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Du Plooy, Philippus Theunis

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1. Background

Cancer of the colon and rectum is the third most common non-cutaneous malignancy in both males and females in the USA, and is also the third leading cause of cancer deaths amongst males and females, following lung and prostate cancer in males, and lung and breast cancer in females respectively[1].

Colorectal cancer is currently staged according to the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) TNM Staging System (table1)[2,3]. According to this system, individual tumours are stratified based on features related to the extent of the primary tumour (T), the involvement of the loco regional lymph nodes (N), and the presence of distant metastasis (M). Based on the three variables, tumours are the further classified into four stages, with stage I disease representing local disease only, stage II locally advanced disease, stage III locoregionally advanced disease (typified by lymph node involvement) and stage IV metastatic (systemic) disease.

Adequate lymph node assessment is essential in the staging of colorectal cancer as it has prognostic and therapeutic significance. Prognostically, five year survival rates for colorectal cancer stage II (without lymph node involvement) and stage III (with lymph node involvement) with surgery alone is approximately 80% and 50%

respectively[4,5]. Therapeutically, all patients with lymph node involvement need adjuvant chemotherapy in view of a definite survival benefit (67% five year survival with surgery and adjuvant chemotherapy vs. 50% with surgery alone in stage III disease)[6]. This is the current recommendation of the National Institutes of health Consensus Conference[7]. Unfortunately, no survival benefit has been shown for patients with stage II disease (T3-T4, N0, M0) treated with chemotherapy. Chemotherapy is therefore controversial in stage II disease.

Two opposing views exist as to the benefit of lymphadenectomy in determining survival: some authors maintain that a full lymphadenectomy has therapeutic benefit in decreasing the tumour burden, whereas others regard cancer as a systemic disease and a thorough lymph node evaluation prognostically important in providing better staging of the disease, and not in itself a method of improving survival [8].

Current guidelines from the American Cancer Society and National Cancer Institute suggest a minimum number of twelve lymph nodes to be examined in resected specimens of colorectal cancer in order to accurately distinguish node negative from node positive disease[2,9,10]. A diagnostic dilemma therefore occurs in the patient with fewer than twelve nodes identified in the resected specimen to stage the patient accurately and to decide if the patient needs adjuvant chemotherapy. A Canadian study demonstrated that the determination of lymph node negativity was based on suboptimal sampling in 73% of cases in Ontario[11]. These results have been echoed in

an American study noting an adequate number of lymph nodes in only 37% of specimens resected [12].

Primary Tumour (T)

TX Primary tumour cannot be assessed
T0 No evidence of primary tumour
Tis Carcinoma in situ (intraepithelial or intramucosal carcinoma)
T1 Tumour invades the submucosa
T2 Tumour invades the muscularis propria
T3 Tumour invades through the muscularis propria into the subserosa or into the nonperitonealized pericolic or perirectal tissues
T4 Tumour directly invades other organs or structures

Regional Lymph Nodes (N)

NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Metastasis in 1 to 3 regional lymph nodes
N2 Metastasis in 4 or more regional lymph nodes

Distant Metastasis (M)

MX Presence of distant metastasis cannot be assessed
M0 No distant metastasis
M1 Distant metastasis

Stage	**Grouping**	**5-year survival**
I	T1-2,N0,M0	>90%
IIa	T3,N0,M0	60-85%
IIb	T4,N0,M0	60-85%
IIIa	T1-2,N1,M0	55-60%
IIIb	T3-4,N1,M0	35-42%
IIIc	T1-4,N2,M0	25-27%
IV	T1-4,N0-2,M1	5-7%

Table 1. American Joint Committee on Cancer/International Union Against Cancer TNM Definitions and Stage Groupings[2,3]

2. Literature review

This literature review on lymph node metastasis in colorectal cancer will focus on the following:

- The prognostic relevance of lymphatic metastasis
- The therapeutic implications of lymph node involvement
- The minimum number of nodes identified in the resected specimen to stage the lesion accurately
- Factors determining nodal yield
- Predictors of lymph node metastasis

2.1. The prognostic relevance of lymph node metastasis

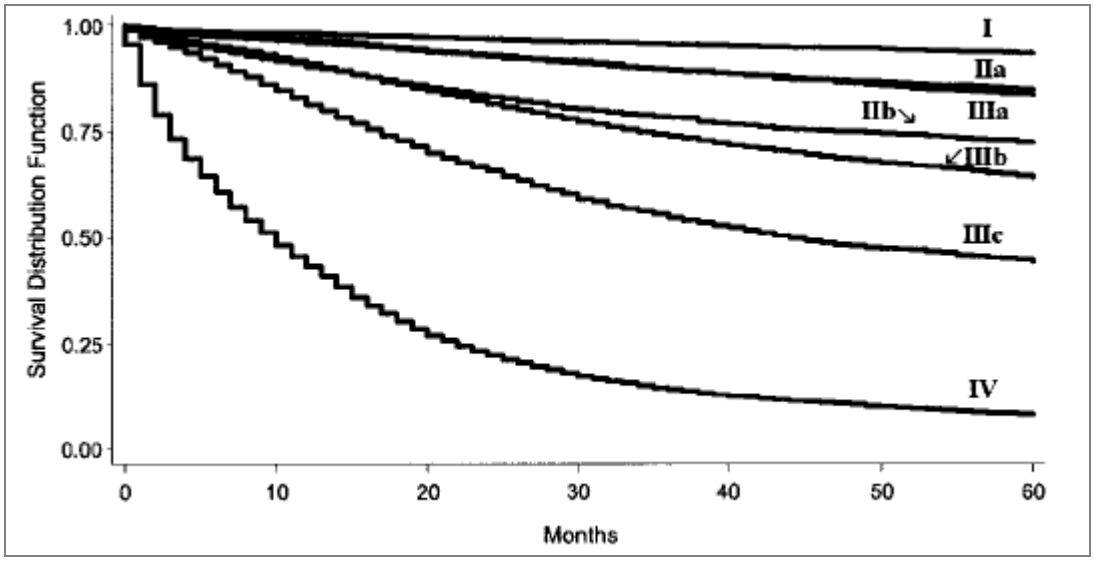
The presence of lymph node involvement in distant metastasis free (M0) colorectal cancer is considered the single most important indicator of overall survival and disease free survival. This is typified by the fact that survival from colorectal cancer decreases from approximately 80% in patients with stage II disease, to 50% in patients with nodal involvement[13]. Furthermore, Swanson et al[14] showed that the number of nodes examined in resected specimens of colorectal cancer directly correlates with overall survival in a subset of patients classified as having no nodal involvement. In a study on 35787 prospectively collected patients in the National Cancer Data Base with T3N0M0 staging, the five year survival rate varied from 64% with one to two nodes examined, to 86%

if more than 25 nodes were examined. Different five year survival rates were noted based on three subsets of nodal involvement being 1-7, 8-12 and 13 or more nodes respectively. The authors recommended that a minimum number of 13 nodes be examined to accurately assess nodal involvement in colorectal cancer.

In a secondary analysis of the INT0089 trial[15], the authors noted the following with respect to survival of node-negative patients with colorectal cancer: five and eight year overall survival rates of 100% and 92% respectively if more than 30 nodes were examined, and 91% and 74% if less than 30 nodes were examined.

By looking at the five year survival rates of 119363 patients with adenocarcinoma of the colon in the SEER (Surveillance, Epidemiology and End-Results) database in relation to the different subsets of stages in the JNCC sixth edition TNM staging system, O'Connell et al[16] showed that five year survival is proportionate to the number of involved nodes present, with one interesting exception (figure 1). Five year stage specific survival rates were 93.2% for stage I, 84.7% for stage IIa (T3N0M0), 72.2% for stage IIb (T4N0M0), 83.4% for stage IIIa (T1/T2,N1M0), 64.1% for stage IIIb (T3/T4,N1,M0), 44.3% for stage IIIc (any T,N2M0) and 8.1% for stage IV (metastatic disease). It would therefore appear that survival is better in stage IIIa than in stage IIb. The authors attributed this difference to the current recommendation of providing adjuvant chemotherapy to patients with stage III disease, but not to patients with stage II disease.

Fig. 1 Five year survival according to AJCC stages I – IV[16] (*with permission from Oxford Journals*)



It has also been shown that the ratio of involved to total nodes examined is prognostic for overall survival. In reviewing the data from the Intergroup trial 0089 of adjuvant chemotherapy for patients with stage II and III colon cancer Berger et al[17] noted that lymph node ratio was an important factor for overall survival, disease free survival and cancer-specific survival if more than ten nodes were removed with the tumour. Similarly, in a study on 7192 patients who had resections performed for colorectal cancer from the California Cancer Registry, Ng et al[18] reported that in both N1 and N2 disease, a lower percentage of lymph nodes involved with metastatic disease was associated with improved survival ($p < 0.0001$).

