IMPACT OF NEOADJUVANT THERAPY ON THE LYMPH NODE YIELD AFTER SURGERY FOR RECTAL CANCER

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CERTIFICATE

I hereby certify that this dissertation on "*Impact of Neoadjuvant Therapy on the Lymph Node Yield after Surgery for Rectal Cancer"* is a bonafide work done by **DR. ANAND RAJA,** in the department of Surgical Oncology, College of Oncological sciences, Cancer Institute (WIA), Chennai, under my guidance and supervision, to my satisfaction.

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1. INTRODUCTION

The number of lymph nodes harvested from the surgical specimen after total mesorectal excision (TME) is a key factor in the contemporary management of rectal cancer. In 1931, Ernest Miles identified the upward zone of lymphatic spread of rectal cancer along the superior haemorrhoidal vessels and the inferior mesenteric vein as being the most constant and therefore the most important route of rectal cancer spread.[1] Subsequently, Grinnell[2] and Dukes[3] reported that both the number of lymph node metastases and their location affects the prognosis in patients with rectal cancer. The number of lymph nodes retrieved indirectly reflects the quality of both surgical and pathologic procedures.[4] In 1990, the Working Party Report to the World Congress of Gastroenterology in Sydney suggested that a minimum of 12 lymph nodes should be recovered for colorectal cancer staging[5]. This was adopted in 1999 by the American Joint Committee on Cancer.[6] The examination of fewer than 12 LNs is considered a surrogate marker of inadequate surgery and/or pathologic examination and is a relative indication for adjuvant therapy.[7-8] Removal of fewer lymph nodes is also associated with a poorer survival and higher rates of local recurrence.[9-12] If the nodal status is not determined accurately, the prognosis may be difficult to determine and it may be incorrect. Many factors influence the number of lymph nodes retrieved. These factors may be related to the patient (sex, obesity), to the surgeon (specimen size and type of lymphadenectomy) to the tumour (size, stage, and localization) and to the pathologist (experience and technique used for harvesting the specimen).

Lymph nodes are fewer in the rectum than in the other parts of the colon[36] Recently, neoadjuvant chemoradiation (NCRT) therapy has been considered the preferred treatment strategy for locally advanced distal rectal cancer.[13] NCRT is thought to decrease the lymph node yield after surgical excision.[14-17] The extreme expression of this phenomenon is observed after complete pathologic response to CRT, when lymph nodes may be totally absent in the rectal specimen. The prognostic significance of this response is still under investigation.[18, 19] Both short and long course preoperative radiotherapy have been associated with a decreased number of lymph nodes retrieved in rectal specimen compared with surgery alone[36].(It decreased from 10 to 8 in the prospective Dutch TME trial[36] using short course radiotherapy18 and from 19 to 14 and 10 to 7 in 2 series using long course radiotherapy However, in these studies no multivariate analysis was performed to avoid any bias of selection). This is probably caused by the immune response and fibrosis in lymph nodes exposed to radiotherapy, which results in diminution in their size, making their identification in the pathology specimen difficult.[20]. Apoptosis after radiotherapy occurs more rapidly in lymph nodes than in tumour itself.[36] In a study by Baxter et al[15], using a minimum of 12 lymph nodes retrieved as the standard guideline, only 20% of patients who received NCRT underwent adequate lymph node sampling. To date, the number of nodes retrieved after NCRT and their prognostic significance has not been fully characterized[15, 21-22].

The increasing use of NCRT in rectal cancer raises questions:

1. Is harvest of less than 12 nodes acceptable?

2. If so, can a cut of value for a minimum number of nodes harvested after NCRT be specified?

3. What are the various factors influencing lymph nodal after NCRT. If the nodal yield is significantly influenced by any variable(s), it may be possible to maximize the number of retrieved nodes and consequently improve the accuracy of staging.

At present, there is no clear consensus on the minimum number of nodes to be harvested after NCRT for rectal cancer to signify adequacy of staging. The impact of the nodal yield on outcomes is also debated.

This study was thus undertaken to evaluate the influence of NCRT on lymph nodal yield, to evaluate the various factors influencing the lymph nodal yield and to evaluate the impact of nodal yield on survival.

This study is also important as despite extensive search, we could not find any data from India with regard to nodal yield in rectal cancer after NCRT.

2. AIMS AND OBJECTIVES

1. To evaluate the influence of neoadjuvant therapy on lymph nodal yield.

2. To evaluate the various factors influencing the lymph nodal yield after neoadjuvant therapy

3. To evaluate the impact of nodal yield following neoadjuvant therapy on survival.

3. REVIEW OF LITERATURE

Adequate dissection of the draining lymph nodes in colorectal cancer is very important. On one hand, it provides adequate staging of the tumour, thus allowing proper prognostication and affects the decision to receive effective adjuvant treatment. It has been shown that the greater the number of lymph nodes removed, the higher the possibility of finding involved ones. On the other hand, adequate resection of lymph nodes improves local control, decreases the incidence of local recurrence and improves survival. The number of nodes retrieved in a surgical specimen reflects the extent of surgical resection and the effort of a pathological study. To stage accurately node-negative rectal cancer, an examination of a minimum of 12 lymph nodes is recommended by The College of American Pathologists.[11]

Today, NCRT followed by surgery is a widely practiced strategy for locally advanced rectal cancers. The average lymph node yield was reported to be reduced in the resection specimen after neoadjuvant treatment compared to primary surgery specimens. Therefore, the UICC/AJCC recommendation of 12 or more locoregional lymph nodes is unlikely to be met in many patients with rectal cancer after neoadjuvant treatment. While this fact has been recognized and documented not only in independent reports, but also in the latest edition of the UICC TNM classification, the factors affecting the nodal yield and the prognostic impact of the reduced number of total lymph node yield after NCRT, however, is not fully determined.

Marks et al[41] concluded that there may be great variability in the lymph node yield after NCRT. An absolute minimum target number of 12 lymph nodes, is not realistic, because 23% of patients with rectal cancer will have 5 lymph nodes or less in their mesentery in a TME specimen after neoadjuvant therapy, and 72% will have less than 12 nodes. Only 28% of the patients had 12 lymph nodes or more in the resected specimen. They analysed the impact of various factors and concluded that age, sex, preoperative clinical T stage, dose of radiation, type of neoadjuvant therapy (chemo-radiation vs. radiation alone), tumour regression, time interval between completion of NCRT and surgery and type of surgery have no effect on the nodal yield.

Habr-Gama et al[38] retrospectively analysed their data on patients with carcinoma rectum after NCRT. They found that 11% of their patients had no nodes harvested in the specimen (ypNx) and the survival rates of these patients was slightly better when compared to patients with ypN0 disease, although this difference was not statistically significant. They also concluded that the absence of recovered nodes after neoadjuvant NCRT and radical surgery is infrequent and does not necessarily correlate to disease understaging or inadequate resection. Rather, when radical surgery is performed, ypNx may be associated with increased sensitivity to chemoradiation and significant tumour downstaging. Patients with ypNx after neoadjuvant CRT and radical surgery should not be considered high-risk patients for recurrence development and that larger series are probably required to fully demonstrate a possible survival benefit among these patients. There was

no association between time interval between completion of NCRT and surgery with the nodal yield.

