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ORIGINAL ARTICLE

Potential pathological understaging of pT3 rectal cancer with less than 26 lymph nodes recovered: a prospective study based on a resampling of 50 rectal specimens

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Abstract The aim of the paper was to establish if the 12 lymph nodes recommended by tumor–node–metastasis (TNM) system are sufficient for a correct staging of rectal cancer. For this purpose, we first compared the mean number of lymph nodes recovered in the same surgical specimen at the routine sampling and at a resampling performed by a second expert gastrointestinal pathologist. The study was performed on 50 cases of pT2N0 and pT3N0 rectal cancers, with a minimum number of 12 lymph nodes recovered at first sampling, histologically negative for metastases. Resampling retrieved a variable number (1 to 24) of nodes missed at first sampling. The final pN0 status was maintained in pT2 patients, whereas in 18.7% of pT3 patients, metastatic lymph nodes were detected if the mean number of lymph nodes increased from 17.8 to 26.8 after the second sampling. Interestingly, all pN1 patients had only a single metastatic lymph node measuring less than 4.9 mm. As we have shown that most (five out of six) missed metastatic lymph nodes were detected in specimens in which a maximum number of 19 lymph nodes had been originally recovered, we strongly suggest a resampling of pT3N0 rectal specimens if less than 20 lymph nodes have been recovered.

Keywords Rectal cancer · Lymph node yield · Perirectal tissue sampling · Quality standard

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Introduction

An accurate histological lymph node evaluation is crucial to ensure correct prognostic information and therapeutic strategies for patients with colorectal cancer [4, 23]. Actually, lymph node metastasis in colorectal cancer is the main criterion adopted by oncologist to recommend for adjuvant therapy [7]. Unfortunately, the routine manual dissection of lymph nodes from the pericolonic/perirectal adipose tissue is a laborious process, as most lymph nodes, being small in size, can be easily missed with the risk of understaging patients. This is supported by the evidence that lymph node metastases can occur in nodes less than 5 mm [1, 14–16]. In this regard, the major problem is the minimum number of lymph nodes that should be recovered for a reliable staging system that could predict clinical outcome, especially for T3N0 patients [6, 8, 23, 26, 28, 30]. In this group of patients, the prognosis seems to be dependent on the number of lymph nodes examined, being poorer if few nodes have been histologically evaluated [11,

19, 22, 23, 26, 30]. It is likely that T3N0 patients with few (six to nine) lymph nodes recovered should be considered at a high risk for missing positive nodes, and adjuvant therapy can be advised [6, 18, 26]. Over time, several authors have proposed a different number of lymph nodes, ranging from 6 to 17 [5, 9, 12, 13, 21, 24], but the tumor–node–metastasis (TNM) system recommends that at least 12 lymph nodes is a sufficient number for an accurate identification of regional metastatic disease [25].

The aim of the present paper was to establish if the 12 lymph nodes recommended by TNM is a sufficient number for a correct staging system of rectal cancer. Previous studies about the minimum number of lymph nodes to be recovered in colorectal cancer have been performed using retrospective analysis and statistical correlation between the number of lymph nodes detected for each specimen and the number of metastases discovered [1, 5, 9, 10, 20, 27]. Conversely, we first planned a prospective study that compared the mean number of lymph nodes recovered in the same surgical specimen at two different moments: the routine sampling and the resampling performed by a second pathologist with expertise in gastrointestinal pathology. The study was performed on 50 cases of patients with pT2N0 and pT3N0 rectal cancer, with a minimum number of 12 lymph nodes recovered at first sampling, histologically proven to be negative for metastases. A correlation was made between the total mean number of lymph nodes recovered, the number of metastases disclosed, and the original pTN stage. The present study first showed that 18.7% of pT3 patients with rectal cancer had been understaged if less than 26 lymph nodes had been recovered.