Recently, the American Joint Committee on Cancer (AJCC) expanded the N-categories for colorectal cancer in the seventh edition of the AJCC Cancer Staging Manual[19]. Accordingly, nodal involvement is subdivided into:

- N1a – 1 involved lymph node
- N1b – 2 to 3 adjacent nodes involved
- N1c – tumour deposit in fat near a lymph node
- N2a – involvement of 4 to 6 nodes
- N2b – involvement of 7 or more nodes

2.2. The therapeutic implications of lymph node involvement

Lymph node involvement in colorectal cancer necessitates adjuvant chemotherapy. Various studies have demonstrated a significant survival benefit in patients with stage III colorectal cancer receiving adjuvant fluorouracil-based chemotherapy. The North Central Cancer Treatment Group[20] in comparing the administration of fluorouracil and levimasole with observation in 401 patients with stage II and III colon cancer, showed a 31% reduction in recurrence rate in the treatment arm of the trial for patients with stage III disease. A subsequent study by the Eastern Cooperative Oncology Group[6,21] of 1269 patients with stage II and III disease also revealed a reduction in risk of local recurrence of 41% and risk of death of 31% compared to surgery alone in patients with stage III disease treated with fluorouracil and levimasole post operatively. No significant benefit though was noted in both studies in administering adjuvant therapy to patients

with stage II disease. Based on this, the National Cancer Institute consensus conference[22] in 1990 recommended fluorouracil based chemotherapy as standard of care in patients with stage III colon cancer. Currently, the combination of fluorouracil, leucovorin and oxaliplatin are favoured for patients with stage III colon cancer[23].

The role of adjuvant chemotherapy in patients with stage II disease is controversial. No single randomized clinical trial has shown a survival benefit for chemotherapy in patients with stage II colon cancer[23]. Evaluating a pooled analysis of seven studies, no significant change was noted in the five year survival rate of patients with stage II colon cancer treated with adjuvant fluorouracil-based chemotherapy (81% five year survival) and those treated with surgery alone (80% five year survival)[24]. In a review of the available literature in 2004, the Cancer Care Ontario Program in Evidence-Based Care advised against the routine use of adjuvant chemotherapy in stage II disease[25].

It has been shown before that five year survival of patients with stage IIb colorectal cancer is worse than that of patients with stage IIIa disease[16], with the authors attributing the difference to the practice of providing adjuvant chemotherapy to all patients with stage III disease, but not to patients with stage II disease. It would therefore appear that there may be a subset of patients with stage II colon cancer who might possibly benefit from adjuvant treatment. In an attempt to identify which subset of patients with stage II disease would benefit from

adjuvant treatment, Petersen et al[26] evaluated 268 patients with stage II disease. Four factors were identified as conferring a poorer survival rate: tumour perforation, peritoneal involvement, venous spread and involved surgical margin. Stage II tumours with none of these features had a five year survival rate comparable to that of stage I disease, whilst the presence of these factors decreased the five year survival rate to 49.8%. In applying Petersen's model to 1625 patients with Dukes B (stage II) and C (stage III) colorectal cancer Morris et al[27] concluded that it discriminated well between high risk and low risk patients with stage II disease. As survival was noted to be worse in the patients with high risk features and Dukes B disease than with Dukes C disease, the authors suggested adjuvant chemotherapy for these patients. Incidentally, this study also noted a direct correlation between the number of nodes identified and survival. Apart from the factors noted by Petersen, bowel obstruction at presentation has also been mentioned as a feature identifying which patients with stage II disease potentially warrant chemotherapy[28]. A survival benefit has been suggested in a retrospective analysis of 318 patients with stage II disease in the ECOG INT-0035 study on fluorouracil and levimasole in patients with stage II disease and T4 stage, bowel perforation, or bowel obstruction[29]. In 625 patients with stage II disease and 415 patients with stage III disease, Sarli et al[30] noted similar five year survival rates of 52.6% for patients with one to three involved nodes (stage III) and 51.3% for patients with stage II disease who had less than nine nodes examined, concluding that chemotherapy be offered to patients with stage II disease when an inadequate number of nodes had been examined. In 2004 the

American Society of Clinical Oncology commented that chemotherapy may be warranted in patients with stage II disease and the abovementioned features[28]. The potential benefit of chemotherapy in this subset of patients though, needs to be further investigated.

2.3. The minimum number of nodes identified for staging accuracy

Current guidelines from the American Cancer Society suggest a minimum number of twelve lymph nodes to be examined in resected specimens of colorectal cancer in order to distinguish N0 from N1 or N2[2,9,10]. This number was based on the 1990 Working Party Report to the World Congress of Gastroenterology in Sydney[31]. Unfortunately, the minimum number of nodes that needs examining to definitively classify a tumour as being node negative remains a subject of debate.

Recommendations on the number of nodes required to stage colorectal cancer accurately varies between six and seventeen (table 2). Caplin et al [32] demonstrated poorer survival in patients with Dukes B colorectal cancer (node negative) when less than six nodes were examined compared to examining seven or more nodes. Scott and Grace[33] concluded that 94% of node positive tumours were correctly identified after examining thirteen nodes. Goldstein and co-workers[34] suggested examination of at least seventeen nodes based on a retrospective review of 750 pathology specimens of colorectal cancer. Tepper et al[35] suggested a number of fourteen nodes to be examined based on their review of 1664 patients. Based on

their examination of 140 patients with Dukes B colorectal cancer resected by the same surgeon, Cianchi et al[36] proposed a minimum of nine nodes to be examined to reliably stage colorectal cancer. Yet, the largest study thus far conducted on the number of nodes versus staging accuracy concluded that a minimum number of thirteen nodes be examined to accurately stage T3 colon cancer as node negative[13]. This study however, was conducted on node negative disease, and the recommendations made were based on the effect of examining more nodes on overall survival.

Table 2. Some studies examining number of nodes and staging accuracy in colorectal cancer.

Study	**No. of patients**	**Stage**	**Site of tumour**	**Reccomendation on number of nodes**
Caplin et al [32]	378	Dukes' B and C	Colon and rectum	≥ 6
Scott and Grace[33]	103	Dukes' B and C	Colon and rectum	≥ 13
Goldstein and co-workers[34]	750	T3	Colon and rectum	≥ 17
Cianchi et al[36]	140	Dukes' B (StageII)	Colon and rectum	≥ 9
Swanson and co-workers[14]	35787	T3N0 (Stage II)	Colon	≥ 13
Tepper et al[35]	1664	II and III	Rectum	≥ 14
Wong et al[40]	196	T2 and T3	Colon and rectum	≥ 14
Kim et al[70]	151	T2 and T3	Colon and rectum	≥ 10

Interestingly, when a mathematical model was applied to calculate the probability of a true node negative result based on the number of nodes examined in a study of 1585 patients with stage II and III colon cancer, who had undergone either left or right hemi-colectomy, and from whom at least ten lymph nodes had been examined, the following calculations were made[15]:

- For T1 and T2 tumours, more than forty nodes need examining to have an 85% probability that the patient is node negative. Examining eighteen nodes gives only a 25% probability of being truly node negative.
- For T3 and T4 tumours, forty and thirty nodes were calculated as needing to be examined to have a 85% probability of nodal negativity respectively
- Examining eighteen nodes in T3 and T4 leads to a probability of node negativity of >25% for T3 tumours and >50% for T4 tumours.

In interpreting the results of this study, a number of factors need consideration. Firstly, the total number of lymph nodes present in the right and left colon mesocolon respectively was quoted from a study by Herrera-Ornelas [37]. It is well known that a great inter-individual variation exists in the number of nodes in the mesentery of the large bowel[38], which would definitely affect the calculation. Secondly, the studies comparing lymph node number examined to five year survival are mostly consistent in their findings that a minimum number of between nine and seventeen nodes examined would fairly accurately predict lymph node negativity. Thirdly, it is well documented that the more advanced the tumour in terms of depth of bowel wall penetration, the higher is the likelihood of

lymphatic spread[37,39]. The risk of lymphatic nodal involvement is therefore lower in T1/T2 tumours, compared to T3/T4 tumours. The study does however emphasize an important point relevant to curative surgery in colorectal cancer, namely to attempt to remove the entire tumour burden with adequate margins, including the regional lymphatic basin.

