

ORIGINAL PAPER

The influence of nodal size on the staging of colorectal carcinomas

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Aims: The reliable identification of node negative colorectal carcinomas (CRCs) has often been linked to the histological examination of a minimum number of lymph nodes. The sizes of the lymph nodes, their metastatic status, and their number were investigated to establish whether these parameters are related, and whether their relation could help in determining the adequacy of staging.

Methods: One thousand three hundred and thirty four negative lymph nodes, 189 metastatic lymph nodes, and 43 pericolic/perirectal tumour deposits measuring ≥ 3 mm from 60 node positive and from 63 node negative patients with CRC were assessed for size.

Results: The mean size (SD) of these structures was 4.5 (2.7) mm. The lymph nodes were significantly larger in the CRCs with metastatic nodes (4.7 v 4.3 mm). Involved nodes were significantly larger than negative nodes (6.3 v 4.2 mm), despite the fact that the largest node was ≤ 5 mm in one third of node positive CRCs. The examination of the seven largest nodes could have adequately staged 97% of node positive CRCs and 98% of all CRCs.

Conclusions: The nodal staging of CRCs is dependent not only on the number of lymph nodes investigated, but also on qualitative features of the lymph nodes assessed, including their size. Lymph nodes are not equivalent and any study neglecting this fact will give grounds for error in the recommendation of a minimum number of nodes for the reliable determination of node negative CRCs. Although pathologists should aim to recover all nodes, a negative nodal status based on only seven nodes can be reliable.

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The nodal status in colorectal carcinomas (CRCs) is an important prognostic feature that is incorporated into all relevant staging systems of this disease,^{1–5} is of primary importance in decisions related to the administration of adjuvant chemotherapy, and represents an objective confirmation of an adequate regional lymph node dissection.⁶ Most guidelines on nodal staging give quantitative recommendations for the determination of nodal involvement, or more precisely for a reliable determination of the absence of nodal metastases.^{7–14} These guidelines suggest that all lymph nodes should be recovered from the mesocolic fat or perirectal tissues and examined microscopically, but on the basis of the most lenient recommendations^{7–10} at least six to seven nodes must be assessed to be reasonably confident that a CRC is truly node negative, rather than an understaged node positive CRC.

“Qualitative features might be important in determining the nodal stage: qualitative features may help in the identification of the lymph nodes most likely to be affected by metastasis, and these nodes could be investigated more thoroughly”

It seems reasonable to state that lymph nodes in a given draining region are not equivalent, and that not only quantitative but also qualitative features might be important in determining the nodal stage: qualitative features may help in the identification of the lymph nodes most likely to be affected by metastasis, and these nodes could be investigated more thoroughly. One such feature, (probably the best) is the fact that a lymph node possesses direct drainage from the tumour site, and is therefore a sentinel node. Although the technique of lymphatic mapping in CRCs has been developed, and gives promising results,^{15–17} less encouraging results have also been reported^{13, 18}; technical issues or tumour characteristics may be responsible for these latter results. Clearly, the value of sentinel lymph node identification in CRCs demands further

investigation. Another qualitative feature possibly permitting a selection of nodes more likely to be metastatic is the distance of the lymph node from the tumour. In a previous study, we demonstrated that metastases are most likely to occur in nodes located in the mesocolic or perirectal tissues of the bowel segment affected by the tumour and the segments 1 cm distal or proximal to the tumour.¹⁹ More distant nodes might become invaded in a later stage because these were associated with metastases of lymph nodes close to the primary carcinoma. As a further possible qualitative feature allowing a selection between lymph nodes more likely to be positive and those more likely to be negative, we investigated the size of the lymph nodes in our present study.

