Lymph node ratio and kras mutation in risk stratification of colon cancer

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Abstract

Background:
Colorectal cancer (CRC) considered to be third prevalent and the fourth leading cause of death among all cancers. Nowadays, there are multiple gene assays provide expected information about prognosis of CRC.

Materials and methods:
This study included 433 patients stage III colon cancer. After surgical resection of tumor, patients with inadequate LN resection (12 LN) excluded from the study. Lymph node ratio (LNR) determined by (metastatic lymphatic node number/total excised lymphatic node number). Different clinicopathological parameters, PFS and OS have been tested and compared with LNR.

Results:
287 (66.7%) patients had Lymph node ratio <0.2 (LNR1), 81 (18.7%) patients had Lymph node ratio 0.2-0.5 (LNR2) while 65 (15%) patients presented with Lymph node ratio >0.5 (LNR3) group. Baseline Carcino Embryonic Antigen (CEA) level in patients with LNR > 0.5 was statistically high than patients with LNR < 0.2 (P 0.04). High grade tumors (3 & 4) found to be associated with more LNR in comparison to low grade tumors (1& 2) however p value was not significant. LNR was considerably higher in larger tumor size (T3&T4) versus smaller tumors (T1&T2), p < 0.001. Left sided colon cancer documented significantly more LNR3 than right sided colonic tumors; (53.8% versus 46.2%); with p value <0.001. 105 patients (24.2%) showed disease recurrence; LNR were higher in patients with recurrent disease (local &/or distant), p 0.002. K-ras mutation assay was possible for 55 recurrent tumors, LNR3 patients had significantly more K-ras wild type than LNR2 and LNR1 with P 0.03. Combined LNR3 and K-ras wild patients had significant poor response to monocolonal anti EGFR (Epidermal growth factor receptor) in comparison to LN ratio (2&1) combined with wild K-ras genotypes; P 0.01. Two years median follow up showed that; LNR3 patients had median PFS of 52 months while not reached in LNR1&2 (p value <0.001). LNR3 patients showed median OS of 55 months while not reached in LNR1&2 (p value <0.001).

Conclusion:
In stage III colon cancer, a high lymph node ratio observed a high CEA, left side tumours, T3 & T4 pathologies, high tumour grade, wild K-ras, low PFS, and OS. When the effectiveness of the LNR has been validated in other trials, it can be used for risk stratification and personalization of r along with k ras.

Key word: Lymph node, Kras, Colon cancer

Introduction:

Carcinoma of colon and rectum considered one of the commonest tumor around the world, with about (1-2 million) newly diagnosed patients per year. It is the 3rd common cancer and the 4th common cause of cancer deaths, exceeded by lung, liver and stomach cancers [1]

It is the second most common tumor in females after breast cancer, and the third in males after prostate and lung cancers. It is more common in the Western countries (55%) with changing rates because of the fast development of the other countries [2]

Western lifestyle with its risk factors as alcohol consumption increase CRC risk. Also, inflammatory bowel diseases as ulcerative colitis increase the risk of CRC and need close surveillance programs. Hereditary syndromes such as: FAP and HPNNC, represent 5% of CRC cases. Familial clustering accounts for about 20% of CRC cases. Sporadic cases represent the vast majority 75% [3].

The Lymph Node infiltration remains the key deter minant of prognosis and adjuvant therapy following a potentially curative resection in non-metastatic CRC. Moreover; pathological assessment of surgically excised lymph nodes has been documented to impact tumor staging and survival outcomes in either negative or positive LN specimens [4]

Node (N) staging of AJCC staging system for CRC (N1; 1-3 LNs, N2; 4 or more LNs) is related to the number of positive nodes resected; with more rise in

number of LN excised there will be more possible high number of positive lymph nodes, which corresponds to a higher N stage [5].

The more the number of resected regional lymph nodes with minimum 12 LNs needed, the better local disease control, accuracy, staging, adjuvant treatment planning, overall survival, prognosis, and the less the mortality [6].

Lymph node ratio (LNR) is defined as the ratio of involved lymph nodes to the total number of removed lymph nodes, so it is less dependent on the number of removed lymph nodes than do N staging. It is divided into three groups: LNR1 (less than 0.200), LNR2 (0.200-0.429), and LNR3 (LNR more than 0.429) [7].