2.4. Factors influencing nodal yield

The number of lymph nodes identified in a resected specimen of colorectal cancer will depend on patient-related factors, surgical factors, pathological factors and in the case of rectal cancer whether neo-adjuvant radiotherapy was administered.

a) Patient related factors

Factors relating to the individual patient include the number of nodes present in the individual, the location of the tumour and the distance of the nodes to the tumour. The total number of lymph nodes present in the mesentery of the large bowel vary from patient to patient[38], and even in the same individual it may vary based on reactive enlargement of nodes that would usually not be detected[40]. Results on the amount of nodes present in the various parts of the large bowel are conflicting: some studies suggest fewer nodes to be present in the rectum than for the rest of the colon[39,41], whereas others have not noted any significant difference[33]. Others have shown that the transverse colon may have fewer nodes

when compared to the rest of the large intestine. Cserni et al[42] observed that the number of lymph nodes in each centimeter of bowel is greatest close to the tumour. Furthermore, lymphatic mapping studies have shown that the anatomical concept of lymphogenic spread does not always hold true: skip-metastasis – where higher level nodes are involved without spread to nodes closer to the tumour – has been found in up to 10% of colon cancer specimens[43]. Involved nodes have also been noted at sites relatively distant from the primary tumour, and without involvement of the local lymphatic system[44,45,46]. Unexpected lymphatic drainage may occur in 4-8% of cases[47].

b) Surgical factors

The extent of the surgical resection will obviously influence the number of nodes in the resected specimen; the longer the segment of bowel resected, the more nodes would be present, and the greater the extent of the mesocolic resection, the more nodes are present. Current guidelines for colon cancer surgery[48] entail resection of the primary tumour with a five centimeter margin of macroscopically normal bowel on either side, with removal of the blood vessels and lymphatics down to the primary feeding arterial vessel of the involved segment of bowel. The lymph node resection should be radical and the lymph nodes should be removed en bloc. Apical nodes should be removed if possible and tagged as such.

With regards to surgical factors influencing nodal yield, additional factors deserve consideration. Firstly, does sentinel node biopsy relate to improved staging in colon cancer, and are there differences in nodal yield between laparoscopic colon and rectal resection versus open resection respectively?

The sentinel node is defined as the first node in the lymphatic basin of the tumour. The use of the sentinel node concept is well established in melanoma and breast cancer[49, 50], unfortunately its role in colorectal cancer remains unclear. Unlike in breast and melanoma surgery, the aim of sentinel lymph node (SLNB) examination in colorectal cancer is not to change the surgical management of the patient or to decrease the incidence of surgery related morbidity, but rather as a potential method of focusing sensitive histopathological techniques (i.e. immunohistochemistry) on the nodes with the highest likelihood of metastatic involvement. Unfortunately, the results of recent studies looking at the predictive value of sentinel node biopsy in colorectal cancer are conflicting. A few studies have reported a high predictive value of SLNB for the nodal status. In a recent study conducted in six Dutch hospitals on 69 patients, the authors reported a sensitivity of 89% (24 of 27 patients), a negative predictive value of 93% (3 of 67 patients) and an accuracy of 96% when comparing the status of the sentinel node with the subsequent histological examination of the resected specimen[51]. Similarly Saha et al[52] noted an accuracy of 95% (53 of 56 patients). Numerous other studies, including large multicentre trials have failed demonstrate this. Bembenek and coworkers[53] in a multicentre prospective trial on 315 patients with

colon cancer demonstrated a false negative rate as high as 46% for SLNB. A recent study from the MD Anderson Cancer Centre showed a high false negative rate (41%) and low sensitivity (59%) for SLNB in colon cancer[54]. At present, the use of SLNB is not able to predict the presence or absence of nodal metastasis with clinically relevant reliability. It may be argued given the heterogeneity of the trials thus far in patient selection, technique of dye injection and histopathological tools used for assessment, as well as the technical problems of identifying the SLN in the obese, and the learning curve that needs to be overcome, SLNB may eventually have a role in colorectal cancer. However, its routine use can not at present be justified given the fact that it has significant cost implications without changing management in a large proportion of patients, as well as the problems related to poor sensitivity and a high false negative rate.

Various studies examining laparoscopic versus open surgery for colon cancer have concluded that laparoscopic surgery is a safe alternative to open surgery[55, 56, 57]. Pertaining to number of nodes retrieved in the resected specimens, the results have been similar between laparoscopic and open surgery. The multicentre COLOR (Colon Cancer Laparoscopic or Open Resection) trial of 627 laparoscopic resections and 621 open colonic resections revealed no statistically significant difference between the two groups in terms of number of nodes resected[56]. This was also echoed in a recent meta-analysis of non-randomized comparative studies of the short-term outcomes of laparoscopic resection for colorectal cancer[57]. Analysing 6438 resections published in the literature, the

authors concluded that the two approaches were 99% similar in terms of adequacy of oncological clearance.

Laparoscopic surgery for rectal cancer has not yet been widely accepted. A few recent studies have however documented equivalence in terms of oncological clearance and short term outcome between laparoscopic and open rectal surgery. Law et al[58] from Hong Kong prospectively compared 310 consecutively performed low anterior resections to 111 laparoscopic resections. No differences in mortality and pathological staging were observed, with short term benefits of laparoscopic surgery in terms of blood loss and shorter hospital stay. Overall survival was better for the laparoscopic surgery group with 5 year survival of 71.1% and 59.3% ($p = 0.029$) respectively for laparoscopic and open surgery. This however was not demonstrated in a Spanish study, comparing 204 patients randomly assigned to laparoscopic surgery (101 patients) and open surgery (103 patients) [59]. Whilst echoing the benefits of laparoscopic surgery in terms of blood loss and length of hospital stay, no difference in recurrence rate or overall survival could be demonstrated between laparoscopic and open surgery. Interestingly, laparoscopic surgery in this study was associated with an increase in mean number of lymph nodes harvested, with a mean number of nodes of 13.63 for laparoscopic surgery versus 11.57 for open surgery ($p = 0.026$). The results of the multicentre COLOR II trial of laparoscopic versus open surgery for rectal cancer are eagerly awaited.

c) Pathological factors

An additional factor determining the number of nodes identified in the resected colo-rectal cancer specimen is the examining pathologist. With no major change in the surgical procedure, it has been shown that the number of nodes retrieved could be increased by a more thorough dissection by the pathologist[34,60,61]. All nodes should preferably be removed and sampled for histology. Yet in trying to identify potential barriers to optimal lymph node assessment by pathologists in Ontario, Canada, Wright et al[62] noted that only 57.9% of practising pathologists were aware of guidelines on lymph node retrieval in colorectal cancer, and only 25% of the minimum number of nodes (12) that need examining to stage the lesion accurately. If fewer than twelve nodes are identified after gross examination of the resected specimen, additional techniques aimed at improving nodal yield should be considered[63]. These include fat clearing or the use of lymph node revealing substances[33,64,65]. Because a significant proportion of metastatic lymph nodes in colorectal cancer are less than five millimeter in diameter[66], it has been suggested that all nodes grossly negative or equivocal for tumour be submitted entirely for microscopic examination[63]. Other strategies aimed at identifying very small amounts of metastatic tumour like immunohistochemistry to cytokeratin[67], and genetic investigations like the polymerase chain reaction (PCR) for carcinoembryonic antigen[68] have not yet been proven to be of prognostic value compared to the existing histopathological investigations[69], and have the additional problem of increased cost and low cost to benefit ratio.

Recently, good results have been demonstated with a technique of injecting methylene blue into the feeding vessel of the resected specimen. Kerwel et al[70] randomized 50 patients into standard pathological assessment for lymph nodes versus methylene blue staining followed by pathological assessment. Subsequent fat clearance was used to identify all of the residual nodes present in the mesentery. They noted an increase in the mean number of nodes in the stained group versus the standard group (30 vs. 17 nodes respectively). Moreover, no patients in the stained group had less than twelve nodes examined, but 7 out of 25 cases in the unstained group had less than twelve nodes examined.

More controversial due to varying false negative rates, is the use of ex-vivo sentinel lymph node mapping and harvest after colorectal resection. This technique involves injecting blue dye submucosally or subserosally around the tumour, with focused harvest of the stained lymph nodes. An Italian study[71] recently successfully identified the sentinel lymph nodes in 97.3 % of cases, with a mean number of five sentinel nodes identified per resected specimen. However, combining ex vivo sentinel lymph node mapping with methylene blue injection, Märkl and coworkers[72] successfully demonstrated the sentinel nodes in only 78 % of cases, with a sensitivity of identifying nodal metastasis in the sentinel nodes of only 75%. Once again an excellent mean number of nodes harvested (42 nodes per specimen) was achieved with methylene blue injection.

d) Neoadjuvant radiotherapy

Pre-operative radiotherapy is frequently employed in the management of rectal cancer, given either as short course radiotherapy for decreased local recurrence rates in lesions that are obviously resectable, or as full course chemoradiation to downstage lesions where concerns regarding the circumferential margin exist[73]. Various authors have shown that neoadjuvant radiotherapy decreases lymph node yield in rectal cancer. Mekenkamp and coworkers[74] noted a reduction in the mean number of lymph nodes retrieved from 8.5 nodes where pre-operative radiotherapy was not given to 6.9 nodes where neoadjuvant radiotherapy was administered. Similarly, a Swiss study examining factors influencing nodal yield in abdomino-perineal resection specimens, documented a reduction in the mean number of nodes identified from 10.5 to 8.0 in patients who recieved pre-operative radiotherapy[75].