MATERIALS AND METHODS

One hundred and twenty three consecutive cases of invasive CRC operated on between 1 March 2000 and 28 February 2001 were evaluated for their nodal status. The surgical procedure consisted of standard radical resection in most cases, and limited resection in a minority of the cases. Lymph nodes were retrieved after one to three days of fixation in 7% buffered formalin. Careful palpation of the mesocolic and perirectal tissues was carried out on a cork board because this method has proved very satisfactory in the recovery of lymph nodes from the axillary fat of patients with breast cancer.²⁰ No fat clearing solution was used in our study. The recovery of lymph nodes concentrated on the tissues around the bowel segment affected by the tumour and the 3 cm long segments proximal and distal to the tumour,¹⁹ but whenever fewer than seven

Abbreviations: CRC, colorectal carcinoma; HE, haematoxylin and eosin; NN, negative node; PN, positive node; TDN, perirectal or pericolic tumour-deposits ≥ 3 mm with no evidence of residual nodal elements

Table 1 pT and pN categories of the tumours involved in the study^{4,5}

	pN0	pN1	pN2	All
pT1	5	0	0	5
pT2	12	1	0	13
pT3	45	32	19	96
pT4	1	4	4	9
All	63	37	23	123

Tumours were not separated according to the presence or absence of distant metastases (M category).

nodes were identified microscopically, a second cut up was performed on the same segments and on the remaining bowel segment removed during surgery. Carcinomas removed by polypectomy and specimens with synchronous CRCs of the same bowel segment were excluded.

Lymph nodes were embedded separately. All lymph nodes were assessed by examining two to five serial ribbons (5–25 µm apart) of basically the same level, representing the cross section estimated to be the largest during sectioning. These were stained with standard haematoxylin and eosin (HE). Negative lymph nodes (NNs), positive lymph nodes (PNs), and mesocolic or perirectal tumour deposits with no evidence of nodal structure ≥ 3 mm (TDNs)^{4,5,21} were recorded separately, and the largest size of each item was measured using the Vernier scale of the microscope.²²

In node positive CRCs all nodes were ranked according to their largest dimension, the first being the largest one. Nodes with an identical measured size received the same sequential

numbers (for example, three nodes each numbered 2 to 4 would indicate that these three nodes were the second, third, and fourth largest ones, and no distinction could be made between them on the basis of size). The sequential number of the largest metastatic node was then considered to assess the chance of identifying a node positive carcinoma as such if only the largest nodes were considered in staging. The results of assessing only the first one to seven largest nodes were finally compared with the results of assessing all the lymph nodes, and the results were expressed as the percentage where these nodes adequately identified a node positive CRC as node positive.

Statistical comparisons of continuous variables between two groups were made by the Student's *t* test. Significance was set at $p < 0.05$.

RESULTS

Of the 123 CRCs assessed, 42 were located in the right colon and 81 in the left colon and rectum. The study involved 64 male (median age, 69) and 57 female (median age, 67) patients (one male and one female patient had two synchronous but distant CRCs). Table 1 details the stages of the CRCs investigated.

In total, 1334 NNs, 189 PNs, and 43 subserosal or perirectal TDNs were assessed. The median number of lymph nodes assessed in the whole study population, and also in the node negative and node positive groups, was 12.

The mean size of all lymph nodes (SD) was 4.45 (2.52) mm; if TDNs were also included, the mean was 4.53 (2.72) mm. The size of lymph nodes in node negative CRCs was first compared with the size of the lymph nodes (comparison 1) and with the

Table 2 Nodal size in millimeters in different patient or lymph node groups

	Groups compared		p Value
	Node negative CRCs	Node positive CRCs	
Comparison 1 Mean nodal size (SD)	4.3 (2.4)	4.6 (2.6)	<0.05
Comparison 2 Mean nodal and TDN size (SD)	4.3 (2.4)	4.7 (3.0)	<0.01
Comparison 3 Mean nodal size (SD)	NNs 4.2 (2.3)	PNs 6.0 (3.4)	<0.005
Comparison 4 Mean nodal size (SD)	NNs 4.2 (2.3)	PNs and TDNs 6.3 (4.1)	<0.005
Comparison 5 Mean smallest nodes (SD)	Node negative CRCs 1.7 (0.8)	Node positive CRCs 1.7 (0.8)	NS
Comparison 6 Mean largest nodes (SD)	Node negative CRCs 7.7 (3.2)	Node positive CRCs 9.9 (5.6)	<0.05

CRC, colorectal carcinoma; NN, negative node; NS, not significant; PN, positive (metastatic) node; TDN, perirectal or pericolic tumour deposit ≥ 3 mm with no evidence of residual nodal elements.