LNR is a marker of aggressive behavior because it is associated with a significantly increased percentage of the lymphatic invasion and poorly differentiated tumour, so it is advisable to use LNR in the CRC staging system as a prognostic parameter [8].

We planned this analysis to examine the prognostic role of LNR on disease free survival and overall survival (primary objective) and to correlate LNR with different clinico-pathological parameters of patients with non metastatic, lymph node-invaded CRC (secondary objective).

Patients and Methods:

We conducted a retrospective cross sectional study included 433 patients with pathologically proven colonic cancinoma recruited from outpatient clinics Oncology Center and Gastrointestinal Surgery Center Mansoura University with a minimum follow up period of at least one year. Data were retrieved from the electronic hospital-based cancer registry database of the Department of Oncology at Mansoura University Oncology Center and Gastrointestinal Surgery Center, Mansoura, Egypt. Only patients aged > 16 and <70 years, ECOG performance status 0-2, stage III colon cancers, with good hematological and adequate bone marrow functions, and adequate liver and kidney functions; were considered eligible.

While, patients whose tumor location was not labeled correctly in the documentation and patients with inadequate LN resection (12 LN) were excluded. Patients with prior or concurrent malignancy, pregnant and lactating females, patients with active or uncontrolled infection or bleeding, patients with distant metastasis were considered ineligible. Patients were evaluated by history and physical examination, laboratory investigations including CBC, serum creatinine and liver functions, CEA and CA19-9, baseline CT chest, abdominal and pelvis, brain CT, bone scan, the International Staging System was used for clinical staging of the disease according to TNM staging system for colon cancer, and tissue samples for pathological, vascular, perineural, lymphatic, serosal involvement and LN analysis. DNA from paraffin-embedded tissue for patients developed metastatic disease during follow up was extracted, and polymerase chain reaction (PCR) and pyrosequencing targeted for *KRAS* codons 12–13 was performed [9]. For the survival analysis, we used the LNR cutoff values as identified by Ren et al., [7]. The endpoints for the survival analyses were progression-free (PFS) and overall survival (OS).

Statistical methods

The relations between qualitative variables were evaluated by the X^2 test or Fisher's exact test, as appropriate. The relations between continuous variables were evaluated by Pearson correlation test. Difference between means of the study parameters were evaluated by the independent sample T test. Survival curves were estimated by the Kaplan–Meier method log-rank for comparing curves. The Cox proportional hazards model was used for multivariate analysis. P value less than 0.05 was considered significant. Two sided statistical tests were used in all of the analyses.

Results:

433 patients were considered eligible in this study. Postoperative LN examination of the patients showed that; 66.7% patients with LNR1 group (less than 0.2), 18.7% patients with LNR2 group (0.2-0.5) and 15% patients with LNR3 group (more than 0.5) as shown in table (1).

Table (1): LNR groups.

LNR group	Frequency	Percent
LNR1 (less than 0.2)	287	66.3
LNR2 (0.2-0.5)	81	18.7
LNR3 (more than 0.5)	65	15.0

There were 287 patients with LNR1 group less than 0.2 (127 males versus 160 females; 44.3% versus 55.7 %), and 81 patients with LNR2 group 0.2-0.5 (40 males versus 41 females; 49.4% versus 50.6%), and 65 patients with LNR3 group more than 0.5 (37 males versus 28 females; 56.9% versus 43.1%); with non-significant p value, table (2).

Table (2): Relation between LNR and patient's gender.

LNR	Male	Female	X^2	P value
LNR1 (less than 0.2)	127 (44.3%)	160 (55.7%)		
LNR2 (0.2-0.5)	40 (49.4%)	41 (50.6%)	3.6	0.1
LNR3 (more than 0.5)	37 (56.9%)	28 (43.1%)		

In patients with high LNR above 0.5, a significantly high Basal CEA level (before operation) was achieved; (mean ± SD 19.96 ± 86,3) compared to low LNR patients of < 0,2 (mean ± SD; 5.56 ± 27.4); p value 0.04, table (3).

Table (3): Relation between LNR and CEA level.

LNR group	N	Mean	Std. Deviation	Std. Error of Mean	X^2	P value
LNR1 (less than 0.2)	287	5.5669	27.41956	1.61853		
LNR2 (0.2-0.5)	81	6.9049	23.44811	2.60535	3.2	0.04
LNR3 (more than 0.5)	65	19.9677	86.32082	10.70678		

Higher grade tumors (3&4) demonstrated more LNR in comparison to low grade tumors (grade 1&2); mean ± SD; 0.217 ± 0.289 versus 0.2 ± 0.295, respectively with no significant p value, table (4).