2.5. Predictors of positive lymph nodes

The probability of having nodal involvement is related to a number of factors:

a) T-stage

It is well documented that increased depth of invasion (T-stage) correlates with increased risk of lymph node metastasis and overall outcome[2,3]. In a recent study

by Kim and coworkers[76], 22.9 % of patients with T1 and T2 disease had positive lymph nodes, compared to 55.6 % of patients with T3 disease.

b) Tumour biology

Various factors relating to the biology of the tumour have been associated with lymph node involvement, predominantly degree of differentiation, presence of lymphovascular invasion, and histological subtype of the adenocarcinoma.
In a Swedish study of 1239 resected specimens of colorectal cancer, Derwinger et al[77] noted poorer differentiation to be significantly associated with both T-stage and lymph node metastasis ($p<0.001$), with a low grade T2 cancer having a 17% risk of lymph node metastasis compared to a 44% risk for a high grade T2 lesion.

Lin et al[78] demonstrated that both intratumoral as well as peritumoural lymphovascular invasion was statistically significantly associated with lymph node involvement, with p-values of 0.030 and 0.014 respectively, with lymphovascular invasion being identified in 31 of 81 samples investigated (38%).

Although the prognostic significance of occult nodal metastasis (i.e isolated tumour deposits in a lymph node or micrometastasis not detected by standard pathological assessment) is unknown, Wasif and coworkers[79] from the John Wayne Cancer Institute demonstrated in a prospective multicentre trial the following predictors of occult nodal metastasis: advanced T-stage (T3/T4),

lymphovascular invasion and poor tumour differentiation. Overall, occult metastases were identified via immunohistochemistry in 23.4% (25/107) of patients initially deemed node negative via standard pathological analysis. The authors recommended performing cytokeratin immunohistochemistry on patients who were node negative on standard hematoxylin and eosin staining, and who exhibited these features as it may influence decisions regarding adjuvant treatment.

c) Number of nodes examined

The impact of number of nodes examined on the yield of positive nodes is controversial. The current recommendation of a minimum of twelve nodes to be examined is based mostly on observational studies,[13,31-36] largely failing to incorporate significant biological factors known to be related to lymph node involvement i.e tumour differentiation, depth of infiltration and presence of lymphovascular invasion into their assessment. Recently, Baxter et al[80] from Toronto developed a logistic regression model to ascertain the odds of having node metastasis while adjusting for confounding factors including tumour grade, age, site of the lesion and race. They demonstrated via statistical analysis on 11044 patients with T3 tumours in the SEER database, that a dramatic increase in odds of node positivity existed with increasing the number of nodes examined to five ($p < 0.0001$). With examining between six and thirteen nodes there was a marginal increase in odds of positive nodes ($p = 0.006$), but when more than

thirteen nodes were assessed, odds of node positivity actually declined (p =0.04). They concluded that in T3 lesions there is a marginal benefit on staging if more than thirteen nodes are examined, but also a significant risk of understaging if less than seven nodes are examined.

d) Presence of Guanylyl cyclase C in lymph nodes

Guanylyl cyclase C (GCC) is the receptor for bacterial heat-stable enterotoxin. It is selectively expressed by intestinal mucosal cells from the duodenum to the rectum, but not by extra-gastrointestinal tissues[81]. Ever since the demonstration of its utility as a sensitive and specific marker for colorectal tumours[82,83], various studies indicating its potential clinical utility have been published. Waldman and coworkers[84] dissected lymph nodes from bowel resections of 33 patients, 28 of whom had colon cancer, and the remainder had benign disease. Bisecting individual lymph nodes and analysing the one half with standard histopathology, and the other half with reverse transcriptase-PCR, they noted a 100% concordance between nodes with histopathologically confirmed metastasis and GCC expression in the nodes. Additionally, GCC expression was detected in 18% of nodes classified histopathologically as not involved with cancer. Furthermore, none of the nodes harvested from the benign disease specimens showed expression of GCC.

In a recent prospective study from the Thomas Jefferson University in 257 patients with pathological confirmed node negative colon cancer, the authors

noted that the presence of GCC in nodes strongly correlated with time to disease recurrence and disease free survival[85]. In this study, only 32 patients had lymph nodes negative for GCC with only 2 (6.3%) of these patients developing disease recurrence during the follow up period. Of the 225 patients with lymph nodes positive for GCC, 47 (20.9%) developed recurrent disease during the follow up period.

A multicentre clinical trial examining the utility of GCC for staging of patients with colorectal cancer is in progress[86]. The aim is to recruit 1000 patients at the time of surgery. Lymph nodes identified per resected specimen are being bisected, with one half being examined by standard histopathological means, and the other half with reverse transcriptase-PCR for GCC. These results would then be interpreted in terms of patient outcome.

2.6 Summary:

Prognosis in colorectal cancer is determined *inter alia* by the number of lymph nodes examined in patients with node negative disease, and by the number of involved nodes in patients with node positive disease, in the absence of distant metastasis. Furthermore, the ratio of involved to total number of nodes appears prognostically important.

Flourouracil-based adjuvant chemotherapy is currently standard of care in patients with stage III colon cancer, with 15 additional lives saved per 100 patients treated.

Adjuvant chemotherapy for stage II disease, though controversial, needs consideration in the presence of high risk features of tumour perforation, peritoneal involvement, venous spread, involved surgical margin and bowel obstruction. Inadequate nodal sampling is considered an indication for chemotherapy by some.

Although the recommendations for the minimum number of nodes that needs to be examined to stage colorectal cancer accurately varies considerably from six to seventeen, the current recommendation by the National Cancer Institute of twelve nodes seems to be the minimum acceptable.

Nodal yield in colorectal cancer is influenced by patient-related, surgery related and pathologist related factors, of which the diligence of the operating surgeon and examining pathologist appear to be the most important.

Neoadjuvant radiotherapy influences nodal yield additionally in rectal cancer.

Factors predicting node positive disease include advanced T-stage, lymphovascular invasion and poor tumour differentiation. The role of the number of lymph nodes examined in predicting lymph node involvement is still somewhat controversial.

Novel ways of assessing the risk of lymph node involvement are being developed. Gyuanylyl cyclase shows a lot of promise, but its exact clinical application needs to be further defined.

3. Patients and Methods

This is a retrospective study, conducted on data from pathology reports in the Johannesburg National Health Laboratory Service data base, of all patients who underwent resection of adenocarcinoma of the colon and rectum between 2000 and 2005. Ethics approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (Medical).

Pathology reports of resected cases of colorectal cancer were reviewed for patient demographics, the location of the tumour, resection type, centre where the surgery was conducted, the indication for surgery e.g. for obstruction, perforation or elective, T-stage of the tumour, number of lymph nodes and number of involved nodes, tumour differentiation, subtype of tumour, and whether radiotherapy was administered pre-operatively (rectal cancer). Results for colon and rectal cancers were reported separately, due to the effects of preoperative radiotherapy on nodal yield in rectal cancer.

In terms of referring hospitals four groups were created. Data from the teaching hospitals – Charlotte Maxeke Johannesburg Academic Hospital, and Helen Joseph Hospital, were reported separately. Due to low numbers, data from the non-academic state hospitals and private hospitals in the database were pooled. Non-academic state hospitals represented in the database include in alphabetical order: Bela-Bela, Boitumelo, Bophelong, Edenvale, Ermelo, Far East Rand, Jubilee,

Klerksdorp, Leratong, Mafeking, Mankweng, Natalspruit, Pietersburg, Philadelphia, Pholosong, Potchefstroom, Rustenburg, Sebokeng, Tambo Memorial, Tembisa, Themba, Tshepong, Tshilidzini and Witbank.

Patients were grouped according to the number of lymph nodes retrieved into the following groups: not recorded, no nodes identified,1-7 nodes identified, 8-12 nodes, 13-18 nodes, and 18 or more nodes identified. These numbers were chosen based on the work of Swanson et al[13] and Goldstein and coworkers[34]. Additionally, patients were subdivided into those with nodal metastasis and those without. To assess adequacy of nodal retrieval, patients were subdivided into those having less than the recommended twelve nodes examined, and those having twelve or more nodes examined.

