
Prognostic Value of Negative Lymph Nodes (NLN) in Rectal Adenocarcinoma

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Abstract: We studied the impact on prognosis by negative LN (NLN) harvest count on rectal cancer patients, using immunohistochemistry to exclude micrometastasis in nodes negative by hematoxylin and eosin (H & E) stain. A prospective study including 114 patients in Mansoura university hospitals between 2005-2008 aged 18 years or older who were diagnosed as having localized invasive adenocarcinoma rectum with stage I, II & III was conducted. Close follow up done in both surgery department and department of clinical oncology and nuclear medicine for 5 years. We examined patient survival in relation to the negative lymph node count. Our results revealed that the number of negative lymph nodes could affect significantly survival curves where the disease free survival and overall survival were significantly better among the group of patients with more than ten negative LNs ($P=0.021$ & 0.012 respectively). In conclusion, the negative lymph node count is associated with improved survival of rectal cancer patients.

Keywords: Rectal Cancer, Negative LN, Survival

1. Introduction

Rectal carcinoma appears to comprise a number of individual disease entities with different physiologic characteristics and probabilities of metastasis [22]. Of all the features of the disease examined, in nonmetastatic colorectal cancer, lymph node status is the strongest pathologic predictor of patient outcome [25, 26]. Many studies have correlated improved survival with increasing number of retrieved lymph nodes [7, 36]. Improved outcomes were referred to more accurate staging, which reflected the true node status of the patient, subsequently lead to appropriately utilized chemotherapy. However, other studies have challenged this hypothesis [4, 32]. Papers reported significantly increased overall survival and disease free survival as the number of lymph nodes retrieved increased regardless of whether the lymph nodes were positive or negative for metastatic disease [7, 32]. The total number of LNs (TLNs) retrieved encompass both positive (PLNs) and negative LNs (NLNs), so the relationship between TLNs and prognosis is confounded by the prognostic effect of the number of positive LNs [32]. The concept of NLN counts has recently attracted attention as a prognostic indicator in

colon [20], gastric [13], esophageal [45], cervical [6] and breast cancer [39] but still little is known with respect to its prognostic significance. This study was designed to clarify this issue.

2. Materials and Methods

A prospective study including 114 Patients In mansoura university hospitals between 2005-2008 included patients aged 18 years or older who were diagnosed with localized invasive adenocarcinoma rectum with stage I, II, III. Tumour stage was based on TNM staging system and American Joint Committee on Cancer this has been recently updated as its seventh edition and is detailed elsewhere [16, 17]. We excluded in situ, metastatic, mucinous tumours, obstructing tumours and cancers on top of inflammatory bowel disease. Patients with less than total 12 LN harvest (College of American Pathologists consensus statement 1999) [8] and those with preoperative radiotherapy also excluded as it lower the number of LN retrieved and fewer LN metastasis [24, 38, 42, 44]. Operative procedures were conducted as usual: low anterior resection or abdominoperineal operation according to the site of the tumour with total mesorectal

excision [18, 27]. The specimen is examined macroscopically to detect exact site of the tumour, its configuration, extent then sections from the tumour prepared as paraffin section stained with H & E for the type, grade and infiltration of the bowel wall. The negative lymph nodes by hematoxylin and eosin stain then stained for CEA and cytokeratin to offer estimates of occult metastatic burden. Immunostaining technique was applied by adding anti CEA antibody to detect micrometastatic deposits of malignant cells in the lymph nodes and anticytokeratin antibody to prove its epithelial nature (figure 1) as some macrophages may absorb soluble CEA and give misinterpretation but cytokeratins were

consistently absent in macrophages [10]. Patients were followed up closely in both surgery department and department of clinical oncology and nuclear medicine for 5 years. Postoperative follow up included: clinical evaluation, serum CEA, abdominal ultrasound every three months, barium enema every six months, yearly colonoscopy and CT scan. We examined patient survival in relation to the negative lymph node count and the cutoff value was 10 NLN [34]. All of statistical analyses were performed using the statistical software package SPSS for Windows, version 16 (IBM Corp, Armonk, NY, USA). Statistical significance was set at two-sided $P < 0.05$.