Sebastian et al[37] retrospectively compared patients undergoing straight surgery to those receiving NCRT followed by surgery and concluded that patients exposed to preoperative standard chemoradiation for colorectal cancer have a significantly lower number of lymph nodes retrieved during surgery compared to patients undergoing straight surgery (14.6 Vs. 17.2). In this study, the average number of lymph nodes in the patients receiving NCRT was above the recommended number of 12. They also evaluated the influence of age, gender, race, surgery type, and neoadjuvant therapy on lymph nodal yield and concluded that neoadjuvant therapy was the only factor affecting the total number of lymph nodes retrieved.

Baxter et al[15] collected data from the Surveillance, Epidemiology and End Results (SEER) cancer registry, which collects information on cancer incidence and survival from 11 population-based cancer registries and three supplemental registries in the United States of America. Their findings reveal that on an average, fewer nodes were examined in patients who had undergone preoperative RT (7 nodes) compared to those who did not (10 nodes); this difference was statistically significant in a multivariate analysis, which controlled for age, race, gender, year of diagnosis, stage, and patient geographic location. In 16% of patients who underwent preoperative RT (vs. 7.5% who did not), no nodes were identified in the resected specimen. This difference remained statistically significant after adjusting

for covariates. Only 20% of the patients who received NCRT had adequate lymph node staging (12 or more nodes harvested in the specimen), compared to 33% who underwent straight surgery. They found that the odds of harvesting adequate number of lymph nodes were less than one-half after preoperative RT (adjusted odds ratio, 0.48; 95% confidence interval, 0.38–0.56). They recommend that until further information is generated, given the difficulty with pathologic staging after preoperative therapy in rectal cancer patients, these patients should have their disease staged before preoperative treatment; this initial stage should then be accepted as correct, unless the pathologist (or the surgeon) finds more extensive disease than initially indicated. Interestingly, the number of recovered nodes was higher for N+ disease than for N0 disease. The authors not only concluded that radiotherapy might decrease the number of recovered nodes but also that the minimum established number of recovered nodes (≥12) may be inappropriate after neoadjuvant therapy. It is important here to remember that this was a population-based study in which the surgical technique and the CRT regimen were diverse

Rullier et al[36] evaluated nodal yield after chemoradiation, the factors affecting the nodal yield and survival analysis. Compared with surgery alone, preoperative chemoradiotherapy decreased the number of lymph nodes retrieved by 24% (13 vs. 17) and the number of positive lymph nodes by 48% (1.2 vs. 2.3). They also demonstrated that the dose of radiotherapy influenced the number of lymph nodes retrieved. Addition of 1Gy decreased the number of lymph nodes retrieved

by 0.21%. Although NCRT had an impact on lymph nodes retrieved, no association was found between the long-term survival and the number of lymph nodes retrieved in irradiated specimen with negative lymph nodes. They performed a cut point analysis in an attempt to arrive at a “number of nodes”, that would predict adequacy after NCRT, but no cut-off value of lymph nodes was observed. The disease-free survival was similar in patients in whom <10 nodes were retrieved and those in whom >10 lymph nodes were retrieved (72% vs. 70% at 5 y). Their results suggested that, in contrast to colon cancer and non-irradiated rectal cancer, prognosis of patients with irradiated rectal cancer is not influenced by the number of lymph nodes retrieved. They recommended that in the absence of specific rectal guidelines, the pathologist should optimize the number of rectal lymph nodes retrieved. When less than 12 lymph nodes are retrieved, a second look at the specimen is therefore indicated as quality control. Only after such further specimen examination, the total number of lymph nodes retrieved, even less than 12, can be considered as accurate.

Wang et al[48] investigated the possible relation between lymph node harvest and primary tumour response in the setting of surgery after NCRT. They used three parameters as indicators of primary tumour response: pathologic (yp) T stage, primary tumour size, and tumour regression grade (graded from 1 to 5). After grading, the number of lymph nodes between the groups was compared. In the neoadjuvant group the tumours had an earlier T stage (P = 0.001), earlier overall TNM stage (P = 0.001), and less incidence of distant metastasis (P = 0.001)

than did tumours in the non neoadjuvant group, but there was no significant difference in N stage (P = 0.591). T stage in the neoadjuvant group represents pathologic, postradiation staging; thus, downstaging may explain the earlier T stage in the NCRT group. The number of lymph nodes was significantly lower in the neoadjuvant group than in the non neoadjuvant group (15.4 vs. 20.6, P =0.011). Significantly fewer lymph nodes were harvested in the early T stage group than in the advanced T stage group, and there was a trend of fewer lymph nodes harvested in the small size tumour group as compared with the large size tumour group. Moreover, there were significantly fewer lymph nodes in the early T stage group and the small size tumour group as compared with the non neoadjuvant group. No significant difference was found between the non neoadjuvant group and either the neoadjuvant advanced T stage group or the neoadjuvant large size tumour group. This finding indicates that the possible relation between lymph node number and tumour characteristics (invasion depth and size) shown in previous studies holds true after NCRT. They observed that fewer lymph nodes were found in the neoadjuvant low TRG group than in the neoadjuvant high TRG and non neoadjuvant groups, supporting the notion that a decreased lymph node number is related to good tumour response. They concluded that although for poor responders the established number of 12 lymph nodes may be justified, this standard may not apply for tumours that exhibit a good response.

Wijesuriya et al [39] analysed their data on nodal yield after NCRT. Tumour regression was seen in all patients after NCRT. Complete regression was observed

in 30% of the patients. The nodal yield was significantly lower in patients receiving NCRT compared to straight surgery (4Vs.9). The median diameter of the largest node was also significantly smaller in the NCRT group than in the control group, at [5mm (range 2–12mm) vs. 9mm (range 4–15mm)]. In 15% of the patients no nodes could be harvested. Tumour deposits were found in similar proportions in the two groups; in 4 of 17 patients, being 11 of 83 (13.25% of lymph nodes examined in the NCRT group vs. 9 of 20 patients, being 33 of 177 18.6% lymph nodes examined in the control group (P = 0.308). They concluded that In conclusion, the results of their study showed that NCRT was effective in down-staging rectal tumours and lymph nodes. This resulted in the retrieval of fewer and smaller lymph nodes from irradiated patients than non-irradiated controls.

Scott et al[49] compared patients who underwent straight surgery, surgery after short course and long course chemo-radiotherapy. The median numbers of nodes found were in each of the three groups were 17, 17.5, and 13.5, respectively (not significant; Mann-Whitney U test). They concluded that both short course radiotherapy and chemoradiotherapy given over four to six weeks can significantly reduce the size of mesorectal lymph nodes, presumably as a result of apoptosis and involution. This will make nodes harder to find, but a careful dissection technique by an interested pathologist will still discover substantial numbers of nodes.

Taflampas et al[33] divided their patients into 3 groups. Group A, the control group, included patients who did not receive preoperative CRT. Group B consisted of patients who underwent the short course of CRT, and Group C included patients who underwent the long course of NCRT. The number of overall LNs removed was lower in Group B and Group C, and the number of positive LNs was higher in Group C, as compared with the control group. Neither trend reached statistical significance in this series. Patients who underwent the long course of CRT, however, were more likely to have fewer than 12 LNs examined (P = 0.03). In this series, the number of LNs (overall and positive) retrieved was not significantly reduced by NCRT. This could be attributed to the fact that the study population included patients with all T stages and that the patients were managed by a dedicated multidisciplinary team. Because of the small number of patients, this study could be considered prone to Type 2 error, and it is possible that if the number was higher, this trend could be proven to be statistically significant. The number of patients with less than 12 nodes harvested in the specimen were 33.68%, 30.23% and 56.66% in groups A, B and C respectively. The mean nodal yield for groups A, B and C were 17.16, 16.18 and 14.37 respectively. The mean numbers of positive nodes for the 3 groups were 1.95, 1.25 and 2.73 respectively.