Table 3 Rates of detection of positive nodes when only the first one to seven largest nodes are examined*

	Number of lymph nodes or TDNs assessed						
	1	2	3	4	5	6	7
Number of largest node(s) assessed	1	2	3	4	5	6	7
Node positive CRCs identified	29–30	41	46–47	52	54–55	55–56	58
% Of node positive cases (n=60)	48–50%	68%	77–78%	87%	90–92%	92–93%	97%
% Of all cases (n=123)	75–76%	85%	89%	93%	95–96%	96–97%	98%
	Number of lymph nodes (but not TDNs) assessed†						
Number of largest node(s) assessed	1	2	3	4	5	6	7
Node positive CRCs identified†	24–25	35	39	44	47–48	49–50	52
% Of node positive cases (n=54)†	44–46%	65%	72%	81%	87–89%	91–93%	96%
% Of all cases (n=123)	76%	85%	88%	92%	94–95%	96–97%	98%

*The accuracy of this approach is expressed as the percentages of cases adequately identified, in comparison with the results of an assessment of the total number of nodes examined in the resection specimens. †Tumours with only TDNs but no PNs classified as node negative. CRC, colorectal carcinoma; TDN, perirectal or pericolic tumour deposit ≥ 3 mm with no evidence of residual nodal elements.

size of the lymph nodes plus TDNs (comparison 2) in node positive CRCs (table 2). The size of the NNs from both node negative and node positive specimens was next compared with the size of the PNs (comparison 3) and with the size of the PNs and TDNs together (comparison 4) (table 2). The sizes of the smallest (comparison 5) and largest (comparison 6) nodes were also compared in node negative and node positive CRCs (table 2).

Table 3 shows the reliability of staging based on the first one to seven largest nodes. Although 70% of the lymph nodes assessed (1072 of 1521 lymph nodes; or 1089 of 1563 if TDNs are also included as lymph nodes) measured ≤ 5 mm, because the PNs were generally larger than NNs, only 45% of the PNs were ≤ 5 mm (44% if TDNs are also included as positive nodes). Twenty CRCs (a third of the cancers with nodal involvement) had their largest metastatic node ≤ 5 mm.

DISCUSSION

The standard assessment of nodal status requires a histological assessment of the lymph nodes recovered from the mesocolic or perirectal tissues. There have been several suggestions as to the minimum number of lymph nodes required for a reliable identification of node negative CRCs—for example, six,⁷ seven,⁸ eight,¹⁰ 12,⁵ 14,¹² 16,¹³ and 17¹⁴ have all been proposed. It seems likely, however, that in addition to the quantitative aspects of the lymph nodes assessed microscopically, certain qualitative features may also be important for the adequate staging of CRCs.

In this study, the microscopic size of the lymph nodes reflecting their largest macroscopic dimension was assessed as a qualitative feature that could possibly predict the metastatic status of a lymph node and could theoretically be included in a model on the accuracy of staging. The mean number of lymph nodes assessed was similar in the node positive and node negative CRC groups, but the mean size of the nodes was unnoticeably but significantly larger in the node positive group. In addition, the largest lymph nodes in the node positive CRC group were larger than the largest nodes in the node negative CRC group. It was found that metastatic lymph nodes are significantly larger than uninvolved nodes. However, there is a considerable overlap between the sizes of PNs and NNs, so that nodal size, as a sole criterion, does not allow a selection between PNs and NNs in general. Nevertheless, size has much to do with the detectability of a lymph node, larger nodes being easier to recover, and it seems that recovering only the six or seven largest nodes could result in a 8–9% or 3–4% false negative staging (table 3).

Because even the definition of an involved lymph node is questionable, two different approaches were applied in the analyses. According to the staging guidelines of the UICC/AJCC or the Royal College of Pathologists,⁴ 5, 21 a tumour nodule ≥ 3 mm in diameter in the perirectal or pericolic fat without histological evidence of a residual node in the nodule is classified as regional lymph node metastasis. However, it has been suggested that these nodules most often are not derived from destroyed metastatic nodes, but are rather intravascular, perivascular, or perineural extensions of the primary tumour, and represent a feature of poor prognosis, independently of their size.²³ Our observations on the nature of these nodules are very much in accord with this last statement, and therefore the UICC/AJCC guidelines were followed in general. These were reflected in the staging (table 1), but TDNs were reported separately and a second analysis was carried out with the omission of these nodules, including only those structures that had residual nodal elements (including the circumscribed capsule of a totally invaded node). Fortunately, the results of the two analyses were very similar.