Table (4): Relation between LNR and tumor grade.

Grade group with positive lymph node ratio	N	Mean	Std. Deviation	Std. Error of Mean	X^2	P value
Grade 1&2	407	0.2012	0.29592	0.01467	0.2	0.7
Grade 3&4	26	0.2179	0.28963	0.05680		

T3 & T4 disease were significantly presented with high LN ratio (Mean ± SD; 0.227 ± 0.3) versus patients with T1& T2 disease groups (Mean ± SD; 0.072 ± 0.148); with a significant p value < 0.001, table (5).

Table (5): Relation between LNR and tumor infiltration in bowel wall.

T. group with positive lymph node ratio	N	Mean	Std. Deviation	Std. Error of Mean	X^2	P value
T1 & T2	71	0.0726	0.14843	0.01761	4.1	`<0.001
T3 & T4	362	0.2276	0.30999	0.01629		

Table (6) showed that patients with LNR1&LNR2 more presented with right sided and transverse colon cancer versus left sided and sigmoid colon cancer (78.8% vs 21.3% & 71.6% vs 28.4%, respectively) while LNR3 was more presented in left sided and sigmoid colon cancer (53.8% vs 46.2%) with significant relation; P <0.001.

Table (6): Relation between primary colon cancer and LNR.

LNR group	Right & Transverse	Left & sigmoid	X^2	P value
LNR1(less than 0.2)	226 (78.8%)	61 (21.3%)		
LNR2 (0.2-0.5)	58 (71.6%)	23 (28.4%)	28.2	<0.001
LNR3 (more than 0.5)	30 (46.2%)	35 (53.8%)		

During follow up period there were 105 patients (24.2%) developed disease recurrent; high LNR was significantly presented in patients developed (local and or distant recurrence); mean ± SD; 0.449 ± 0.367 versus patients did not developed recurrent disease; mean ± SD; 0.18 ± 0.28; p value 0.002, table (7).

Table (7): Relation between LNR and tumor recurrence.

Recurrence	N	Mean	Std. Deviation	Std. Error Mean	P value
No recurrence	373	0.1835	0.28185	0.01459	0.002
Recurrent disease (local ± distant recurrence)	105	0.3183	0.34854	0.04500	

Assessment of K-ras was possible in 55 patient specimens (out of 105 patients developed recurrent disease). Kras wild was detected in 29 patients while 26 patients were mutant. Wild Kras form has been presented significantly in LNR3 patients (55.2%) versus LNR2 (24.1%) and LNR1 (20.7%) versus Kras mutant was presented in LNR3 (23.1%) versus LNR2 (30.8%) and LNR1 (46.2%) with P 0.03 (figure 1). The response to monocolonal anti EGFR for LNR2&1 with wild Kras genotype patients was significantly better than LNR3 with wild Kras group, P 0.01 see table 8.

Table (8): Response to monocolonal anti EGFR antibodies (Kras wild patients) in relation to LNR.

	Poor Responder (Progressed)	Responders (SD, PR &CR)	X^2	P
LNR1&2 (13)	4 (30.8%)	9 (69.2%)	7.5	0.01
LNR3 (16)	13 (81.3%)	3 (18.8%)		

After two years median follow up, patients with LNR3 group (over 0.5) reported median PFS of 52 months (95 percent CI; 37.5-66.4) while PFS had not been reached in LNR1&2 (log rating 33.01; p <0.001). Also LNR3 group (over 0.5) reached a median OS about 55 months (95% CI; 35.5-74.5) while OS was not reached in patients group with LNR1&2 (log rank 37.1; p value <0.001), figure (2&3).