Wichmann et al[22] prospectively analysed the perioperative database of a single institution to study the importance of lymph node retrieval after preoperative radiochemotherapy in rectal cancer. They found that the nodal status in patients who underwent straight surgery without receiving neoadjuvant chemoradiation

and patients who underwent surgery after neoadjuvant chemoradiation were 19.1 and 13.6 respectively ($p < .05$). The numbers of positive nodes were 1.4 and 3.1 in the NCRT and straight surgery arms. The assessment of the number of lymph nodes within the resected specimen in relation to the size of the primary cancer revealed that only in patients with a primary cancer of pT3 significantly more lymph nodes were resected from those who did not receive NCRT, while the differences observed in patients with primary cancer stages pTx, pT0, pT1, pT2, and pT4 did not reach statistical significance. These findings indicate that the number of lymph nodes did not directly relate to the primary cancer size and that possible variations in the distribution of cancer sizes did not account for the significant differences regarding lymph node. The percentage of patients with fewer than 12 LNs examined was significantly higher in the patients who underwent CRT (11.9% *vs.* 7.1%). They found that though no significant differences regarding the ratio of anterior and abdominoperineal resections were observed between both groups, they detected significant differences regarding the number of lymph nodes resected with the procedures. In patients who did not receive preoperative radiochemotherapy, anterior rectal resection resulted

in 19.3 lymph nodes per specimen, while specimens after abdominoperineal resection contained 16.1 lymph nodes ($P<.05$). In patients who did receive

preoperative radiochemotherapy, 14.0 and 14.1, lymph nodes per specimen, respectively, were detected ($P<.05$ vs. corresponding control patients).

Rinkus et al[40] in their study revealed as opposed to straight surgery, the nodal harvest was significantly lower in patients receiving NCRT (mean10 Vs. 6.8,p value=0.003). The mean number of positive nodes was 0 in patients receiving NCRT and 4 in those undergoing straight surgery (p 0.005). The patient survival decreased as the number of nodes harvested increased though this was not statistically significant (p =0.006).There was no difference in nodal yield when stratified according to tumour size. The type of surgery did not have an impact on the nodal yield.

Kim et al[21] conducted a study to assess factors associated with the number of nodes retrieved and the impact of the number of lymph nodes in rectal cancer in patients who received NCRT with radical surgery. In 3.48% of the patients, no nodes could be harvested from the specimen. The mean number of nodes was 19.5 in the ypN(-) (pathological node negative) patients and 23.2 in ypN(+) (pathological node positive) patients (p=0.03). However, multivariate linear regression showed that ypN(+) status was not associated with an increased number of nodes retrieved (p=0.76). An advanced ypT classification (ypT3 and ypT4) and a tumour diameter greater than 4 cm were associated with an increased number of nodes retrieved. Intergroup comparison showed that there was no difference of cancer specific and recurrence free survival among ypNx patients and a subset of ypN(-) patients based on the number of nodes retrieved. In this study, using a 12 lymph nodes guideline, 77 patients (29.8%) had insufficient lymph nodes retrieval (0–11 nodes retrieved. Patients receiving NCRT had significantly

lesser number of mean nodes retrieved compared to straight surgery patients (21 Vs. 24.5). They concluded that in a neoadjuvant setting, ypN(+) disease was an independent risk factor for oncological outcomes. However, an absence of nodes (ypNx) or a decreased number of nodes retrieved in ypN(-) patients do not represent an inferior oncological outcome. The number of nodes does not seem to impact survival and recurrence seems in ypN(-) patients.

Ha et al[42] retrospectively analysed data in Korean population for the adequacy of lymphadenectomy and the factors affecting nodal yield after NCRT. In patients treated without preoperative chemoradiotherapy, percentage of patients with at least 12 lymph nodes harvested was 85.6% (185/216), compared with 60.2% (240/399) in patients treated with preoperative chemoradiotherapy. Preoperative chemoradiotherapy decreased the odds of retrieving at least 12 lymph nodes by up to 75%. The odds also increased with increasing stage. Univariate analysis revealed that age, BMI, preoperative chemoradiotherapy, location, and stage significantly influenced the number of lymph nodes retrieved. On multivariate analysis, age, BMI, preoperative chemoradiotherapy, and stage were the independent factors that influenced the number of lymph nodes retrieved. The mean number of lymph nodes retrieved (adjusted by age, BMI, and stage) was significantly lower in patients treated with preoperative chemoradiotherapy compared with those not treated (14.5 vs. 21.5; P <0.001). There was no correlation between CRM and number of lymph nodes (P =0.637). There was no correlation between specimen length and number of lymph nodes (P

= 0.109). In this study increasing age is a significant factor for decreased lymph nodes retrieval. This study confirmed that the number of lymph nodes retrieved significantly decreases with increasing BMI. Gender was not co-related with nodal yield. In this study, more lymph nodes were evaluated in patients with stage II and III than in those with stage I. Preoperative chemoradiotherapy was the most prominent factor affecting the number of lymph nodes. The reduction rate by preoperative chemoradiotherapy was 32.6% (7/21.5). The overall and disease-free survival rates were not different regardless of whether the patients group had the minimum number of lymph nodes retrieved or not (82.8% vs. 81%, *P* =0.68, 77% vs. 76.9%, *P* = 0.73 respectively). When applying adjusted cut-off value of 8 lymph nodes in patients who underwent preoperative chemoradiotherapy, they also did not find any association between the survival and the number of lymph nodes (<8 vs.8 or more lymph nodes; 79.1% vs. 85.1% in the overall Survival, *P* = 0.33, 76.6% vs. 79.3% in disease-free survival, *P* = 0.38).

Luna-Perez et al[29] conducted their study in the Mexican population to determine the prognostic significance of retrieved lymph nodes in specimens of locally advanced rectal adenocarcinoma treated with preoperative chemoradiotherapy plus surgical resection with total mesorectal excision. Overall number of retrieved lymph nodes in patients receiving NCRT was 2,554 (average 12.1/specimen, range 1–59); mean of 22 lymph nodes was obtained with use of fat clearing technique, whereas mean of eight lymph nodes was obtained in specimens studied with manual technique ($p < 0.001$); 252 (9.8%) of these