The size of lymph nodes has been investigated in several studies on the staging of CRCs, and the metastatic status of “small lymph nodes” has received special attention.^{24–27} We

found that lymph nodes adjacent to the bowel segment affected by the tumour and the 1 cm long segments proximal and distal to it are usually larger than those situated further away. In addition, significantly fewer nodes were recovered from areas more distant from the tumour affected bowel segment.¹⁹ The term small is by no means subjective, but the results reported here suggest that the generally used < 5 mm is inadequate to define “small nodes”. The mean (SD) nodal size in the whole series of more than 1500 lymph nodes was 4.45 (2.52) mm, suggesting that small nodes would be those that are < 1.9 mm (mean – SD). Nodes of this size are certainly difficult to detect by palpation. Pathologists in our institution feel that lymph nodes ≤ 3 mm may be difficult to detect by palpation. Considering the mean size of lymph nodes, it is not surprising that most of the lymph nodes were < 5 mm and, despite the generally larger size of metastatic lymph nodes, as many as a third of node positive CRCs had no PN > 5 mm.

“It appears very probable that, after a certain limit, increasing the number of lymph nodes recovered for histopathological assessment is unlikely to improve the staging significantly”

A few studies have correlated nodal size with metastatic status in CRCs. By allotting nodes to three size categories with cut off limits at 5 and 10 mm, Kotanagi *et al* found only a non-significant trend for PNs to be larger than NNs.²⁵ They also found that in a quarter of their 34 patients with node positive CRCs the mean diameter of the PNs was smaller than that of the NNs. In contrast, Mönig *et al* reported results similar to ours. The metastatic nodes were on the whole larger than non-metastatic ones; most of the nodes measured < 5 mm, with 53% of the PN < 5 mm, and the overlap between the sizes of PNs and NNs was considerable.²⁸

Fat clearing is said to improve the nodal staging of CRCs,¹¹ 24, 27 but it is not required in routine staging. Adequate formalin fixation is also of value in improving the lymph node retrieval from pericolic or perirectal fat.²⁹ 30 In a short term internal audit relating to the recovery of as many lymph nodes as possible from the axillary fat of patients with breast cancer,²⁰ we were able to find extremely small lymph nodes in the axilla, and this experience enabled us to detect small nodes around CRCs too (comparison 5; table 2). Our nodal retrieval concentrated on the nodes located in the pericolic or perirectal fat adjacent to the bowel segment affected by the tumour and 3 cm proximal and distal to it. The mean number of lymph nodes was therefore lower than the 17 attained with our earlier practice of trying to recover all palpable nodes from resection specimens.¹⁹ The proportion of node positive CRCs in this series (48.8%) is very similar to the rate of 48.3% reported from Italy, where the average nodal yield (41.1) was greater than that seen here.³⁰ Such a high yield was reached without fat clearing agents, with adequate fixation, and macroscopic examination on a cork board. The same group also reported its experience with conventional palpation based lymph node retrieval: the mean node number found with this method was close to ours (11.3), but the rate of nodal involvement was significantly lower (30.4%). The depth of tumoral infiltration, a factor known to influence nodal involvement, was similar in the Italian series (with more nodes assessed) to our own, whereas in the other Italian series with a lower rate of nodal involvement there were more pT1 and pT2 tumours; this could partly explain the differences in nodal involvement rates. It is not the purpose of this study to evaluate different fat clearing techniques, but a large series documented nodal involvement in 28.2% of 864 patients after the examination of a mean of 27 nodes/patient.³¹ On the other hand, without increasing the number of lymph nodes investigated greatly (11.4 *v* 10.2), Poller found that the proportion of node positive CRCs rose from 45% to 64% when