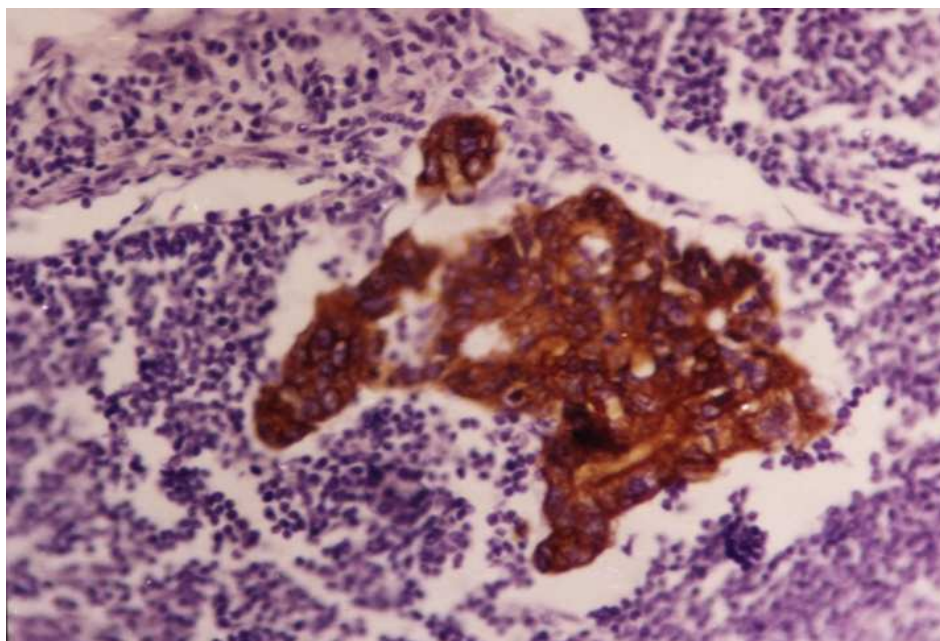


Figure 1. Grade II adenocarcinoma with focal deposits in LN positive for Cytokeratin (AntiCytokeratin antibody).

3. Results

A total of 114 patients fulfilled selection criteria during the 9-year study period were identified, including 61 male (53.5%) and 53 female (46.5%) patients. Peak age among patients was between 50 and 60 years (48.2%) ranging from 18 to 72 years. The main presentation was bleeding per rectum (65%). Tumours of the upper rectum represents 43%, midrectum 17% while 40% of tumours in the lower rectum.

All patients with upper rectal tumours underwent anterior resection and those having lower rectal tumours together with two patients with midrectal cancer had abdominoperineal resection. There were 17 patients (15%) with stage I, 72 patients (63%) stage II, and 25 patients (22%) with stage III rectal cancer. Moderately differentiated tumours were the commonest (48%), and the least were poorly differentiated (11%). Grossly, annular stenosing lesion was there in 50% of cases. The mean number of total lymph nodes (TLN) harvest was 14.4 LN and that of positive (PLN) and negative lymph nodes (NLN) were 3.7 LN & 10.7 LN respectively. The proportion of well & moderate differentiation gradually decreased from stage I to stage III (100% to 64%). Patients

with stage III cancer had PLNs (mean of 7.6). There were fewer NLNs in patients with stage III cancer (mean of 9) than those with stage II (mean of 11.1) or stage I (mean of 11.5) cancer (table 1).

Table 1. Demographic and pathological characteristics of our patients.

| Characteristic | No (114) | % |
|------------------------------|----------|-------|
| Sex | | |
| Male | 61 | 53.5% |
| Female | 53 | 46.5% |
| Age | | |
| <50 | 36 | 31.6% |
| 50-60 | 55 | 48.2% |
| >60 | 23 | 20.2% |
| Clinical presentation | | |
| Bleeding/rectum | 74 | 65% |
| Constipation | 21 | 18.4% |
| Mass | 11 | 9.6% |
| pain | 8 | 7% |
| Site of the tumour | | |
| Upper rectum | 49 | 43% |
| Mid rectum | 19 | 17% |
| Lower rectum | 46 | 40% |