contained metastases. Lymph node metastasis was observed in 72 of 210 specimens (34.2%). Patients were grouped according to number of retrieved lymph nodes per specimen: those with 1–10 lymph nodes (*n* = 119/210) and those with 11 or more lymph nodes (*n* = 91/210). Five-year cancer-specific survival in the former group was 48%, and conversely 69.6% in the latter group (p = 0.02). Patients with pT0–2,N0,M0 had 1% of local recurrences, whereas there were 12.2% and 19.7% local recurrence in patients with pT3–4,N0,M0 and pT2–4, N_, M0, respectively (p = 0.001). Five-year survival in 1–10 lymph-nodes group and in the 11 or more lymph group according to N (1–2) status was as follows: N1 (28 and 69%, respectively) (p = 0.04), whereas in N2 it was (0 and 19%, respectively) (p = 0.74). Results of the current study suggest that the final stage is dependent on number of retrieved lymph nodes and is a powerful prognostic covariate for local recurrence and survival as demonstrated in multivariate analysis. In this series, significant differences in 5-year survival were found in N1 group of patients according to number of lymph nodes retrieved per specimen. In N1 group with 1–10 lymph nodes, it was 28%, whereas it was 69% in the group with 11 or more lymph nodes (p = 0.04). However, no difference was found in patients with N2. These results corroborate the number of retrieved lymph nodes per specimen properly staged and support the fact that appropriate staging could allow patients to be stratified for more aggressive adjuvant chemotherapy therapy such as the combination of 5-FU continuous infusion or oxaliplatinum in patients with metastatic lymph nodes after preoperative chemoradiotherapy. Furthermore, the

group of under sampled patients should be considered for adjuvant chemotherapy. They concluded that In conclusion, retrieval of at least 11 lymph nodes in surgical specimen is a powerful tool to stage patients with rectal adenocarcinoma treated with chemoradiotherapy and surgery due to the fact that proper staging is the main predictor for local recurrence and survival.

Beresfordet al[50] prospectively studied 161 patients who were deemed unresectable at presentation to short course radiation followed by surgery. The probability of survival at 36 months increased from 47% in the baseline group (any positive nodes) to 62% among those node-negative but less than three nodes recovered (HR =0.72), and to 70% among those node-negative but with three or more nodes recovered (HR=0.48). The risk associated with presence of three or more negative nodes is significantly reduced below the baseline. We also studied disease-free survival (DFS). For node positive patients, 3-year DFS was just 16% (median = 26months). For those node-negative patients in whom zero, one and two lymph nodes were identified, this increased to 26% (median = 32 months). Log-rank comparisons of these two groups showed no significant difference (probability, Chi-square = 0.123). If three or more negative nodes were found, 3-year DFS was 58% (median not reached). This was significantly better than for the node positive group on log-rank comparison (P = 0.005), but failed to reach significance when compared with the zero-, one- and two- node-negative group (P = 0.43).

Lindebjerg et al[51] investigated the prognostic impact of tumour response evaluation by the tumour regression grade system combined with the ypN-status. Patients with post-treatment positive lymph node metastases had a significantly lower survival rate compared with patients who were ypN-stage negative (63% and 87% respectively, P = 0.007). The 5-year survival rate of patients, who were lymph-node negative and had a major pathological response (TRG 1–2) was 100%, compared with 60% in patients with a major pathological response and positive lymph nodes. The difference was highly significant (P < 0.01).

Maschuw et al[17] evaluated the role of short course chemoradiation on nodal yield. The median number of lymph nodes in the surgical specimens taken from patients who had undergone hypofractionated preoperative radiotherapy (case group) was 12 (range 1–19). In the control group, the median number of lymph nodes was 15 (range 4–36). They detected a significant difference (p=0.0005) in the number of lymph nodes between the two groups. In addition, the histopathological tumour stage, T2 vs. T3, did not have any significant influence on the number of detected lymph nodes (OR=0.446, CI 0.178–1.127, and p=0.093).They hypothesized that the decreased detection of lymph nodes after short-term preoperative radiotherapy might not be explained by a reduction of lymph node numbers but only by a harder detection of lymph nodes by the routinely performed pathologic examination. They concluded by questioning if 12 nodes could be taken as adequate after NCRT.

Chang et al[52] evaluated the prognostic impact of pathologically identified persistent LN metastasis after preoperative radiotherapy for locally advanced rectal cancer in the SEER dataset of the Unites States. Among patients who received radiation therapy preoperative radiotherapy was associated with a greater proportion of patients who had lower N stage compared to post-operative radiation ($p < .001$), a lower total number of LNs examined (median 6 vs. 9, $P < .001$), and a lower number of positive LNs identified (median 2 vs. 3; $P < .001$). Preoperative radiation therapy was also associated with an increased likelihood of having no LNs reported upon resection ($P < .001$) (18.6% in patients with NCRT compared to 6.2% in patients without NCRT). They argued that lymph node status following preoperative radiotherapy is a significant predictor of outcome. Persistence of LN metastasis was associated with an increased risk of long-term cancer-related mortality and may serve as a marker for a tumour's more aggressive biologic behaviour and the consequent need for improved adjuvant chemotherapeutic regimens.

Doll D et al[35] investigated the total lymph node yield and the number of tumour-positive lymph nodes in the resection specimens of two patient groups with T3 rectal carcinoma. One group of patients was primarily resected and received adjuvant radiochemotherapy postoperatively, the other patient group received neoadjuvant radiochemotherapy before surgery. Total lymph node yield was significantly lower in the neoadjuvant group compared to the primary-surgery group 12.9 Vs. 21.4 nodes ($p<0.0001$). Forty patients (39%) in the neoadjuvant

group did not meet the UICC/AJCC consensus recommendation of a total lymph node yield of 12 or more. No lymph node metastases were found in 75/102 (74%) resection specimens after neoadjuvant therapy and in 63/114 (55%) primary-surgery specimens. For tumour-positive lymph nodes, a mean number of 1.0 ± 2.4 were detected after neoadjuvant treatment, while the primary-surgery group had a mean number of 2.3 ± 4.4 ($p = 0.014$). A lymph node harvest of 12 or more did not correlate with better survival compared with a lymph node count of less than 12 ($p = 0.524$). The 5-year survival rate was 88% in the tumour-free lymph node group and 63% when one to three positive lymph nodes were found on pathologic examination. For patients with more than three tumour-positive lymph nodes, survival dropped to 39% after 5 years (Fig. 2). Thus, the number of tumour-positive lymph nodes significantly influenced overall survival of patients with neoadjuvant therapy for rectal carcinoma ($p<0.0001$). In the study the authors could rule out that the differences in total lymph node yield in the after-neoadjuvant-therapy specimens compared to that in primary-surgery specimens was due to confounding factors that had previously been suggested to influence total lymph node yield. Possible confounding factors such as gender, age, tumour location, and type of surgery [Miles procedure versus (low) anterior resection] were statistically evenly distributed in both of our study groups. They concluded that the number of lymph nodes in a radiated specimen should not be compared to the number of lymph nodes in a primary-surgery specimen. Neither does a reduced total lymph node yield in a neoadjuvantly treated rectal cancer specimen necessarily indicate

inadequate lymphadenectomy or inadequate pathologic workup. They also concluded that the recommendation for a total lymph node yield of 12 or more should be revised for rectal carcinoma following neoadjuvant radiochemotherapy treatment, as a reduced lymph node yield in a rectal carcinoma specimen after neoadjuvant radiochemotherapy has no prognostic relevance.

Marcos et al[20] looked at the effect of preoperative chemoradiotherapy (CRT) on the number of lymph nodes retrieved in the mesorectal specimen. Other clinicopathological factors were also studied. Patients receiving preoperative CRT had a lower median number of lymph nodes retrieved than those who had surgery upfront (16 vs. 19, $p = 0.004$). Only 64% of patients receiving neoadjuvant therapy had 12 or more lymph nodes removed, in comparison to 88% for those having no preoperative therapy ($p = 0.003$). Other factors that were associated with a lower yield of lymph nodes included female sex and tumour location in the lower rectum. Multivariate analysis of the data also showed a significant association with the above-mentioned factors. Patient age, tumour grade, T stage and type of surgery (APR vs. LAR) had no impact on lymph node yield. Timing of surgery any time after 3 weeks of finishing CRT resulted in similar numbers of lymph nodes retrieved.

