surgery. Mean age was 65.1 ± 10.8 years, with 88.7% above 50 year. Females represent 35.9% of patients.

Most of the tumors had locally advanced with more than one third (34.5%) of studies presented with clinical obstruction that required palliative colostomy before chemoradiation course, 61.3% of disease were less or equal to 5 cm from anal verge, mean length of the tumor were 5.65 ± 2.3 cm.

Most of our cases (78.9%) enrolled in the study presented with advanced Clinical stage as defined by MRI criteria. Pretreatment CEA was above 3 ng/ml in 42.2% of patients. Mean hemoglobin was 13.3 ± 1.7 g/dl, mean white blood cell (WBC) 8.12 ± 3.1 , mean neutrophil count 5.8 ± 2.4 , mean lymphocytic count 1.75 ± 0.8 . Mean Neutrophil-Lymphocytic Ratio (NLR) was 4.1 ± 2.87 . NLR of 58.4 patients were >3 and $41.5 \leq 3$ (Table 1).

Surgery was done in about 70% of patients. Out of them 33% of patients were Dukes C, only one quarter (24.6%) received adjuvant chemotherapy. Tumor gross residual was shown in 35.9% while microscopic residual showed in 43% of specimens. Only 8 patients

Variables	Frequency	%
Age		
<50	126	88.7
≥ 50	16	11.3
Sex		
Male	51	35.9
Female	91	64.1
Dukes Stage		
A	43	30.28
B	52	36.62
C	47	33.1
Grade		
1	10	7
2	111	78.1
3	21	14.8
Distance from anal verge		
>5 cm	55	38.7
≤ 5 cm	87	61.2
CEA		
>3	82	57.7
≤ 3	60	42.2
LN removed		
>12	53	37.3
≤ 12	89	62.6
Positive LN	96	67.6
Negative LN	46	32.4
NLR		
>3	83	58.4
≤ 3	59	41.5
Resection Type		
R0	125	88
R1	15	10.6
R2	2	1.4
Excision Margin		
Negative	134	94.4
Positive	8	5.6
Colostomy		
No	93	65.5
Yes	49	34.5
Pathological response		
Tumor absent	81	57
Tumor present	61	43
Post-operative adjuvant chemotherapy		
Yes	35	24.6
No	107	75.4

Table 1: Patient characteristics.

(5.6%) showed positive excisional margin. 62.6% of patients had ≤ 12 resected lymph nodes while 37.4 of patients >12 of them about 67.6% with positive node. CRM was not involved in 88% of patients (Table 1).

Nearly 24% of patients experienced tumor recurrence with 82% was alive at time of data collection. Follow up duration ranged from zero up to 63 months (interquartile range 23 months) with a median of 13 months. Disease free survival ranged from 0.3 to 75.9 with a mean of 21.6 (18.8, 24.5), while survival ranged from 0.3 to 74.7 with a mean of 26.7 (23.9, 29.5) and a median of 25.06 (Table 2).

In order to study neutrophil lymphocytic ratio as a predictive factor for disease free survival and overall survival, other factors like age, sex, tumor length, tumor distance from anal verge, number of resected lymph nodes, positive lymph nodes, excision margin, tumor differentiation and circumferential resection margin with their impact on disease free survival and overall survival were also studied (Table 3).

Regarding disease free survival, the univariate analysis, only involved excisional margin seen in 8 patients and positive CRM seen in 15 patients showed significant impact on DFS (p value 0,01, 0,001, respectively). On the other hand, similar results were not being able to achieve for NLR ratio DFS with all studied factors (Figure 1). None of the studied factors showed impact on overall survival in a univariate analysis. However, Neutrophil lymphocytic ratio at cutoff 3 showed statistical significance (Figure 2), patients group with NLR ratio ≥ 3 showed less survival with a mean of 52.5 month compared to those with NLR ratio <3 that showed mean survival of 60.7 (p value 0.05) (Table 4).

Discussion

In our retrospective analysis of locally advanced rectal cancer (LARC) patients. We explore the benefit of the NLR as prognostic factor that predict the response of current treatment CCRT and surgery. In order to reach to this evidence we studied 142 patients treated by stranded protocol (neoadjuvant CCRT, surgery, \pm adjuvant chemotherapy).