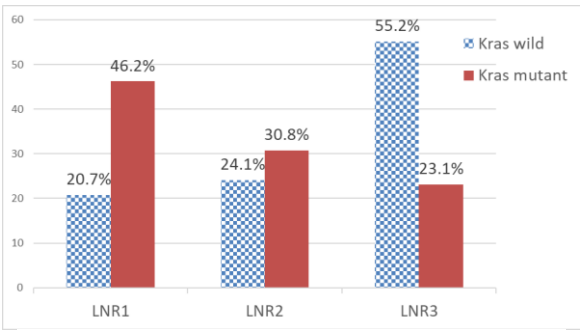

Figure (1): LNR and Kras distribution in metastatic cases

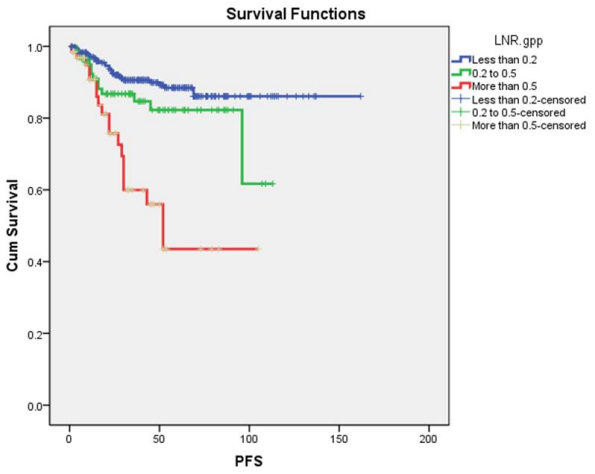

Figure (2): Progression Free Survival (PFS)

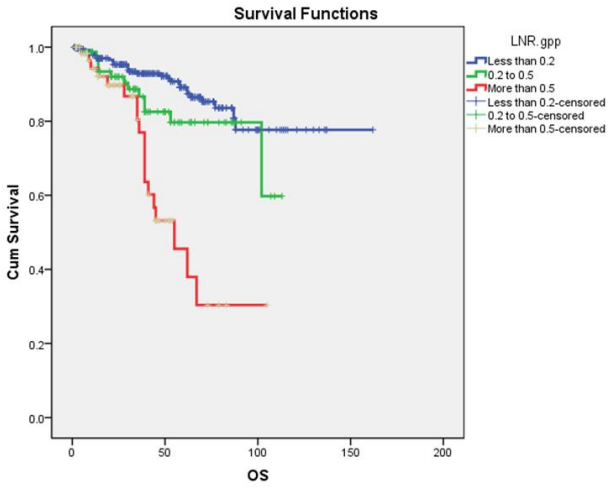

Figure (3): Overall Survival (OS)

Discussion:

CRC is considered as the 3rd commonest cancer and the 3rd commonest cause of cancer-related death around the world [8]. The classic TNM staging system for CRC concludes that lymph node stages depends on the number of positive lymph nodes, But it does not include the examined tumor-free LNs and the extranodal extension that shown to have a prognostic value, while LNR is considered as a tumor aggressiveness sign; as it is associated with higher percentage of lympho-vascular invasion and poor tumor differentiation. Thus; LNR could be included as an important staging and prognostic parameter in node positive CRC [10].

A previous study has suggested LNR; which is defined as the ratio of involved lymph nodes to the total number of resected lymph nodes; as a better prognostic parameter compared to the N stage, This study has showed great changes in rates of survival in the pN1 (LN; 1-3) and pN2 (LN; ≥4) categories depending on the value of LNR [5].

The present study evaluated the prognostic value of LNR in non metastatic colon cancer in relation with other prognostic parameters such as biomarkers e.g. CEA, we found a significant high preoperative CEA level was associated with high LNR and this was in agreement with a previous report evaluated the relation between LNR and CEA level and showed high CEA level in patients with high LNR , with a significant p value ≤0.001[11].

CRC is the second most common cancer in females and the third most common in males. Incidence rates vary ten folds in both sexes worldwide, In some regions with previously low incidence rates as; Eastern Europe and East Asia; have been shown increasing in number of CRC cases, due to changes in risk factors as; lifestyle and diet **[12].**

The current study evaluated the relation between LNR and patient's gender and showed no significant relation between LNR and difference in patient's gender and this was in consistance with Zare and his colleages that showed no significant p value between different study groups [13].

In our results LNR was significantly high in left sided colon cancer versus right sided colon cancer patients while other report [5] found no significant relation between Left sided versus right sided colon cancer in relation to LNR; and this difference may be due to different number of included patients in both series (433 versus 921 patients) and the difference in LNR cutoff values in both studies. However our results could demonstrate a group of left sided colon cancer that may have a worse behavior.