Sprenger et al[53] prospectively determined the effects of chemoradiation on mesorectal lymph node retrieval with a meticulous histopathological evaluation. All patients were participants of the randomized phase III German Rectal Cancer

Trial CAO/ARO/AIO-04 and received standardized 5-FU-based long-term CRT, curative surgical resection including TME, and extensive macroscopic and histopathological diagnostics. A total number of 2,021 lymph nodes were recovered (range, 12–81; median, 30.0). Twenty patients (31.3%) had persistent nodal metastases in cumulative 53 lymph nodes. The mean number of involved nodes was 2.65 per patient (median, 1.0; range, 1–8 nodes). Among these 53 lymph node metastases, 15 manifested as micrometastases (not larger than 0.2 cm). Three additional patients (4.6%) showed evidence of isolated tumour cells (ITC, not larger than 0.02 cm) in one lymph node each. According to the current TNM classification, the latter were classified as “ypN0” or “ypN0 (i+)” respectively, characterizing ITC as cells without yet known specific metastatic attributes. Lymph node size including non-metastatic and metastatic nodes was below 0.5 cm in all but one case. The majority of nodes ranged between 0.1 and 0.2 cm. There was no significant correlation between the numbers of detected mesorectal lymph nodes and patient-dependent variables (gender and age). Tumour-related variables (tumour size, ypTNM status, number of lymph node metastases, histopathological tumour regression grade, tumour differentiation, lymph and blood vessel invasion, and perineural invasion) did also not affect the number of available lymph nodes within the perirectal tissues. The 44 patients without nodal involvement had a median number of 30 detected nodes, whereas patients with lymph node metastases had 29.5 nodes. Interestingly, in patients with solely micrometastatic involvement, the median retrieval accounted for only 24.5 nodes. They stated that

an appreciable number of mesorectal micrometastases in lymph nodes below 0.5 cm are not being detected by manual lymph node recovery and standard pathological diagnostics. They concluded that the diligence and accuracy of the pathologist—beside the surgeon's obligation to supply high-quality TME specimens—is essential for sufficient lymph node retrieval and valid nodal staging after NCRT.

Latkauskas et al[54] assessed the role of preoperative radiochemotherapy on the number of lymph nodes detected in the tumour-bearing specimen. The mean nodal yield in patients receiving NCRT and those not receiving it was 6.29 Vs. 13.5. The average mean number of positive nodes in the two groups were 2.11 and 5.12 respectively (p=0.007). A total number of patients who had positive lymph nodes were 88 (64%) in the control group in comparison with 8 (21%) patients in the study group (P<0.05) Sufficient number of lymph nodes detected in the tumour-bearing specimen were found only in 5% of patients receiving NCRT.

Linebarger et al[55] evaluated the effect of obesity on nodal yield and concluded that "lymph node retrieval was not affected by BMI."

4. MATERIALS AND METHODS

A retrospective analysis of 331 patients who underwent curative resection for rectal cancer in the Department of Surgical Oncology, Cancer Institute (WIA), Chennai between January 1, 1991, and December 31, 2005 was done. Basic demographics, tumour location, type of surgery and postoperative staging were retrospectively recorded. The rectum was defined as follows: low rectum (0 to 5 cm from the anal verge), mid rectum (5 to 10 cm), and high rectum (10 to 15 cm). 225 patients received NCRT during the study period. 106 patients underwent straight surgery. NCRT was administered in patients with clinical stage of cT3/cT4 or cN+ disease. Diagnosis was based on the findings clinical examination, endoscopy and computed tomographic scans.

Neoadjuvant therapy consisted of 2 cycles of intravenous 5 fluorouracil at a dose -325mg/m^2/day for 5 days (concurrently with radiation) and a 1 cycle of Mitomycin C at a dose 6 mg/sq.m for 1 day (concurrently with radiation). External beam irradiation was administered up to a total dose of 5000 cGy (25 fractions, 200 cGy per fraction) administered by a 4 field box technique over a period of five weeks. The field included the tumour site within the pelvis as well as the lymphatic draining area reaching up to the L-5 to S-1 superiorly and the ischial tuberosities inferiorly. Laterally the radiation field extended 1.5 cm beyond the bony pelvis. On the posterior aspect the radiation encompassed the entire sacrum.

RESPONSE TO TREATMENT

Patients were restaged at three to four weeks four weeks after completion of NCRT to assess tumour response. Tumour response assessment included the same clinical and radiologic studies used at initial staging. Clinical response to neoadjuvant chemoradiation was judged on surface area of abnormality (tumour size) and intramural involvement (induration). Clinical response was defined as:

- ***Complete:*** No residual tumour, no surface abnormality, no induration.
- ***Good***: barely perceptible, 75% or greater reduction in surface abnormality and induration.
- ***Moderate***: 25% to 75% reduction in surface abnormality and induration.
- ***Minimal***: less than 25% reduction in surface abnormality and induration.
- ***No chang*e**: No change in surface abnormality and induration.

Surgery was performed at 4 to 6 weeks after completion of NCRT. Surgery consisted of a total mesorectal excision (TME) based resection, either abdominoperineal resection or low anterior resection with en bloc resection of any adjacent organ involvement. Lymph nodes up to the origin of the inferior mesenteric artery are harvested.

Exclusion criteria included patients who had metastatic disease on presentation, recurrent cancer, and previous pelvic irradiation. Also excluded were patients who underwent local excision of their rectal cancer and patients who underwent palliative resections for symptom relief.

HISTOPATHOLOGY

All the surgical specimens were fixed in 10% formalin solution and routinely processed for paraffin embedding. Lymph nodes were retrieved by the use of a gross examination and manual palpation. Serial sectioning of the specimen perpendicular to the longitudinal axis was performed at 3–5 mm intervals. Routine histologic examination was performed using hematoxylin and eosin staining. Histologic processing of the specimens was the same for all patients. The specimens were dissected out by a dedicated pathologist without use of fat clearing solutions by standard techniques. Patients were staged according to the American Joint Committee on Cancer guidelines[24]. Pathology reports included tumour size in cm, histology type, pathology stage, total number of regional lymph nodes present in the resected specimen, and number of lymph nodes with cancerous cells. For comparison purposes, sub analysis of the number of lymph nodes retrieved during surgery of patients based on type of surgery was also performed.

STATISTICAL ANALYSIS

Statistical analyses were performed using statistical software package SPSS version 11.0 (SPSS, Chicago, IL). Categorical variables were analysed using the chi square test, and continuous variables were analysed using the correlation by Spearman's rank correlation coefficient. Survival analysis was performed by using

life table analysis. A p value of less than 0.05 was considered as statistically significant.