Several studies had demonstrated that NLR can serve as a marker of systemic inflammatory and immune response and can predict clinically useful outcomes for multiple cancers, including LARC. Taken together, these results indicate that the NLR is a simple laboratory variable for risk stratification in LARC patients [10].

The limit of NLR significance is difference in many studies mostly they

Variable	Mean \pm St. deviation
Hb/g/dl	13.3 \pm 1.7
WBC/ 10^9	8.12 \pm 3.1
Neutrophil/ μ l	5.8 \pm 2.4
Lymphocyte/ μ l	1.75 \pm 0.8
Platelets/ μ l	300.5 \pm 102.4
Mean NLR	4.1 \pm 2.87

Table 2: Disease free survival range.

Variable	Frequency	%
Disease Free survival		
No recurrence	109	76.8
Recurrence	33	23.2
Overall survival		
Alive	117	82.4
Dead	25	17.6

Table 3: Free survival and overall survival.

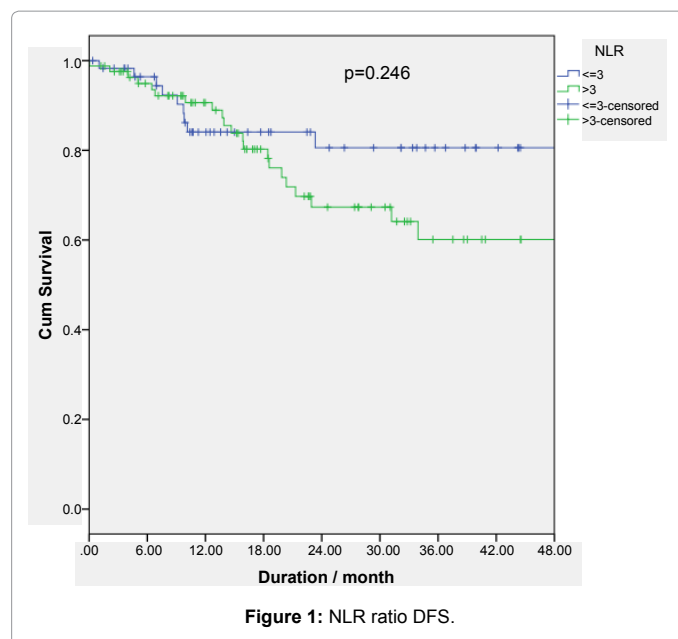


Figure 1: NLR ratio DFS.

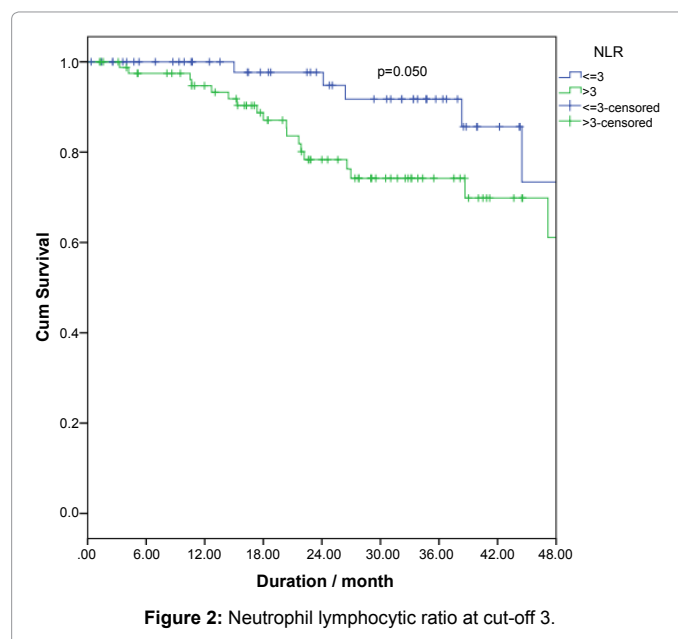


Figure 2: Neutrophil lymphocytic ratio at cut-off 3.

categorize it at <5 versus ≥ 5 [11-14] although a cutting point at <2 versus >2 was used by Liu et al. [13]. Also, two studies used receiver operating characteristic (ROC) analysis to dichotomize NLR points of ≤ 4 versus >4 and ≤ 3 versus >3 [15]. We used point of ≤ 3 versus >3 at which NLR was statistical significant prognostic factor for OS this result were similar to that reported by Chiang et al. [15]. Until now all studies investigating the optimal cut point of NLR did not determine a specified point. More studies are needed to reach to final conclusion [16].