Data was entered into an electronic database (Microsoft Excel), and multivariate analysis performed via StatSoft, Inc. (2008). STATISTICA (data analysis software system), version 8.0. Results for the number of nodes present per resected specimen, was reported as means with standard deviations. P-values were calculated using both Pearson and M-L Chi square tests for each set of recorded variables.

4. Results

A total of 365 resected specimens of colorectal cancer were identified in the database, with rectal cancer accounting for 27.67% of specimens (101 cases), including 55 low anterior resections (LAR) and 46 abdomino-perineal resections (APR). The remainder of the cases represented colon cancer specimens: 117 right hemicolectomies (RHC), 11 transverse colectomies (TVC), 49 left hemicolectomies (LHC), 49 sigmoidectomies (Sig), 24 unspecified colonic resections (NOS) and 13 subtotal and total colectomies (SC).

At least 21% of cases in this series presented as emergencies: on 54 reports the history provided indicated obstruction as a presenting feature, and 24 cases had perforation highlighted.

Patient demographics are outlined in table 3. Males and females were nearly equally represented, being 50.5% and 47.3% respectively. Unfortunately in 50.9% of patients the race was not documented, making a proper assessment of racial distribution impossible. The mean age of patients in this series was 56.6 years. A significant proportion of patients with right sided colonic cancers were less than 35 years of age (12/117), with patients less than 50 accounting for 33.0% of right hemicolectomy specimens ($p = 0.067$)(figure 2). This trend would probably reach statistical significance in larger series.

Table 3. Demographic features

Sex	**Number**	**Percentage**
Male	187	50.5
Female	175	47.3
Not documented	3	
Race		
White	78	21.4
Black	92	25.2
Coloured	6	1.6
Asian	3	0.8
Not documented	186	50.9

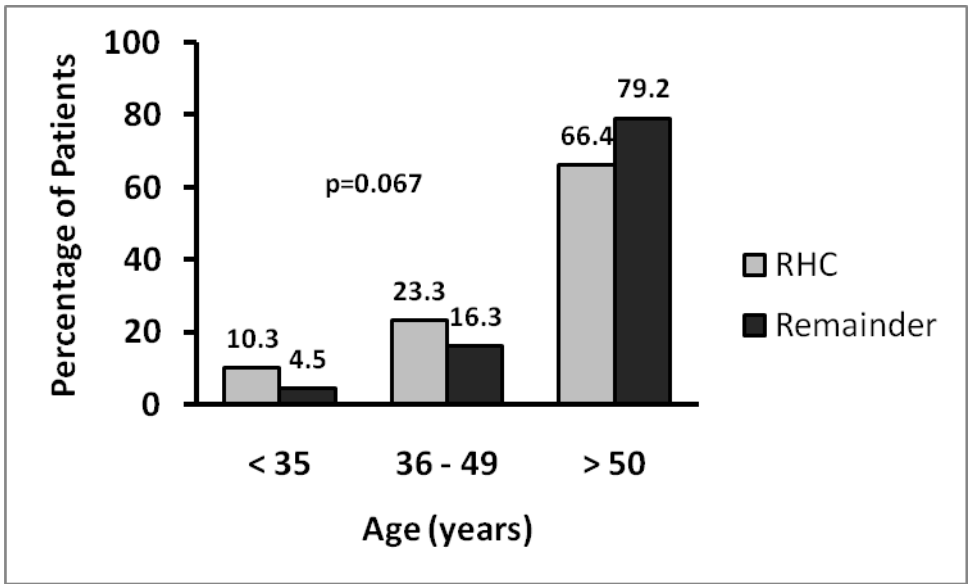


Figure 2. Age characteristics between right hemicolectomy (RHC) specimens and the other resection subtypes combined.

In only 13 reports mention was made of metastatic disease elsewhere, 11 reports clearly stated no metastasis detected elsewhere, and in the remainder no mention to the presence or absence of metastatic disease was reported.

Charlotte Maxeke Johannesburg Academic Hospital contributed the most cases with 159 specimens, with Helen Joseph hospital contributing 71 resections, the non academic public hospitals combined equating for 121 cases and private hospitals for only 14 cases in the series.

The vast majority of patients - 68.6% - presented with T3 disease, with T4 disease present in 16.8% of cases, and T2 and T1 in 9.2% and 1.4% of cases respectively.

The mean number of nodes identified in this series overall is 8.9 nodes per resected specimen, with a standard deviation of ± 6.7 nodes. Statistically significant differences in the mean number of lymph nodes identified could be seen amongst the various resection subtypes ($p < 0.001$) (table 4). Right hemicolectomy, low anterior resection and subtotal and total colectomy specimens have mean values above the overall mean, with left hemicolectomy, transverse colectomy, sigmoidectomy and abdomino-perineal resection having means below average. The lowest means (5.4 nodes per specimen) was identified in the subgroup where the surgeon failed to mention on the pathology report which segment of colon was resected.

Table 4. Mean number of nodes identified with standard deviation per resection subtype ($p < 0.001$) NOS = Not otherwise specified.

Resection	No. patients	Mean number of nodes identified
Right hemicolectomy	117	10.3 (± 6.8 SD)
Transverse colectomy	11	7.6 (± 8.2 SD)
Left hemicolectomy	49	7.7 (± 5.0 SD)
Sigmoidectomy	49	6.5 (± 4.8 SD)
Low anterior resection	55	10.3 (± 6.7 SD)
Abdomino-perineal resection	46	7.2 (± 5.2 SD)
Subtotal/total colectomy	13	18.1 (± 10.5 SD)
Colectomy NOS	24	5.4 (±6.4 SD)
Overall	365	8.9 (± 6.7 SD)

Investigating the effect of where the surgery was conducted on nodal yield, no statistical significant difference was noted between having the surgery at Charlotte Maxeke Johannesburg Academic Hospital, Helen Joseph Hospital, the private hospitals or at the non academic state hospitals respectively ($p = 0.2675$) (table 5). Even subdividing the surgeries into colon and rectal surgery did not result in a statistically significant difference in nodal yield between the hospital groups, with a p-value of 0.31 between the different hospital subgroups for rectal surgery (table 6).

Table 5. Mean number of lymph nodes identified per hospital group overall. Jhb = Charlotte Maxeke Johannesburg Academic Hospital. (p = 0.268)

Hospital	**Jhb**	**Helen Joseph**	**Non academic**	**Private**
No patients	159	71	121	14
No nodes	1.9%	4.2%	9.6%	0.0%
1 – 7 nodes	37.8%	37.1%	42.1%	28.6%
8 – 12 nodes	37.2%	37.1%	22.8%	35.7%
13 – 18 node	13.5%	20.0%	17.5%	7.1%
> 18 nodes	9.6%	1.4%	7.9%	28.6%
Mean no	9.7 (± 7.8 SD)	8.0 (±5.1 SD)	8.0 (± 6.7 SD)	13.4 (± 10.2)

Table 6. Mean number of nodes identified per hospital group for rectal surgery. Jhb = Charlotte Maxeke Johannesburg Academic Hospital. (p = 0.31)

Hospital	**Overall**	**Jhb**	**Helen Joseph**	**Non academic**	**Private**
Nr Patients	101	50	27	19	5
Mean no nodes	8.9	8.9	8.2	8.9	12.2
Std deviation	± 6.2	± 6.5	± 5.3	± 6.3	± 7.3

In terms of numbers of lymph nodes examined in the subset of patients in whom no nodal spread could be demonstrated, an adequate number of nodes were examined in only 29.2 % of patients (figure 3).