For analysis purposes, specimens from a group of patients that received standard neoadjuvant therapy were compared to specimens of patients that did not undergo preoperative chemoradiation

A first analysis included all patients (n=331) and assessed the lymph nodal yield between the patients undergoing straight surgery and NCRT and the influence of clinical and pathologic variables on the number of both lymph nodes retrieved and positive lymph nodes in the rectal specimen after NCRT. Variables evaluated were age, sex, body mass index(BMI), tumour stage (pT and pN), preoperative serum CEA levels, distance of the tumour from the anal verge, histology, circumferential extent, type of neoadjuvant therapy (chemoradiation vs., radiation alone), dose of radiation, response of the primary tumour, interval between completion of NCRT and surgery and the type of surgery. (APR vs. LAR)

Cut point analysis was performed on the patients receiving NCRT in an attempt to identify the number of lymph nodes retrieved which would have prognostic significance. In order to eliminate the confounding effect of nodal positivity on survival, the cut point analysis was performed on pathologically node negative patients (p N0).

A second analysis was performed in patients treated by preoperative chemoradiotherapy and curative surgery (n=225) to evaluate the impact of the number of lymph nodes retrieved on 5-year overall and disease-free survival.

The median duration of follow-up of the patients was 49 months (range 1-200 months). Differences were considered statistically significant at p value of 0.05)

5. RESULTS

PATIENT CHARACTERISTICS

From 1991 to 2005, 313 patients were identified, 225 of them received neoadjuvant therapy and 88 patients underwent straight surgery.

Patient characteristics are described in table 1

TABLE 1

(Patient Characteristics)

Characteristic	Neoadjuvant Therapy
Age	52 (Range :21-74)
Sex	
Males	131(58.2%)
Females	94(41.8%)
CEA	9.6ng/ml
BMI	21.4
Distance from anal verge **Mean**	
Lower third	197(87.5%)
Middle third	25(11.11%)
Upper rectal	3(1.33%)
Histopathology	
Adenocarcinoma	190(84.44%)
Signet ring cell adenocarcinoma	7(3.11%)
Mucinous adenocarcinoma	

	28(12.44%)
Circumferential	
No	22(9.34%)
yes	203(90.66%)
Neoadjuvant therapy	
Radiation only	17(7.6%)
Chemoradiation	208(92.4%)
Radiation dose	182(80.88%)
>50 Gy	43(19.12%)
<50Gy	
Response to neoadjuvant therapy	
Complete pathological response	32(14.22%)
Residue present	193(85.77%)
Interval between completion of neoadjuvant therapy and surgery	
Mean	10.6
<8 weeks	159 (70.3%)
>8weeks.	66(29.7%)

RECTAL SPECIMEN

Among the patients who received neoadjuvant therapy, the T stage distribution was as follows: pT0 14.22% (n= 32) , pT1-2 28% (n=63), pT3 51.55% (n=116), pT4 6.22% (n=14). 14.22% of patients (corresponding to pT0) achieved complete pathological response.

LYMPH NODE YIELD

The median number of nodes retrieved after neoadjuvant was significantly lower than that retrieved after straight surgery, 7(Range 0 to 24) vs. 12 (Range 2-47), ($p<0.001$).

The number of positive nodes was also significantly lower in the patients receiving neoadjuvant chemo radiation compared to straight surgery (mean 1.5 vs. 2.7 with a range of 0-16 and 1-29 respectively).(p value 0.05)

Only 17.77% (n=40) of the patients receiving NCRT had 12 or more nodes retrieved, whereas 53.4% (n=47 of 88) of patients with straight surgery had more than 12 nodes retrieved.

In 12 patients (4.88%) no nodes could be retrieved from the specimen.

FACTORS AFFECTING LYMPH NODE YIELD

Various independent variables were analysed to evaluate their influence on nodal yield.(Table 2). Among patients who received neoadjuvant therapy, the only factors that influenced nodal yield on univariate analysis were age and type of neoadjuvant therapy. There was a linear inverse correlation with age. As age increased the number of nodes decreased (Spearman's coefficient -0.288, p value<0.001). Patients who received only neoadjuvant radiation also had significantly lower nodes harvested as compared to patients who received neoadjuvant chemoradiation (mean 4.8 Vs. 7.4, p value 0.03).

TABLE 2

(Factors Affecting Nodal Yield)

Characteristic	N	Number of nodes	P value
Treatment			<0.001
Straight surgery	88	13	
NCRT	225	7	
Gender			0.93
Males	131(58.2%)	7	
Females	94(41.8%)	7	
Serum CEA			0.28
<5	109(66.9%)	6	
5-10	25(15.3%)	6	
>10	29(17.8%)	6	
BMI			0.63
<20	87 (38.7%)	7	
Equal or >20	138(61.3%)	6	
Distance from anal verge			0.59
Low rectal	197(87.5%)	7	
Mid rectal	25(11.11%)	6	
High rectal	3(1.33%)	4	
pT stage			0.26
T0	32(14.2%)	6	
T1-2	63(28.0%)	6	
T3	116(51.6%)	7	
T4	14(6.2%)	7	
Histopathology			0.99
Adenocarcinoma	190(84.44%)	7	

Signet ring cell adenocarcinoma	7(3.11%)	8	
Mucinous adenocarcinoma	28(12.44%)	7	
Circumferential			0.88
No	117	7	
yes	108	7	
Radiation dose			0.91
>50 Gy	182(80.88%)	7	
<50Gy	43(19.12%)	7	
Neoadjuvant therapy		4	0.03
Radiation only	17(7.6%)	7	
Chemoradiation	208(92.4%)		
Response to neoadjuvant therapy		7	0.69
Complete pathological response	32	7	
Residue present	193		
Method of Surgery			0.55
Open	190		
laparoscopic	35	7	
		7	

T STAGE AND NODAL YIELD

There was no significant difference between the individual pathological T stage and the nodal yield but if stages pT0 to pT3 were grouped together and analysed against pT4 the number of nodes in the pT4 stage were significantly higher (mean 11 vs. 6.96 in the T4 and T0-T3 respectively, $p = 0.02$).

SURVIVAL ANALYSIS

We were not able to demonstrate a significant difference in the 5-year overall survival between patients in whom ≥12 nodes were retrieved when compared to those in whom <12 nodes were retrieved (52.5% Vs. 64.7%, p=0.063)

CUT POINT ANALYSIS

The cut point analysis was performed in an attempt to identify the minimum number of retrieved nodes that would predict a difference in survival after NCRT.(Table 3)

When the entire group of 225 patients were analysed, cut point analysis did not identify any number of retrieved nodes that predicted a difference in survival.

The 5 year overall survival in patients with no nodes retrieved was significantly higher than those in whom at least one node was retrieved (89.5 % vs. 65.1 %, $p < 0.05$).

There was no significant difference when patients with no nodes retrieved in the specimen were stratified according to the pT stage.