| Characteristic | No (114) | % |
|--|---|------|
| Type of surgery | | |
| Anterior resection | 66 | 58% |
| Abdominoperineal resection | 48 | 42% |
| Gross examination | | |
| Annular stenosing lesion | 57 | 50% |
| Ulcerative lesion | 48 | 42% |
| Cauliflower mass | 9 | 8% |
| pathological stage | | |
| stage I | 17 | 15% |
| stage II | 72 | 63% |
| stage III | 25 | 22% |
| Pathological grading | | |
| Well differentiated | 47 | 41% |
| Moderate differentiated | 55 | 48% |
| Poor differentiated | 12 | 11% |
| Well&moderate differentiated tumours /stage | | |
| stage I | 17/17 | 100% |
| stage II | 69/72 | 96% |
| stage III | 16/25 | 64% |
| | 14.4 LN (range from 12-35) in all stages | |
| Mean No. of LNs dissected (TLN) | | |
| | 13.7 LN in stage I | |
| | 13.8LN in stage II | |
| | 16.6LN in stage III | |
| | 3.7 LN (range from 0-8) in all stages | |
| Mean No. of positive LNs (PLN) | | |
| | 7.6 LN in stage III | |
| | 10.7 LN (range from 12-33) in all stages | |
| Mean No. of negative LNs (NLN) | | |
| | 11.5 LN in stage I | |
| | 11.1 LN in stage II | |
| | 9 LN in stage III | |

We examined the effect of the negative lymph node count (NLN), as the total lymph node count included positive lymph nodes, which determined tumour stage and affected patient prognosis. Negative lymph node count was a variable independent of tumour stage, so the prognostic effect of the negative lymph node count (independent of tumour stage) could be measured more accurately.

Five years of follow up by frequent clinical examination, radiological studies, endoscopy revealed that recurrence had occurred in different sites in thirty patients (26%). Nine of them had only liver secondaries (three were candidates for successful resection). Sixteen patients had locoregional recurrences, six patients with anastmotic recurrence (reoperated upon by abdominoperineal resection), four patients showed abdominal nodal recurrence, two patient had recurrence at the wound of the perineum after abdominoperineal resection (both groups recieved chemotherapy), two patient had both liver and lung secondaries, two patient had abdominal mass and wound recurrence (both groups were not amenable for further intervention). The last five patients had liver and locoregional recurrences (resectable in one case). During follow-up, there were a total of 19 deaths, 16 were related to cancer recurrences. Kaplan Meier plots were used to assess disease free survival and overall survival according to the number of negative lymph nodes. Both five-year disease free

and overall survivals were significantly higher in patients with more than 10 negative LN than those with less than 10 negative LN (P = 0.021 and 0.012 respectively) (table 2) and (figures 2, 3).

Table 2. The relation between survival and the number of negative LN.

| survival | | No. of negative LN | | P value | | |
|------------------------------|-------|--------------------|---------|---------|------|-------|
| | | <10 NLN | >10 NLN | | | |
| | | no. | % | No. | % | |
| Disease free survival | Yes | 36 | 62.1 | 48 | 85.8 | 0.021 |
| | No | 22 | 37.9 | 8 | 14.2 | |
| | Total | 58 | 100 | 56 | 100 | |
| Overall survival | Yes | 42 | 72.4 | 53 | 94.6 | 0.012 |
| | No | 16 | 27.6 | 3 | 5.4 | |
| | Total | 58 | 100 | 56 | 100 | |

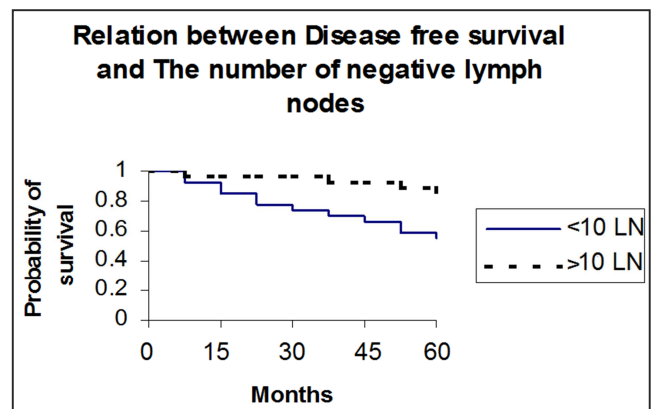


Figure 2. The relation between Disease free survival and the number of negative LN.