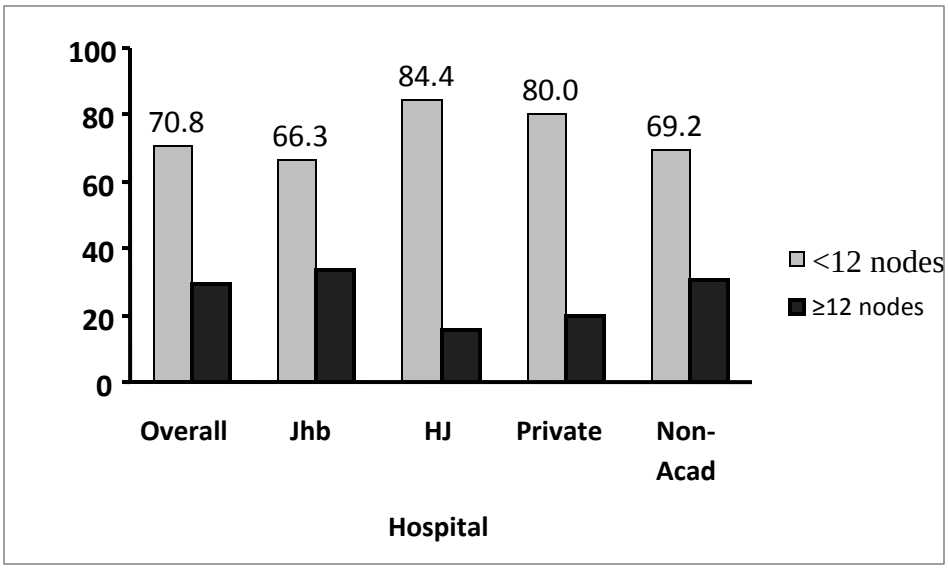


Figure 3. Percentage of node negative patients with < 12 and ≥ 12 nodes examined per hospital group. Jhb = Charlotte Maxeke Johannesburg Academic Hospital, HJ = Helen Joseph Hospital, Private = private hospitals, Non-acad = non academic state hospitals.

The indication for the surgery e.g. whether the surgery was done for elective or emergency indications (obstruction or perforation) did not result in a statistically significant difference in the mean number of lymph nodes retrieved, with a mean number of 8.8 nodes per specimen for the elective group, and 9.3 nodes per specimen for the emergency group (table 7).

Table 7. Mean number of nodes identified per surgery indication. (p = 0.58)

Indication	**No patients**	**Mean**
Elective	287	8.8 (± 6.6 SD)
Emergency	78	9.3 (± 7.3 SD)

Radiotherapy is frequently employed in the management of rectal cancer. To assess the overall effect of radiotherapy on lymph node yield, all cases of rectal cancer were included. On 25 pathology reports pre-operative radiotherapy was highlighted. Radiotherapy resulted in a reduction in the mean number of nodes identified from 9.5 to 5.0 (table 8).

Table 8. Effect of radiotherapy on mean number of nodes examined (p = 0.033)

	Overall	**Radiotherapy**	**No radiotherapy**
No. patients	101	25	76
Mean no nodes	8.9 (± 6.2 SD)	5.0 (± 5.0 SD)	9.5 (±6.4 SD)

Increased depth of penetration (T-stage) of the tumour corresponded with a statistically significant increase in the presence of lymph node involvement for both colon and rectal cancer respectively, with T3 lesions associated with lymph node involvement in 47% of colonic cancer specimens, and 59 % of rectal cancer specimens (figure 4). Conversely, T2 lesions were only noted as having lymph node involvement in 18.8% of colon and 18.2 % of rectal cancer respectively. These findings were statistically significant, with p-values of 0.041 and 0.0024 for colon and rectal cancer respectively, in spite of the low number of T2 cancers present in the series (table 9). Excluded from the calculation were cases in which no lymph nodes were examined pathologically and those cases where no T-stage was assigned by the pathologist. These comprised 23 cases in the colon cancer group and 2 cases in the rectal cancer group.

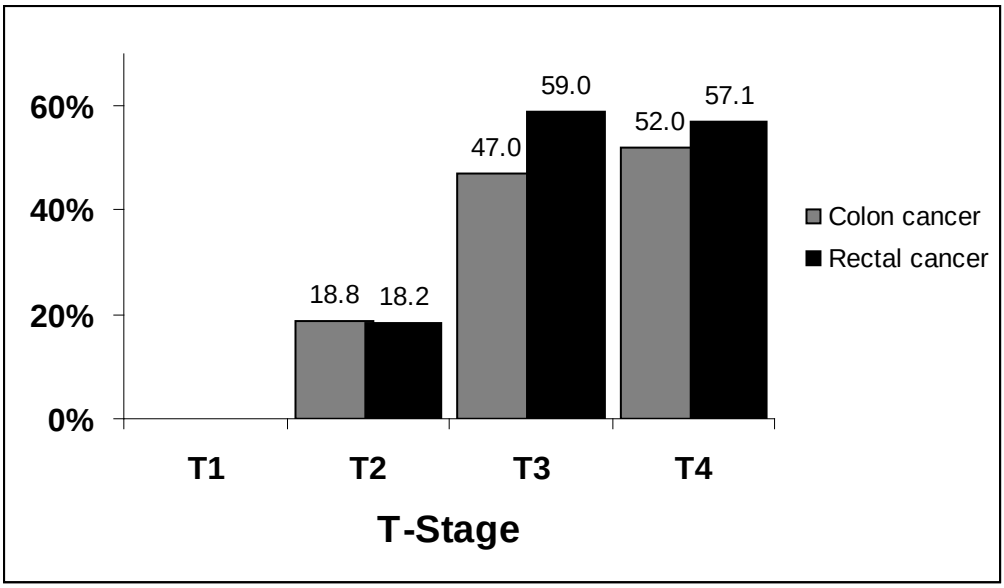


Figure 4. Percentage of patients with node positive disease based on T-Stage for colon and rectal cancer respectively.

Table 9. Number of node positive patients according to T-stage for colon and rectal cancer respectively.

	Colon cancer		Rectal cancer	
T-Stage	**No. patients**	**Node positive**	**No. patients**	**Node positive**
T1	3	0	2	0
T2	11	2	22	4
T3	177	83	61	36
T4	50	26	7	3

In assessing the effect of number of lymph nodes examined on overall node positive yield, patients were subdivided into groups according to number of nodes examined. These groups were then cross referenced against the number of patients with positive nodes for that given subgroup (table 10). Excluded from the calculations was the subset of patients where no nodes where examined. Plotting a

graph of the numbers of nodes examined against the percentage of patients with nodal metastasis identified for that subset of nodes examined, a significant increase in the proportion of patients with positive lymph nodes could be seen for examining up to eight to twelve nodes for both colon and rectal cancer (figure 5). Beyond twelve nodes examined, the curve starts tapering off, and beyond eighteen nodes examined the proportion of patients with positive nodes identified actually decreases.

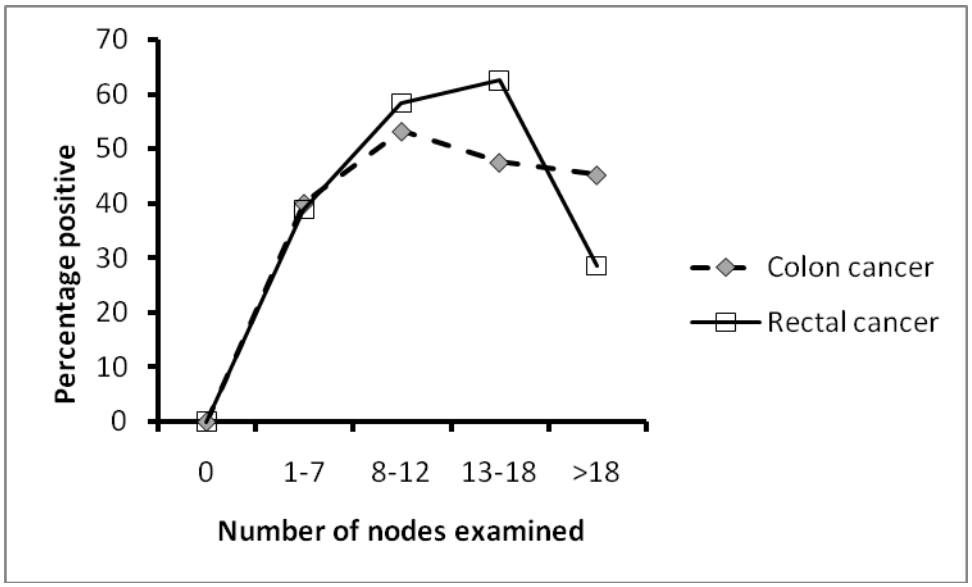


Figure 5. Percentage of patients with positive nodes based on the number of nodes examined for colon and rectal cancer respectively.

Table 10. Number of patients with positive lymph nodes according to the number of nodes examined for colon and rectal cancer respectively.

	Colon cancer		Rectal cancer	
Nodes examined	**No. patients**	**Node positive**	**No. patients**	**Node positive**
0	17	0	4	0
1-7	100	40	36	14
8-12	79	42	36	21
13-18	40	19	16	10
>18	22	10	7	2

Dividing adenocarcinomas into mucinous and non-mucinous subtypes did not result in a statistically significant difference in the rate of positive nodes (table 11). However, given the relatively small number of mucinous tumours in this series - 38 in total- no differentiation between colon and rectal cancers was made, and in larger series statistical significance may be demonstrated.