There was no difference in the 5 year overall or disease free survival when patients who were pathological N0 were stratified according to nodal yield. (Table 4)

TABLE 3

Cut Point Analysis

Number of nodes	n	5 year DFS	P value
0	12	90	0.048
1 or more	213	61	
1 or less	22	88.7	0.01
2 or more	203	59.8	
2 or less	35	77.6	0.055
3 or more	190	59.7	
3 or less	58	70.4	0.15
4 or more	167	59.7	
4 or less	79	69.6	0.11
5 or more	146	58.7	
5 or less	91	65.5	0.31

6 or more	134	60.4	
6 or less **7 or more**	108 117	72.9 61.4	0.51
7 or less **8 or more**	129 96	61.5 60.4	0.40
8 or less **9 or more**	145 80	61.4 60.5	0.46
9 or less **10 or more**	168 57	63.4 60	0.28
10 or less **11 or more**	180 45	63.8 57.1	0.21
11 or less **12 or more**	185 40	64.7 52.5	0.063

TABLE 4

(Survival of Patients with pN0 Stratified with Nodal Yield)

	12 or more	**7-11**	**4-6**	**1-3**
5 year OS	82.2	83.3	70.6	76.8
% year DFS	84	79.5	65.7	70.8

p value for 5 year OS : 0.53

p value for 5 year DFS : 0.45

PATHOLOGICAL N0

To eliminate the confounding effect of positive nodes on survival, the cut point analysis was performed on 124 patients in whom nodes were harvested but all were staged as pathological N0. (Table 5)

TABLE 5

(Cut-Point Analysis with Patients with pathological N0)

Number of nodes	n	5 year DFS	P value
1	8	100	0.24
2 or more	116	72.9	
2 or less	16	85.9	0.43
3 or more	108	72.8	
3 or less	33	70.8	0.45
4 or more	91	76.1	
4 or less	49	71	0.43
5 or more	75	76.9	
5 or less	55	68.3	0.058
6 or more	69	79.9	
6 or less	62	68.3	0.18
7 or more	62	81	

7 or less **10 or more**	73 51	71.6 78.8	0.44
8 or less **9or more**	82 42	71. 81.8	0.23
9 or less **10 or more**	96 28	70.8 88.4	0.09
10 or less **11 or more**	102 22	72.2 85.6	0.25
12 or less **12 or more**	104 20	72.7 84	0.30

For stages pT0-pT2 the overall survival was significantly higher in patients in whom 6 or more nodes were harvested (91.7% vs. 68.6%), (p=0.04). If all patients were grouped together (pT0 to pT4), there was a non-significant trend towards better 5 year overall survival in patients with 6 or more nodes harvested compared to those with < 6nodes(79.9% vs. 68.3%), p=0.058. (Table 6)

TABLE 6

	5 or less	**6 or more**	**P value**	**Chi Square test**
T0-T2	68.6	91.7	***0.04***	4.39
T3-T4	67.9	65.9	0.89	0.02
All T stages together	68.3	79.9	***0.058***	1.80

6. DISCUSSION

For rectal cancer, TNM staging has two distinct drawbacks. T staging does not include consideration of the circumferential resection margin, which clearly affects local recurrence and overall survival.[32] Second; N staging does not incorporate the effect of preoperative CRT on the LN harvest. [33]

LYMPH NODE YIELD

After colorectal cancer surgery, the number of nodes retrieved in a specimen reflects the extent of lymphadenectomy and the thoroughness of the pathological examination.[21] On the one hand, it provides adequate staging of the tumour, thus allowing proper prognostication and affects the decision to receive effective adjuvant treatment. [12, 25-26]. On the other hand, adequate resection of lymph nodes improves local control, decreases the incidence of local recurrence[27-28] and improves survival.[29-31] The number of lymph nodes that should be removed in order to be considered adequate is not universally agreed upon [20].The last edition of TNM classification of the American Joint Committee on Cancer states that "It is important to obtain10-14 lymph nodes in radical colon and rectum resections in patients without neoadjuvant therapy[34]

LYMPH NODE YIELD AFTER NCRT

NCRT has been considered the preferred treatment strategy for locally advanced distal rectal cancer.[13] NCRT is thought to decrease the lymph node yield

after surgical excision. Baxter et al[15] analysed 5647 patients who underwent surgery for rectal cancer from the SEER database of which 1034 patients received NCRT. On average, fewer nodes were examined in patients who underwent preoperative RT (7 nodes) vs. those who did not (10 nodes); this difference was statistically significant on multivariate analysis.[15]. Wichmann et al.[22] evaluated the effect of preoperative CRT on LN retrieval. Their study concluded that patients who underwent a long course of CRT had significantly fewer LNs examined than patients in the control group (13.6 vs. 19.1). The nodal yield after NCRT has varied widely across trials. In the study by Baxter et al[15,] only 20 % of the patients had nodal yield more than 12. It ranged between 12.5% and 45%. [20] In the studies by Doll, Rullier and Sebastien et al the mean nodal yield though less after NCRT was greater than 12.[35-37]). The decrease in nodal yield was also seen in patients receiving short course radiation. [16] In the study by Taflampas et al[33], patients who received NCRT were more likely than patients without NCRT to have reduced nodes, but it was not statistically significant. Sprenger et al[53] stated that if the surgery is appropriately performed and due diligence is given by the pathologist to dissect the specimen, then adequate number of nodes can be dissected out in the specimens after NCRT. Scott et al[49] compared patients undergoing straight surgery, surgery after short and long course radiation. Although the number of nodes harvested after NCRT was decreased compared to straight surgery, it was not statistically significant. Taflampas et al[33] concluded that preoperative CRT does not seem to significantly influence the number of LNs harvested after TME for

rectal cancer regardless of T stage. However, preoperative CRT significantly reduces the percentage of patients with at least 12 lymph nodes examined. Our study demonstrates that that the nodal harvest is significantly decreased for patients receiving NCRT compared to patients undergoing straight surgery (7.2 vs. 13.5). Only 17.77 % of the patients had nodal yield more than 12. This is consistent with the effects of radiation which may lead to lymphocyte depletion, atrophy, and stromal fibrosis and ultimately results in significant normal lymph node size reduction. Our results are consistent with reports by other workers wherein reduced number of nodes is harvested after NCRT and radiation likely contributes to this effect.

POSITIVE NODES AFTER NCRT

The impact of NCRT on the yield of positive nodes is controversial. The Dutch trial[16], where short course radiation was used did not have any difference in the yield of positive nodes between NCRT and straight surgery. (1.6 vs. 1.9; P=0.11). Studies by Wichmann[22] and Rullier[36] et al, where long course radiation was used, there was a significant difference in favour of NCRT Vs. straight surgery (1.4 vs. 3.1; P<0.05, and 1.2 vs. 2.3, p=0.001, respectively). The discrepancy may result from the different schedules of preoperative radiotherapy used in these series. Indeed, long course preoperative radiotherapy induces tumour and nodal down-staging, whereas short course does not.[36]This should be however interpreted with caution as the studies by Wijesuriya[39] and Rinkus[40] et al where long course radiation was used, did not reveal any significant difference in the

yield of positive nodes. Mark[41] et al observed a relationship between the number of lymph nodes retrieved and the number of positive lymph nodes. Higher the number of lymph nodes retrieved, higher the number of positive lymph nodes (P<0.001). Our study revealed a significant difference in the yield of positive nodes in favour of NCRT (1.5 vs. 2.7, p =0.05).

FACTORS INFLUENCING NODAL YIELD

Given the importance of knowing the extent of lymph node yield, various factors have been analysed by various authors to evaluate their influence on nodal yield. If so, it may be possible to maximize the number of retrieved nodes and consequently improve the accuracy of staging.

Marks[41], Habr-Gamaa[38] and Rullier[36] et al found no significant difference between nodal yield and age. Other studies have demonstrated that increased age is associated with decreased lymph nodes retrieval.[42] It has been suggested that node size decreases with increasing patient age, meaning that nodes become increasingly difficult to identify.[43] Our study reveals that age is inversely correlated with the nodal yield. (Spearmans coefficient -0.288, p value <0.001).