Hung et al. [17] studied the preoperative NLR of patients with CRC found that increasing NLR is independent prognostic factor for OS. This finding matched our result as NLR has significance effect on OS at cut point of 3, patient group with NLR ratio ≥ 3 showed less survival rate with a mean of 52.5 month compared 60.7 months among NLR ratio <3 (P value 0.05) [16-18].

Variables	Overall Survival			Disease-free survival		
	Frequency	2 years survival	p-value	Frequency	2 years survival	p-value
Age						
>50	126	71				
≤ 50	16	82	0.2			
Gender						
Female	51	64	0.3			
Male	91	77.5				
Distance From Anal Verge						
<5 cm	68	76	0.1	68	68	0.4
≥ 5 cm	74	92		74	76	
Colostomy						
No colostomy	93	63	0.3			
Yes Colostomy	49	87				
Tumor Size						
T2	15	77	0.8			
T3	98	70				
T4	29	79				
Microscopic Picture						
Tumor Absent	81	83	0.08	81	75	0.9
Tumor Present	61	87		61	70	
Total Nodes						
≤ 12	89	85		89	72	0.9
>12	53	84	0.9	53	73	
Positive Lymph Nodes						
Positive	96	85	0.3	96	76	0.2
Negative	46	83		46	69	
Excision Margin						
Free	134	86	0.06	134	74	0.01
Involved	8	100		8	47	
Differentiation Grade						
1	10	86		10	67	
2	111	88	0.5	111	75	0.4
3	21	76		21	63	
Resection Margin						
R0 CRM Not Involved	125	86		125	79	0.001
R1 CRM<5 mm	15	85	0.7	15	31	
R2 CRM Involved	2	100		2	0	
Neutrophil-Lymphocytic Ratio						
≤ 3	59	98	0.05	59	81	0.2
>3	83	78		83	67	

Table 4: Univariate analysis of clinic-pathological factors 2 years DFS and OS.

Contrary to our result Walsh et al. [19] and Kwon et al. [4] found a bivariate but no multivariable significance association between NLR and overall survival in patients with CRC. Liu et al. found a multivariable but no bivariate association. While Leitch et al. found no association in both analyses [11,12]. Similar analysis was not possible in this study due to limited number of statistically significant variables.

Dou et al. found by univariate and multivariate analysis Lymphocyte ratio were correlated significantly with 5 year DFS. In contrast to what we found in our series NLR was not affect the DFS this may be attributed to heterogeneous group of patients and or shorter follow up period [20].

On other hand, Shen et al. had similar result to our study in multivariate analysis where NLR has no effect on DFS while in univariate analysis has little difference. Although there was no significant difference in the DFS between the NLR groups, the difference may be significant with a longer follow-up time and a larger patient population [10].

Overall survival is considered the most important outcome for patients as it include potential mortality arising from both the LARC and from treatment as well as from other causes like (e.g. infection or iatrogenic illness). Moreover, the major concern for health care provider and patients is how long they survive, not what causes their death. It is striking that these different studies have yielded such inconsistent results on the association between NLR and overall survival; however this may be arise from the mixed nature of these other populations, particularly in terms of tumor stage.

The relatively poor (though statistically significant) correlation between NLR and OS could have been influenced by the fact patients coming to surgery tend to be relatively fit, therefore reducing number of unwell patients who would be more likely to have high NLR levels. Furthermore, in our practice, the proportion of patients having emergency surgery is very low.

Conclusion

Future clinical trials are needed to elucidate that potential mechanism of inflammatory response against tumor cells. Almost all studies done to explore relation of NLR to PFS and OS were retrospective including our study. There were relatively limitations with this kind of study like small number, one single institution and heterogeneity of patients. In addition to we did not have a chance to consider medical conditions that may affect the host's immune condition, because of insufficient data. These limitations should be considered when we conclude the significance of NLR ratio as predictor factor for survival. Our result concluded that Pretreatment NLR is a simple, easily accessible laboratory finding for identifying patients with poorer prognosis who were treated by CCRT.

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