Table 11. Number of patients with involved lymph nodes per histological subtype ($p = 0.13$)

Subtype	**No patients**	**Node positive**	**Percentage**
Mucinous	38	19	50.0
Non-mucinous	306	140	45.7

Degree of differentiation of the tumour was statistically significantly associated with node positive disease for colon cancer ($p=0.049$) (figure 6). Of the 30 cases with well differentiated tumours, only 26% had positive lymph nodes; whereas

50% of cases with poor differentiation were node positive (table 12). The strength of association was less with rectal cancer (p=0.17), possibly reflecting the low numbers of cases with poor tumour differentiation (5 cases) in this subset of patients; statistical significance may be demonstrated by a larger series (table13). The same would apply for the moderate to poorly differentiated subset amongst the colon and rectal cancer specimens, totalling 8 colon cancer specimens and 9 rectal cancer specimens respectively. For statistical analysis purposes, cases with no lymph nodes documented by the pathologist, as well as cases where the pathologist failed to indicate degree of differentiation were excluded from the calculations. This accounted to 25 colon and 8 rectal cancer specimens respectively.

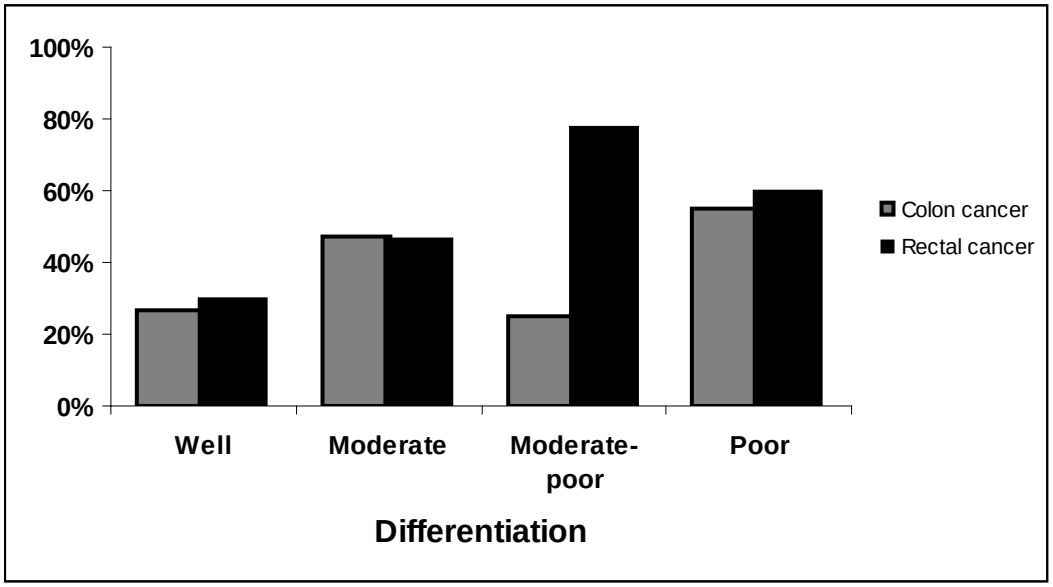


Figure 6. Percentage of patients with positive lymph nodes based on tumour differentiation for colon and rectal cancer respectively.

Table 12. Influence of degree of differentiation on numbers of node positive disease for colon cancer. p = 0.049

Differentiation	Node negative	Node positive	Percentage positive
Well	22	8	26.7%
Moderate	80	72	47.4%
Moderate-poor	6	2	25.0%
Poor	22	27	55.1%

Table 13. Influence of degree of differentiation on numbers of node positive disease for rectal cancer. p = 0.17

Differentiation	Node negative	Node positive	Percentage positive
Well	7	3	30.0%
Moderate	37	32	46.4%
Moderate-poor	2	7	77.8%
Poor	2	3	60.0%

The identification of lymphovascular invasion by the tumour was associated with a significant increase in the numbers of positive lymph nodes. Lymphovascular invasion was present in 75.7% of colonic tumours with nodal involvement, and absent in only 41% of node positive cancers (figure 7 and table14). For rectal cancer, lymphovascular invasion was identified in 68.4% of node positive cases and absent in 41% of node positive cases (figure 7). Excluded from the calculation were cases where the pathologist mentioned that lymphovascular invasion is either suspected or where there was uncertainty if it was present. Cancers where the pathologist failed to mention lymph nodes identified were also

excluded. This accounted for 25 exclusions in the colon cancer group, and 4 exclusions in the rectal cancer group.

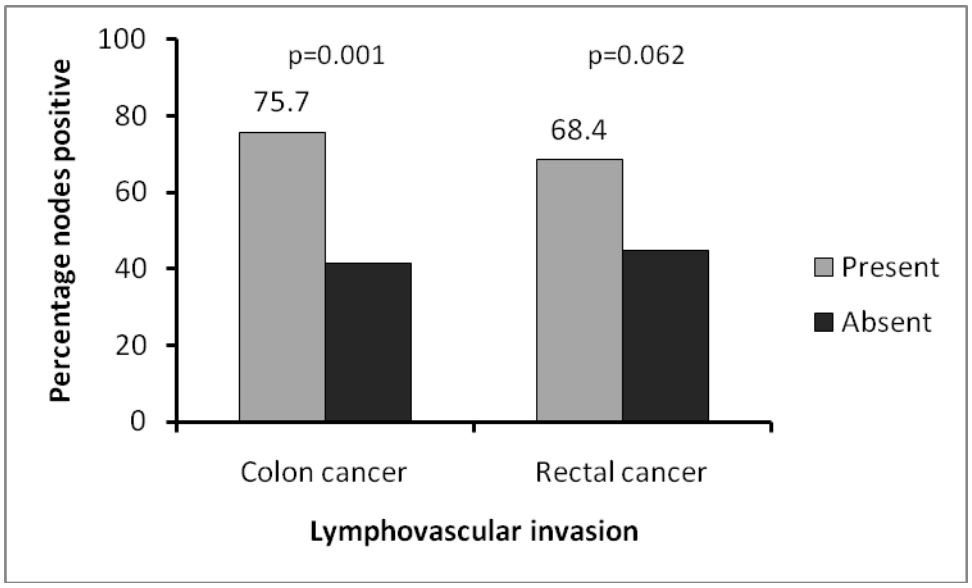


Figure 7. Percentage of patients with positive lymph nodes relative to the presence or absence of lymphovascular invasion for colon and rectal cancer respectively.

Table 14. Relationship of node positivity with the presence or absence of lymphovascular invasion.

Lymphovascular invasion	**Nr patients**	**Node positive**	**Percentage**
Colon cancer			
Present	37	28	75.7%
Absent	202	84	41.6%
Rectal cancer			
Present	19	13	68.4%
Absent	76	34	44.7%

5. Discussion

Patients in this series were nearly equally distributed between the sexes, with a slight male predominance. Unfortunately, due to poor data capturing on the pathology forms, no good indication of the racial distribution of the individual cancers can be given. This data would appear to agree with a recently published series by Cronje et al.[86] reporting that 41% of cancers in black patients in South Africa occurred in patients less than 50 years old. This series, though relatively small, also indicates a similar trend in predominantly right sided lesions occurring at a younger age.

The mean number of lymph nodes identified in this study was 8.9 nodes per specimen. This is less than the recommended twelve nodes per specimen. Nodal yield in resected specimens of colorectal cancer is recognized as one of the tools to assess quality of surgical resection and pathological evaluation[87]. This study indicates adequate lymph node evaluation in the subset of patients staged as node negative, in only 29.2 % of patients. This is significantly worse than some recent international published reports. Mitchell et al.[88] in a retrospective study of cancer resections of five hospitals in the United Kingdom, found only two of the five hospitals studied harvesting means of twelve of more nodes per resected specimen. Overall 53.7% of patients staged as Dukes B (no lymph node involvement) had twelve or more nodes examined. This was echoed in a large American study[87] amongst 1296 hospitals, noting compliance to the suggested minimum number of nodes harvested in only 38% of hospitals in the United States. However, they documented mean number of nodes examined per resected specimen of colon cancer of sixteen for National Cancer

Institute Comprehensive Cancer Centers, thirteen nodes for Veterans Affairs hospitals, fourteen lymph nodes for other academical institutions and twelve nodes for community hospitals.

Significant differences existed amongst the various resection subtypes in terms of mean number of nodes resected, varying from 18.1 nodes per specimen for subtotal and total colectomies to 6.5 nodes per sigmoidectomy specimen. These probably relates to the difference in length of bowel resected: the longer the resected segment the more lymph nodes should be present. It would however appear, that more lymph nodes are harvested for right hemicolectomy specimens (mean = 10.3 nodes per specimen) than for left hemicolectomy resections (mean = 7.7 nodes per specimen). As it has never been reproducibly shown that that a segmental variation in the number of nodes present in the colon and rectum exist, these differences cannot be explained with the present data. The low mean number of nodes (5.4 nodes per specimen) present in the subgroup of resections where no indication as to the segment involved was given, may be due to poor surgery, however, additional factors such as marginal status would have to be considered first before definitive conclusions can be made.