The influence of gender on nodal yield has been studied by Marcos[20] et al where male sex was associated with higher nodal yield. Others have documented higher nodal yield in females[44]. Our study similar to results by Hyung[42] et al did not have any association between gender and nodal yield.

Gorog[45]et al found a smaller specimen with consequent decreased nodes in obese patients. Hyung[42] et al found fewer number of nodes in patients with increasing body mass index (BMI). Linebarger et al[55] evaluated the effect of obesity on nodal yield and concluded that: "lymph node retrieval was not affected by BMI". In the current study, there was no correlation between obesity, BMI and nodal yield.

Tumour location (p=0.095) and tumour stage (p=0.093) did not significantly influence the number of lymph nodes in the study by Maschuw et al.[17] Thorn et al found more nodes in mid rectal tumors.[46] Our study did not reveal any correlation between location of tumour and nodal yield

Marks[41], Habr-gamma[38] and Marcos[20] et al found no correlation between lymph node yield and the interval between completion of NCRT and surgery, whereas Sermier[47] et al found a significant inverse correlation. There was no correlation between nodal yield and time interval between end of NCRT and surgery in our study.

Pathological T stage, in the present study, were not significant factors. Baxter[15] and Marks[41] et al agree with our conclusion where as others have stated to the contrary. Wichmann[22] et all stated that in the subgroup of patients with pT3, patients treated with NCRT had higher nodal yield compared to straight surgery. No other differences could be made out. Wang[48] and Kim[21] et al stated that the higher the stage, the greater is the nodal yield.

There was no impact of circumferential vs. non-circumferential tumours on the nodal yield in our study. Ha et al[42] also concluded the same.

The dose of radiation did not have an impact on nodal yield. This is to be expected as it has been shown in studies that even short course radiation is associated with a significant decrease in the nodal harvest[16].

Response of the primary (complete pathological response vs. residue present) did not have an impact on the nodal yield. Marks et al[41] also came to the same conclusion.

Marks[41] Habr-Gama[38], Wichmann[22] and Marcos[20] et al stated that the type of surgery did not influence the nodal yield. We also arrived at the same conclusion. This is expected since the lymph node bearing tissues: the mesorectum and inferior mesenteric artery stalk, are similarly removed in both operations.

Marks et al[41] stated that there was no difference in nodal yield between patients who received neoadjuvant radiation alone compared to patients who received neoadjuvant chemoradiation. In the current study patients who received radiation alone had higher nodal yield compared to patients who received chemoradiation. This may be a bias as there very few patients who received radiation alone.

Mesenteric fat clearance was not used at our institution. This technique has been shown to increase lymph node yield. [58]

SURVIVAL

39 % of the patients in the study by Doll et al[35] had fewer than 12 nodes harvested and according to the current definition would be defined as under staged. They however stated that the 5-year survival rate in patients with neoadjuvantly treated rectal carcinoma was independent of the number of resected lymph nodes in the resection specimen, with identical overall survival rates for patients with a total lymph node yield less than 12 and with 12 or more. They go on to state that the number of lymph nodes in a radiated specimen should not be compared to the number of lymph nodes in a primary-surgery specimen. Neither does a reduced total lymph node yield in a neoadjuvantly treated rectal cancer specimen necessarily indicate inadequate lymphadenectomy or inadequate pathologic workup. In their study despite the number of nodes not being a prognostic factor, nodal positivity was strongly co-related with adverse outcomes with 5-year survival rates of 88, 63, and 39% for patients without lymph node metastases, one to three tumour-positive lymph nodes, and more than three tumour positive lymph nodes, respectively.

Luna-Perez[29] excluded patients in whom no nodes were harvested and grouped patients into 10 nodes or less and 11 or more nodes and estimated survival. Five-year cancer-specific survival in the former group was 48%, and conversely 69.6% in the latter group. They concluded that In conclusion, retrieval of at least 11 lymph nodes in surgical specimen is a powerful tool to stage patients

with rectal adenocarcinoma treated with chemoradiotherapy and surgery due to the fact that proper staging is the main predictor for local recurrence and survival.

Other investigators also have validated that number of metastatic lymph nodes is a prognostic factor. Jass et al[56] reported 379 patients with surgically treated rectal cancer. Five-year survival among patients with a score of III (number of metastatic lymph nodes [1–4 vs. >4], character of invasive margin, peritumoral lymphocytic infiltration, and local spread) was[57], whereas in patients with a score of 4–5 the rate was 27%. Bognel et al. (29) reported 339 patients treated with surgery; 161 received radiotherapy either pre- or postoperatively or in combination. Five-year disease-free survival with 1–2 lymph node metastases was 46, and 26% with 3 or more nodes.

Habr-Gama et al[38] stated in their paper that patients in who no node was harvested had survival better than patients who were staged pathologically as No although this was not significant and argued that in fact, some of these patients with ypN0 disease could be initially (before CRT) N+ that could have possibly benefited from adjuvant therapy.

In our study patients who had no nodes retrieved in the specimen had survival better than patients in whom any node was retrieved (including patients who were pathologically N+ and N-).

We performed cut point analysis to arrive at a nodal “number” that would help define a minimum number of nodes harvested after NCRT. The lymph node

yield was not co-related with survival as there was no survival difference irrespective of the nodal yield.

In order to exclude the survival patients in whom no nodes were harvested, they were excluded from analysis and a cut point analysis was repeated. Here also lymph node yield was not co-related with survival.

To exclude the strong negative prognostic effect of pathological node positivity, the cut point analysis was repeated excluding patients with ypNx and the ypN+ subgroups. (n = 124). In this subgroup of patients, there was a survival difference for patients with more than 6 nodes for early t stages (pT0-pT2).

Our data clearly supports that in patients with carcinoma rectum who receive NCRT, reduced nodal yield is to be expected. No minimum number of nodes can be set as standard that denotes adequacy of surgery. The decreased nodal yield could be due to the biological effects of radiation on the node bearing tissues, it may shrink the nodes and make them unrecognizable, may cause fibrosis in the nodes or may even cause death in nodes bearing metastasis, all these leading to reduced nodal yield. Our data also brings to light the strong prognostic significance of no nodes being harvested in the specimen. It could be that these patients are exquisitely sensitive to radiation and that that may account for the survival advantage.

In the EORTC 22921 study the progression free survival and the overall survival curves start to diverge at 2 and 5 years after randomization. [59] Their non-

protocol subgroup analysis proposed a subgroup of patients who might benefit from adjuvant chemotherapy. They suggested that only patients who achieve complete pathological response or patients who were downstaged to ypT1-2category after NCRT benefited from adjuvant chemotherapy, whereas those with residual ypT3-4 did not.[60] Although the above study is subject to potential sources of bias, if we extrapolate our data where a survival advantage is seen for the subgroup of patients who were downstaged to T0-2 , and in whom a minimum of 6 nodes were harvested, it leads us to suggest that this is the subgroup of patients that might benefit from adjuvant chemotherapy

7. CONCLUSION

1. Patients with rectal cancer undergoing NCRT had a reduced nodal yield after surgery.

2. The factors co-related with nodal yield were age and type of neoadjuvant therapy.

3. The cut-off of 12 nodes had no prognostic significance after NCRT.

4. Patients in whom no nodes were harvested had survival better than patients in whom nodes were harvested.

5. For node negative patients harvest of 6 nodes for early pT0 to pT2 tumours had prognostic significance.

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