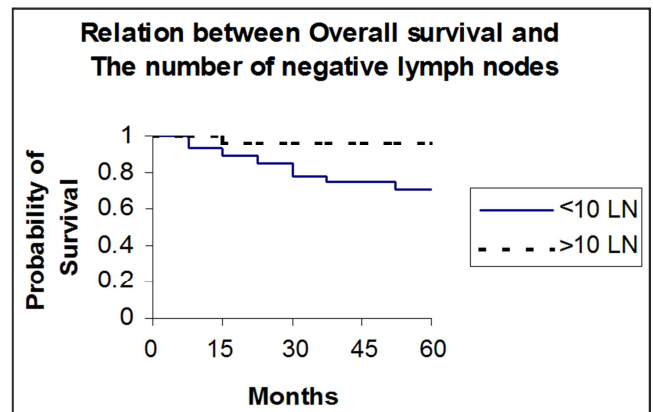


Figure 3. The relation between Overall survival and the number of negative LN.

4. Discussion

Metastasis to regional LNs is one of the most important prognostic factors of colorectal cancer [2, 23], and accurate assessment of lymph node status in patients with colorectal cancer is clearly essential in nearly all pathological staging systems for colorectal cancer. For our research, we studied the prognostic significance of the number of negative lymph nodes in stages I–III rectal cancer patients .The number of

NLNs has been confirmed as an independent prognosis factor in colon [20], rectal cancer [34]. Qingguo Li and his colleagues [34] found that the number of NLNs had a weak or negligible correlation with PLN counts, which means that it was a prognostic factor independent of current metastatic LNs count-based staging. They also found a cutoff limit, after which an increase in the number of NLNs examined will not have any influence on the accuracy of staging and survival, that cutoff value is 10 NLNs and it was applied in our study. We observed a significant relation between negative lymph node count and survival, where the disease free survival and overall survival were significantly better among the group of patients with more than ten negative LNs ($P = 0.021$ & 0.012 respectively).

In previous studies [11,12,14,21,24,37,38,40,42,43,44], the number of recovered lymph nodes or the number of negative lymph nodes has consistently been associated with longer survival in colorectal cancer. The reasons behind the relationship between the number of NLNs and survival have not been determined, although several theories have been postulated. It may be the case that more adequate surgical excision of the tumours and lymph nodes positively affecting survival as there is less likelihood of leaving tumour cells behind and it reduces the chances of iatrogenic spread of cancer cells [29, 30]. The second theory stated that more lymph node harvest will allow accurate staging and identify a metastatic focus with molecular detection [35], or the use of immunostaining [9, 10]. However, this was obvious in stage I and II with less effect in stage III [34], also a greater number of detected lymph nodes could reflect better surgical resection or pathology [3,5]. This finding is matched with our results as lower grade tumours are less in stage III rectal cancer, also stage III patients have positive LNs and fewer negative LNs than stage I & II. However we did not test separate stages as we have fewer patients and most of them have stage II tumours (69 out of 114 patients) so it will not be statistically of value. The third theory considered the host lymphocytic reaction to the tumour, which is associated with LN count [15]. It was found that lymphocytic reaction to tumour cells has been associated with longer survival in colorectal cancer [28,31] as it may be an indicator of host immune response to tumour cells [20,31]. Greater lymphocytic reaction has been associated with high microsatellite instability proportion [1, 19] which, in turn, is associated with longer patient survival [33]. On the other hand, Shuji Ogino with his researchers [41] proved that independent of patient characteristics and other related molecular variables the effect of the negative lymph node count was apparent in all stages of disease although the benefit was significantly greater among patients with earlier pathologic stage.

5. Conclusion

Our study shows that the number of NLNs was an independent prognostic factor for rectal cancer patients. For survival benefit, we suggest at least 10 NLNs should be

retrieved from rectal cancer patients.

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