The bulk of the surgery conducted in the study population was done in academic institutions, with Charlotte Maxeke Johannesburg Academic Hospital and Helen Joseph Hospital combined accounting for 63.0% of cases, the non-academic state hospitals for 33.2% and private hospitals for 3.8% respectively. No difference in mean number of lymph nodes retrieved could be demonstrated between the relevant

hospital groups, neither were there differences in terms of mean number of nodes identified for rectal surgery specifically. Additionally there was no difference in mean number of nodes examined between emergency and elective surgery. No conclusions regarding the centre where colorectal surgery should be performed can be made based on this study. It must be emphasized that mean number of nodes is only one of the features assessing quality of resection, and, as it is influenced by other variables, should not be used in isolation to determine the centre where colorectal surgery should be conducted. Additional studies exploring proximal, distal, mesorectal and mesocolic resection margins for the individual hospital groups and resection subtypes in conjunction with nodal yield could clarify the matter.

Neo-adjuvant radiotherapy accounted for a reduction in the mean number of nodes identified from 9.5 nodes per specimen in the rectal cancer group where no pre-operative radiotherapy was indicated on the pathology report, to 5.0 nodes in patients who had received radiotherapy ($p = 0.033$). However, seeing that pre-operative radiotherapy is indicated in a significant subset of patients with rectal cancer, especially mid-rectal, distal rectal and locally advanced cancers[73], and that the majority of the patients with rectal cancer in this study presented with T3 and node positive disease, it can be assumed that a significant proportion of patients in the group where radiotherapy was not indicated on the pathology form did in fact receive pre-operative radiotherapy, confounding the results. However, this contamination implies that the effect of radiation therapy is even greater on the amount of lymph nodes retrieved in the rectal cancer group of this study.

The following factors were strongly associated with lymph node metastasis in this study:

1. **T-stage**

   A large number of cases presented as T3 disease (68.6%), with 46.9 % of T3 colonic lesions and 59.0% of T3 rectal cancer specimens being node positive compared to only 2 of 11 (18.2%) colonic and 4 of 22 (18.2%) rectal T2 cancers. This is similar to the figures noted in the SEER database by Baxter and coworkersl[80], where 65% of patients in their series presented with T3 disease and amongst these patients 41% had nodal involvement. Similarly, the overall prevalence of node positive disease in this study is 44.4%. This is significantly higher than an earlier South African study by Boytchev et al[89], noting lymph node metastasis in 31% of their study patients. The high number of cases in this study presenting with T3 and T4 disease (85.4% combined), combined with the possible contamination of results with stage IV disease probably equates for the overall higher incidence of lymph node metastasis.

2. **Lymphovascular invasion**

   Lymphovascular invasion was present in 75.7 % of patients with node positive colon cancer and 68.4 % of node positive rectal cancer respectively.

3. **Degree of differentiation**

Degree of differentiation correlated with lymph node involvement for colon cancer, with 55.0 % of poorly differentiated lesions associated with lymph node metastasis in colon cancer, while only 26.7 % of well differentiated tumours had involved lymph nodes ($p = 0.049$). This difference was not apparent in rectal cancer ($p = 0.17$). However, only 5 cases in the rectal cancer group were poorly differentiated, confounding the statistical analysis. These findings seem to concur with the findings of a recent Swedish trial[73] investigating the effect of tumour differentiation on lymph node involvement, finding an overall significant association between degree of differentiation and nodal metastasis ($p = 0.001$). This trial however did not differentiate between colon and rectal cancer in their report.

The following factors were not statistically significantly associated with lymph node metastasis:

1. **Histological subtype**

Dividing the adenocarcinomas into mucinous and non-mucinous subgroups did not result in statistically significant association with positive lymph nodes in the mucinous subgroup. However, only 38 patients in total had mucinous tumours, and 19 of these were node positive. Secondly, because most of the tumours in this

series were T3 lesions, the risk of nodal metastasis in the non-mucinous group was significant, thereby potentially lessening the effect of the mucinous subtype on nodal metastasis.

2. Numbers of nodes examined versus node positivity

This study does not document a significant trend of increased node positivity by examining more than 13-18 lymph nodes, and actually reveals a decrease in the proportion of patients with nodal involvement if more than 18 nodes are examined. This study agrees with the work of Baxter and coworkers[76] on the risk of nodal involvement in T3 disease, indicating that in terms of number of nodes examined, a benefit in terms of detecting nodal involvement exists by examining up to 18 nodes. Examining more than 18 nodes does not result in a significant increase in the number of positive nodes identified for either colon or rectal cancer.

A large number of factors may influence both the number of nodes present in the mesentery of an individual patient and the odds of having nodal involvement. Therefore, attempts at improving accuracy of staging based on numbers of identified lymph nodes in resected specimens may be too simplistic. It is debatable whether the improved survival demonstrated in node negative patients based on numbers of nodes examined truly reflects increased staging accuracy, rather than better tumour biology in this subset of patients.

Weaknesses of this study:

This is a retrospective analysis, which depended on the thoroughness with which the pathology request form was completed. This was incomplete in many instances. Examples include the 50.9 % of cases where the race of the patient was not documented, and the 24 colectomy specimens where the resection subtype could not be identified.

The number of lymph nodes identified in the mesentery of a colorectal cancer specimens will vary according to the factors outlined before: patient related, surgeon related, pathologist related factors and prior radiotherapy. It can therefore only be speculated as to the exact reasons for the lower than acceptable mean number of nodes identified in this series. Furthermore, by including patients with node positive disease in the overall assessment of mean number of nodes identified, it could be argued that the examining pathologist would only remove and examine the grossly positive nodes from the specimen in cases where there are grossly involved nodes present, thereby lessening the total number of nodes examined, and if one metastatic node is identified, it may not change patient management. This however, significantly reduces our ability to predict overall prognosis based on ratio of node positivity, as well as having a tool to assess quality of surgery. Bilimoria et al[84] also included both node negative and node positive patients in their assessment quality of colonic surgery in a national hospital survey in the United States. The focus should be therefore to identify all the nodes present in the resected specimen.

In only 13 reports metastatic disease elsewhere was mentioned, and in only 11 cases definite mention of absence of metastatic disease was stated; in 341 patients the presence or absence of metastatic disease is not known, potentially confounding the results. It can be assumed however, that excluding the patients where surgery was conducted for emergency indications, most of the patients would have been staged appropriately pre-operatively.

6. Conclusions and recommendations:

The overall substandard nodal assessment of 8.9 nodes per resected specimen of colorectal cancer amongst the patients in this study is concerning. Specifically, only 29.2 % of patients where the adequate number of nodes assessed would have the greatest clinical significance – the node negative subgroup – had more than twelve nodes examined; indicating that in the remaining 70.8% of patients, firm recommendations regarding adjuvant chemotherapy could not have been made. This would significantly influence the overall quality of care provided to the patients in this series, including patient survival and disease recurrence.

No difference could be demonstrated in terms of both mean number of nodes resected overall, and mean number of nodes examined for the patients with no nodal metastasis identified between having surgery at the two academic hospitals or at the non-academic public hospitals respectively. No firm conclusions in terms of the centre where colorectal surgery should be conducted can be made based on this study.

Factors associated with lymph node metastasis in this study include advancing T-stage, the presence of lymphovascular invasion and the degree of differentiation of the tumour.

The following recommendations can be made:

- Case by case discussion between the surgeon and pathologist examining the resected specimen.
- Should less than twelve nodes be present in the resected specimen, the examining pathologist should re-examine the specimen to identify more nodes.
- In the presence of factors predicting nodal involvement i.e. T3 or T4 disease, presence of lymphovascular invasion and poor tumour differentiation, serious consideration should be given to identifying as many as possible nodes. In this subset of patients, should less than twelve nodes be identified, additional means to detect micrometastatic disease i.e. immunohistochemistry should be considered.
- The use of the described methylene blue technique of identifying lymph nodes is worth consideration, especially in the South African context, since a cheap and easy method has been shown to dramatically increase the nodal yield.

- The introduction of quality control measures aimed at improving patient care. This would necessitate the creation of a colorectal (and other cancer) database, allowing frequent auditing of results and peer review.
- The need for a study looking at the present quality of colorectal cancer surgery between the academic and non-academic hospitals respectively.

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