Tumor grade describes the degree of differentiation of the cancer cells and how they look like and behave compared to normal cells which is mainly done to adenocarcinomas ranging from grade 1 to 3 (from lower to higher grade) while grade 4 applied to undifferentiated carcinomas. In that way tumor grading provides an idea about tumor behavior, growth, response to treatment and so predicts prognosis [4]. The current study showed no significant relation between different tumor grade and LNR and this was in agreement with a previous report showed no significant relation between LNR and different tumor grade groups; p value 0.16 [5].

CRC is commonly primarily diagnosed with metastatic disease, also may relapses with either local or distant metastases which frequently affects liver, lung, peritoneum, bone and brain respectively. That worse patient prognosis with poorer outcome than early diagnosed disease.

We found a significant relation between high LNR and development of distant recurrent disease and this was in agreement with a previous study conducted on 379 colon cancer and 160 rectal cancer patients and the authors found that high LNR was associated with disease recurrence, p value 0.002 [14] and this findings may consider LNR one of the prognostic and predictive markers for colon cancer.

In this study high LNR was associated with poor PFS and OS. Our result was similar to a report

conducted on 922 patients underwent curative colon cancer resection and the authors found that increasing LNR were independent predictors of decreased overall and disease-free survival for patients undergoing curative co lon cancer resection [5].

Another report by Ogino et al [15] found that patients with 0-3, 7-12 and > or =13 negative lymph nodes showed a significant reduction in cancer-specific and overall mortality. In a study by Johnson et al [16] increasing the negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer which is consistence with our results. They subdivided patients into groups of those with ≤3, 4-7, 8-12, or ≥13 negative lymph nodes. 5-year cancer mortality was 27% in stage IIIB with ≥13 negative nodes versus 45% in those with ≤3 negative lymph nodes (p<0.0001). In patients with stage IIIC cancer, those with ≥13 negative nodes had a 5-year mortality of 42% versus 65% in those with ≤3 negative lymph nodes evaluated (p<0.0001) [16].

In a large series, authors noted that in stage IIIB patients the 5-year survival rate for LNR1 to LNR4 was 63.5 percent, 54.7 percent, 44.4 percent, and 34.2 percent respectively (P <0001, including 24,477 stage III patients). In stage IIIC patients 49.6 percent, 41.7 percent and 25.2 percent, respectively survival for five-year period in those with LNR2 to LNR4 (p <.0001). Authors have concluded that LNR is an independent survival indicator [17].

In agreement with our results, Zheng with his collages found that the 5-year OS of 245 patients with CRC was 54.0%, and the 5-year recurrence-free survival rate was 48.5%.Univariate analysis showed that number of positive lymph nodes, number of negative lymph nodes and LNR were significantly associated with the 5-year OS (*P*<0.05) [18].

Occhionorelli and his collages also, demonstrated that N, LNR and **log odds of positive lymph nodes** (LODDS) are all related to 5-year OS and DFS in 202 CRC cases with statistical significance. DFS rate was 80.83% for LNR0, 71.43% for LNR1, 51.52% for LNR2 (P= **0.0001).** OS rate was 56.67% for LNR0, 48.98% for LNR1, 21.21% for LNR2 (P= **< 0.0001) [19].**

Lymph nodes have been proposed as easier to locate in patients with a good immune response to the cancer and have improved prognosis in these patients [20]. The underlying biology of the tumour will affect parallel lymphatic node production and prognosis. The prognosis and improved lymph node recovery were found for example in MSI and wild-type KRAS/BRAF [21]. This was consistent with our findings; K-ras wild form in patients with high LNR was significantly presented.

Conclusion:

Patients with stage III colon cancer, a high lymph node ratio observed a high CEA, more tumors in left colon left , larger tumor size (T3 & T4), grade 3&4 tumors, K-ras wild cancers, poor PFS, and OS. When the effectiveness of the LNR has been validated in other trials, it can be used for risk stratification and personalization of r along with k ras.

Conflict of interest; all authors declared that he/she had no conflict of interest.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Ethical approval: In line with the institutional and/or national research committee and the Helsinki declaration of 1964 and their later amendments or related ethical principles all procedures conducted in this study were subject to ethical standards.

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All Data Availability for this work are available upon request to corresponding author

Authors' contributions:

Aya Waheed: Conceptualization, Methodology, Software

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Tamer Akl: Project administration; Resources, Writing- Original draft preparation

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Elkhodary TR: Supervision, Software, Validation

ElBaiomy MA: writing- Reviewing and Editing

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