Take home messages

- The nodal staging of colorectal carcinomas (CRCs) is dependent not only on the number of lymph nodes investigated, but also on qualitative features of the lymph nodes assessed, such as their size, their distance from the tumour, and whether or not they are sentinel nodes
- Lymph nodes are not equivalent and any study neglecting this fact will give grounds for error in the recommendation of a minimum number of nodes for the reliable determination of node negative CRCs
- Although pathologists should aim to recover all nodes, when the sizes and number of nodes are considered simultaneously, a negative nodal status based on only seven nodes can be reliable

only the formalin fixation parameters were improved.²⁹ These figures demonstrate that the adequacy of staging varies with the methods applied. It appears very probable that, after a certain limit, increasing the number of lymph nodes recovered for histopathological assessment is unlikely to improve the staging significantly. The assessment of the lymph nodes beneath the tumour is probably more important in some instances^{19, 29}; increasing the nodal yield with chemical agents should be restricted to problematical cases in which very few nodes can be detected macroscopically.^{11, 32, 33} It should also be noted that some specimens will fail to have an acceptable number of lymph nodes. The detection of lymph nodes by palpation seems sufficiently reliable if adequate fixation is allowed and sufficient care is taken during the cut up,^{29, 30} and this is very likely to reveal node positive CRCs in most cases, because involved nodes are generally larger than uninvolved ones. Our data demonstrate that the inclusion of the seven largest nodes could adequately stage up to 98% of all, and 97% of node positive CRCs. Increasing the number of nodes for histopathological assessment would perhaps improve the accuracy of staging slightly, but this potential improvement must be balanced against the available resources. The hypothesis that the examination of more and more nodes improves staging is true only up to a certain point. The large database on 2427 patients treated at the William Beaumont Hospital (NS Goldstein, unpublished observations and personal communication, 2001) suggests that the retrieval of 24 nodes for each specimen on average (range, 1–139) identifies node positive tumours in 46.3% of the patients with pT3 tumours (53% in our series), and the rate of node positive CRCs increases as the number of lymph nodes examined rises, reaching 74% with 25 or more nodes examined. Our data suggest that PNs are larger than NNs, and this is in keeping with the results of Mönig *et al.*²⁸ PNs are therefore easier to detect by palpation. Following the detection of these nodes, the addition of further nodes will probably not improve the staging accuracy, but can bias any mathematical analysis in which all nodes are pooled together as if they are equivalent.

In summary, ever more data suggest that an adequate, HE based histopathological nodal staging of CRCs is dependent not only on the number of lymph nodes investigated, but also on the qualitative features of the lymph nodes assessed. PNs tend to be larger, but reactive nodes may also be large. In addition, many PNs are < 5 mm, although, considering the mean size of the lymph nodes assessed in this series and other series, this does not seem to be “small” in the context of CRCs. Because, in general, PNs are larger than NNs, they may be easier to detect by palpation. Features such as size and location relative to the tumour are important during the retrieval of lymph nodes from pericolic or perirectal fat and these features must be considered. Lymph nodes are not equivalent and any study neglecting this fact will give grounds for error in the recommendation of a minimum number of nodes for the reli-

able determination of node negative CRCs. Although pathologists should aim to recover as many lymph nodes as possible, when the sizes and number of nodes are considered simultaneously, it seems that even a negative nodal status established on the basis of seven lymph nodes can be reliable; this relatively low number is also supported by survival data.⁸

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ECHO

New marker for pyelonephritis?



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In a study of children with acute pyelonephritis plasma and urine concentrations of soluble adhesins E-selectin (sE-selectin) and soluble intercellular adhesion molecule 1 (sICAM-1) do not predict renal scarring, despite a previous report of raised plasma concentrations in children with kidney damage associated with vesicoureteric reflux. An unexpected finding, though, is that because of the consistent presence of sE-selectin in urine early in infection, it might be a useful marker for acute pyelonephritis.

sE-selectin concentration in plasma and urine was higher in samples taken up to a week after onset of pyelonephritis (acute samples) than in later samples or healthy controls or controls with fever unrelated to urinary infection. sICAM-1 concentration in plasma was higher in the acute and later samples than in both controls whereas in urine it was significantly higher only in acute samples compared with controls with unrelated febrile illness. Although abnormalities of renal parenchyma were evident on DMSA imaging in three quarters of children within one week after presentation, persisting in a few up to six weeks, they did not correlate with the plasma and urinary sE-selectin and sICAM-1 profiles.

The study compared 40 children with a first episode of acute pyelonephritis, who had plasma and urine samples taken one week and six weeks after presentation, with 21 healthy controls and—to check for specificity—a further 18 controls with a fever but no urinary infection, all matched for age and sex

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