Materials and methods

Between January 2001 and December 2005, two surgical pathologists of different institutions (Istituto Nazionale per lo Studio e la Cura dei Tumori of Milan and San Luigi–Santi Currò Hospital of Catania), with expertise in gastrointestinal pathology (S.A. and P.G.), performed a resampling of the perirectal adipose tissue from 50 cases of pT2 and pT3 rectal adenocarcinomas (mid and lower one third of the rectum) to ascertain if some lymph nodes had been previously missed at first sampling. The cases to be resampled were selected on the basis of the following strict criteria:

(1) All patients underwent rectal resection with total mesorectal excision.
(2) The sampling of surgical specimens was performed according to the guidelines for rectal cancer, including the examination of the distal resection margin and the circumferential resection margin of the mesorectum, the latter by sampling a 1-mm-thick slice of adipose tissue from the whole surface of this margin [2].
(3) Only tumors with a minimum number of 12 lymph nodes recovered routinely by manual dissection and later proven to be histologically negative for metastasis were included in the study.

In practice, surgical specimens are sent unfixed to a pathologist, opened longitudinally, straightened without stretching, and pinned to a corkboard. All surgical samples were fixed in 10% buffered formalin for 24 h. Lymph nodes were isolated by manual dissection without fat-clearing technique, as it has been shown to be a reliable and

Table 1 Comparison between the number of lymph nodes recovered, metastasis detection, and pN-staging before and after the second sampling in patients with pT2 rectal cancer

Case number	1° Sampling Met./LNs	pTN	2° Sampling Met./LNs	Met./total LNs	Final stage
1	0/16	pT2 N0	0/8	0/24	pT2 N0
2	0/33	pT2 N0	0/6	0/39	pT2 N0
3	0/12	pT2 N0	0/5	0/17	pT2 N0
4	0/13	pT2 N0	0/14	0/27	pT2 N0
5	0/15	pT2 N0	0/8	0/23	pT2 N0
6	0/20	pT2 N0	0/10	0/30	pT2 N0
7	0/12	pT2 N0	0/4	0/16	pT2 N0
8	0/25	pT2 N0	0/3	0/28	pT2 N0
9	0/19	pT2 N0	0/14	0/33	pT2 N0
10	0/17	pT2 N0	0/10	0/27	pT2 N0
11	0/17	pT2 N0	0/8	0/25	pT2 N0
12	0/13	pT2 N0	0/5	0/18	pT2 N0
13	0/35	pT2 N0	0/1	0/36	pT2 N0
14	0/17	pT2 N0	0/1	0/18	pT2 N0
15	0/32	pT2 N0	0/3	0/35	pT2 N0
16	0/23	pT2 N0	0/4	0/27	pT2 N0
17	0/14	pT2 N0	0/7	0/21	pT2 N0
18	0/12	pT2 N0	0/10	0/22	pT2 N0

Met. Number of metastatic lymph nodes, *LNs* number of lymph nodes recovered

reproducible technique [1, 3, 20]. After the routinary sampling by one of the pathologists of the two different anatomical pathology sections, the residual perirectal adipose tissue of each case in which at least 12 lymph nodes had been recovered was maintained in formalin. Histological examination of lymph nodes was routinely conducted without performing serial sections. Only the cases histologically proven to be negative for lymph node metastases were selected, and the corresponding residual perirectal adipose tissue was retrieved from formalin and resampled by the two different pathologists (one for each institution) with expertise in gastrointestinal pathology. Definitive histological diagnosis was delayed for 24–36 h and rendered after performing the second sampling. The pathologic stage of the entire series (first + second sampling) was defined according to the TNM system [25]. Distal and circumferential margins were tumor-free in all cases selected. All patients are still in follow-up.

Results

The routinely pathologic sampling of the 50 surgical specimens selected revealed that 18 tumors were pT2N0, and the remaining 32 tumors were pT3N0 (Tables 1 and 2). The median number of lymph nodes from the first routine sampling was 19.1 (range 12–35) and 17.8 (range 12–33) in the pT2N0 and pT3N0 group, respectively (Table 3).

In all cases, the pathologist performing the second manual sampling identified new lymph nodes that had been previously missed, with a number varying from 1 to 24 lymph nodes (Tables 1 and 2). The maximum number of lymph nodes missed in a single surgical specimen at the first sampling was 14 in the T2 group and 24 in the T3 group (Tables 1 and 2). The total (first + second sampling) mean number of lymph nodes recovered was 25.8 (range 16–39) for the T2 group and 26.8 (range 13–39) for the T3 group (Table 3).

Table 2 Comparison between the number of lymph nodes recovered, metastasis detection, and pN-staging before and after the second sampling in patients with pT3 rectal cancer

Case number	1° Sampling Met./LNs	pTN	2° Sampling Met./LNs	Met./total LNs	Final stage	∅ Metastatic lymph nodes (mm)	∅ Metastases
1	0/13	pT3 N0	1/21	1/34	pT3 N1	4.6	3 mm
2	0/14	pT3 N0	1/11	1/25	pT3 N1	4.9	3.2 mm
3	0/18	pT3 N0	1/5	1/23	pT3 N1	3.4	2.5 mm
4	0/19	pT3 N0	1/10	1/29	pT3 N1	4.8	2.7 mm
5	0/25	pT3 N0	1/11	1/36	pT3 N1	2.4	2.4 mm
6	0/12	pT3 N0	1/8	1/20	pT3 N1	2.4	1.8 mm
7	0/13	pT3 N0	0/12	0/25	pT3 N0	–	
8	0/12	pT3 N0	0/14	0/26	pT3 N0	–	
9	0/12	pT3 N0	0/1	0/13	pT3 N0	–	
10	0/19	pT3 N0	0/3	0/22	pT3 N0	–	
11	0/22	pT3 N0	0/15	0/37	pT3 N0	–	
12	0/12	pT3 N0	0/5	0/17	pT3 N0	–	
13	0/12	pT3 N0	0/4	0/16	pT3 N0	–	
14	0/12	pT3 N0	0/9	0/21	pT3 N0	–	
15	0/12	pT3 N0	0/9	0/21	pT3 N0	–	
16	0/24	pT3 N0	0/6	0/30	pT3 N0	–	
17	0/21	pT3 N0	0/12	0/33	pT3 N0	–	
18	0/16	pT3 N0	0/22	0/38	pT3 N0	–	
19	0/33	pT3 N0	0/1	0/34	pT3 N0	–	
20	0/22	pT3 N0	0/11	0/33	pT3 N0	–	
21	0/18	pT3 N0	0/21	0/39	pT3 N0	–	
22	0/22	pT3 N0	0/10	0/32	pT3 N0	–	
23	0/20	pT3 N0	0/5	0/25	pT3 N0	–	
24	0/28	pT3 N0	0/11	0/39	pT3 N0	–	
25	0/13	pT3 N0	0/5	0/18	pT3 N0	–	
26	0/30	pT3 N0	0/2	0/32	pT3 N0	–	
27	0/14	pT3 N0	0/24	0/38	pT3 N0	–	
28	0/24	pT3 N0	0/8	0/32	pT3 N0	–	
29	0/12	pT3 N0	0/1	0/13	pT3 N0	–	
30	0/12	pT3 N0	0/2	0/14	pT3 N0	–	
31	0/18	pT3N0	0/5	0/23	pT3N0		
32	0/17	pT3N0	0/4	0/21	pT3N0		

Met. Number of metastatic lymph nodes, *LNs* number of lymph nodes recovered, ∅ maximum diameter

Table 3 Correlation between mean number of lymph nodes of the first and second sampling with pN-stage

PT stage	Mean number of LNs (1° sampling)	pN-Stage	Mean number of LNs (1°+2° sampling)	Final pN-stage
T2 (18 pts)	19.1 (range 12–35)	N0	25.8 (range 16–39)	N0 (18 pts)
T3 (32 pts)	17.8 (range 12–33)	N0	26.8 (range 13–39)	N0 (26 pts) N1 (6 pts)

pts Number of patients, *LNs* number of lymph nodes recovered

No metastasis was found in the lymph nodes recovered in the second sampling of patients with pT2 stage (Tables 1 and 3), whereas lymph node metastasis was detected in 6 out of 32 (18.7%) patients with pT3 stage (Tables 2 and 3). Accordingly, the overall final pN stage remained unchanged in 88% of patients and, more specifically, in 100% of pT2 and 81.3% of pT3 patients (Tables 1, 2 and 3). In the pT3N1 patients, the second sampling increased the mean number of lymph nodes recovered from 16.6 (range 12–25) to 27.8 (range 20–36; Table 4). Notably, only one metastatic lymph node was detected for each single case of this subgroup (Table 2). The maximum diameter of the single metastatic lymph node, measured on the histological slide by micrometer ocular, was 4.9 mm (2.4 to 4.9 mm; Table 2).

Residual lymphatic tissue was identified in five out of six metastatic cases. Metastasis size, measured on the histological slide by micrometer ocular, ranged from 1.8 to 3.2 mm (Table 2). Only in one case was a small (2.4 mm) round-shaped neoplastic nodule in the perirectal tissue interpreted as lymph node metastasis according to the TNM system [25].

It was noteworthy that most of the cases (five out of six) with missed metastatic lymph nodes were detected in specimens in which a maximum number of 19 lymph nodes had been previously recovered at the first sampling (Table 2).

Discussion

It is widely known that a meticulous search of lymph nodes in the perirectal adipose tissue is crucial for a correct staging system. In all cases of the present series of rectal adenocarcinomas, we first showed that, even if at least 12 lymph nodes are identified during the first routine sampling, a variable number of lymph nodes, ranging from 1 to 24, are missed. Although this finding seems to be alarming, we point out that a correct overall pN stage, including pT2 and pT3 patients, was achieved in a high percentage of cases (88%) at the first routine manual dissection. All pT2 patients maintained their pN0 stage despite the mean number of lymph nodes recovered increased from 19.1 (first sampling) to 25.8 (second sampling). This could be explained by a lower metastatic biological potential for pT2 colorectal cancers, indicating that these tumors can be correctly staged with 12 lymph nodes recovered.

In regard to pT3 patients, we showed that there is an 18.7% chance of identifying metastatic lymph nodes if the mean number of lymph nodes recovered in the same surgical specimens increased from 17.8 at the first sampling to 26.8 after the second sampling. The mean number 26.8 of lymph nodes in our study is near to that obtained by Leibl et al. [20] who found that 80 and 90% of specimens with lymph node metastases had 26 (or fewer) and 30 (or fewer) lymph nodes recovered, respectively. Notably, the above-mentioned study of Leibl et al. and our present findings support the validity of Goldstein's [10] hypothesis who, performing statistical analyses in a large series of colorectal cancers, suggested that the predictive probability of identifying the single lymph node metastasis in a theoretical surgical specimen of colorectal cancer with a single lymph node metastasis increases up to 80% if about 30 lymph nodes are recovered.

In the present study, metastases occurred in small-sized (<5 mm) lymph nodes, and interestingly, all patients had only a single metastatic lymph node. These findings raise the controversial issue about the minimum number of lymph nodes to be recovered for each specimen. In agreement with other authors, we believe that an ideal minimum number of lymph nodes to be recovered does not exist [8, 10], likely being only a statistical concept that cannot be applied for each single patient. In fact, the number of lymph nodes can greatly vary according to individual biological variation (tumor stage and associated inflammatory response), quality of surgical excision, and the pathologist performing the sampling of peri(colon)-rectal adipose tissue at different pathologic institutions [17, 20, 22, 27–29]. The latter seems to be crucial, as the present study showed that a resampling of perirectal adipose tissue by a second pathologist with expertise in gastrointestinal pathology has retrieved meta-

Table 4 Correlation between pN-stage and mean number of lymph nodes in understaged pT3 patients

No. of patients	Mean number of LNS (1° sampling)	pN-stage	Mean number of LNS (1°+2° sampling)	Final stage
6	16.6 (range 12–25)	N0	27.8 (range 20–36)	N1

No. Number, *LNs* number of lymph nodes recovered

static lymph nodes that had been missed at the first sampling in 18.7% of pT3 patients. However, we admit that the manual isolation of lymph nodes from the perirectal adipose tissue is objectively difficult, despite the pathologist's diligence, as many lymph nodes are small in size (<5 mm) and can be easily missed or crushed during sampling. This is further supported by one case of our series in which the single metastatic lymph node of 2.4 mm in maximum diameter was missed at first sampling, despite 25 lymph nodes had been recovered. Accordingly, we suggest that pathologists should handle with care the perirectal adipose tissue during manual lymph node dissection even if they had been able to detect a number of lymph nodes larger than recommended (12 lymph nodes) by the TNM system. Practically, as we have shown that most missed metastatic lymph nodes were detected in specimens in which a maximum number of 19 lymph nodes had been originally recovered, we strongly suggest a resampling of pT3NO specimens if less than 20 lymph nodes have been recovered.

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