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ORIGINAL REPORT

# Isolated Tumor Cells in Regional Lymph Nodes as Relapse Predictors in Stage I and II Colorectal Cancer

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## ABSTRACT

### Purpose
Lymph node (LN) involvement is the most important prognostic factor in colorectal cancer (CRC), and pN-positive status identifies patients who require adjuvant chemotherapy. Approximately 15% to 20% of patients without nodal metastases (pN0) develop recurrent disease. In this study, we tested the prognostic significance of isolated tumor cells (ITCs) in LNs of patients with pN0 CRC (stages I and II).

### Patients and Methods
ITCs in LNs regional to CRC were assessed in 312 consecutive patients with pN0 CRC who were followed up clinically and/or endoscopically for at least 6 months after surgery (mean, 67 months; median, 64 months; range, 8 to 102 months). LNs were dissected from gross surgical specimens according to a standardized protocol (with a mean of 17 LNs per patient; range, five to 107 LNs). In all, 5,313 pN0 LNs were collected and assessed by using cytokeratin immunostaining in two serial histology sections from each LN, which amounting to a total of 10,626 specimens. The correlation between ITC status and cancer recurrence was tested by using univariate and multivariate statistics.

### Results
ITCs were documented in 185 of 312 patients (59%). CRC relapsed in 31 of 312 patients (10%), and 25 of 31 recurrences (81%) were documented among ITC-positive patients. CRC recurrence rates among ITC-positive and ITC-negative patients were 14% (25 of 185 patients) and 4.7% (six of 127 patients), respectively. In both univariate and multivariate analyses, ITC status was the only variable significantly associated with cancer relapse (Cox model; hazard ratio, 3.00; 95% CI, 1.23 to 7.32; $P = .013$).

### Conclusion
In patients with pN0 CRC, cancer relapse was significantly associated with ITCs in regional LNs. ITCs should be considered among the clinicobiologic variables that identify high-risk patients who can benefit from adjuvant chemotherapy.

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## INTRODUCTION

In colorectal cancer (CRC) with no extranodal metastases, metastatic regional lymph nodes (LNs) distinguish adenocarcinomas in pathologic (p) TNM (pTNM) stages I and II (ie, pN0) from those in stage III (ie, pN1/2) and identify patients eligible for adjuvant chemotherapy.[1,2]

Within 5 years of surgery, up to 20% of p stage I and II patients develop extranodal metastases.[3] The early identification of such a high-risk subgroup at the time of surgery would enable adjuvant therapeutic strategies to be implemented, potentially lowering the cancer recurrence rate.

It has been suggested that recurrences in patients with p stage I and II disease may result from p understaging of the tumor.[4,5] On the basis of this assumption, current guidelines require that no less than 12 LNs be histologically evaluated.[1,6]

There are three main situations in the spectrum of LN colonization as follows: metastases, (ie, metastatic implants larger than 0.2 cm), micrometastases (ie, metastases that range between 0.02and 0.2 cm in largest diameter), and isolated tumor cells (ITCs; ie, single or small nests of distinct tumor cells that are never larger than 0.02 cm and are detectable only by immunohistochemistry [IHC] or molecular biology).[1,7]

The variable prevalence of ITCs in LNs reported in the literature supports the claim that current histologic criteria are bewildering and/or inconsistently applied,[8-20] and consequently, the

influence of ITCs on prognosis has not been unequivocally established. Although some studies found that ITCs can adversely affect outcomes,[21-32] other studies were unable to identify such a correlation.[8-13,15,33-46]

In patients with CRCs, the clinical impact of ITCs in LNs on the outcomes of patients is difficult to determine because the interobserver agreement (when it is tested) in detecting ITCs by using IHC is inconsistent,[47-49] available studies were based on small (mostly retrospective) series of patients (ranging from 19 to 174 patients) and/or adopted nonstandard methods,[4,5,8,9-17,19,21-26,30,31,33-40,42,43,46,50] and LN micrometastases and ITCs were considered together.[8,9,13-15,18,27-31,46,51]

This long-term follow-up study focused on the prevalence and prognostic impact of ITCs in regional LNs obtained from 312 consecutive patients with p stages I and II CRC.

## PATIENTS AND METHODS

### Patients at Enrollment

Between October 2003 and August 2005, 767 patients underwent radical surgical treatment for CRC (all stages) at the Padova University School of Medicine and Teaching Hospital. At the time of the surgical treatment, informed consent was obtained from all the patients involved. The surgical procedure was standardized according to the location of the cancer, minimizing any variability in the lymphadenectomy technique. p cancer staging (pTNM) was based on the seventh edition of the TNM classification.[1]

Of the original series of 767 prospectively enrolled patients, only the 312 (43.5%) consecutive patients with pTNM stages I or II (a-b-c) were considered in this retrospective study. None of these patients were given neoadjuvant therapy. No LN metastases or micrometastases were detected in any of the patients by conventional histology (hematoxylin and eosin staining). The study population included 177 men (57%) with a mean age of 69 years (range, 34 to 91 years) and 135 women (43%) with a mean age of 69 years (range, 38 to 90 years); demographic and clinicopathologic data of patients are given in Table 1.

### Follow-Up of Patients

All patients were followed up every 6 months for the first 2 years after their surgical treatment and every 12 months from the third to the fifth year thereafter. The follow-up included a physical examination and carcinoembryonic antigen assay, colonoscopy, and computed tomography. The mean follow-up was 67 months (median, 64 months; range, 8 to 102 months). None of the patients considered were given adjuvant therapy (before any cancer relapse). The disease-free interval was calculated as the time in months that elapsed between surgery and recurrence or the latest follow-up (according to the protocol).

### Pathology

For the handling of gross surgical specimens and LN collection, all gross surgical specimens were fixed in 5% to 10% formalin (for 18 to 24 hours). Regional LNs were defined as established by the seventh edition of the TNM classification.[1]

For all patients considered, at least three cancer samples were obtained (range, three to eight samples, depending on the size of the cancer); the peri-intestinal fat was dissected from the intestinal wall, distinguishing between fatty tissue more or less than 3 cm from the neoplasia. The fatty tissue was sliced into gross sections (0.1 to 0.15 cm thick). Any LNs encountered by serially slicing the peri-intestinal tissue were collected, and regional LNs were divided into two groups (ie, LNs found $< 3$ or $> 3$ cm from the cancer).

### Histology and IHC

Cancer samples and LNs were embedded in paraffin and histologic sections (0.005 mm thick) were obtained and stained with hematoxylin and eosin.

The primary tumor histology (ie, histotype and grade of cancer differentiation) was consistent with internationally established criteria.[52] Any cancer necrosis (absent [$\leq$ 10% of the neoplastic histology sample] *v* present [ 10% of the neoplastic histology sample]), vascular intramural invasion (present *v* absent), and perineural invasion (present *v* absent) were also recorded (Table 1).

Two additional histologic sections, 0.075 to 0.1 mm apart, were obtained from each of the 5,313 LNs (which gave rise to 10,626 sections in all). The MNF116 anticytokeratin antibody (clone MNF116 mouse monoclonal-AB; working dilution 1:100; Dako, Copenhagen, Denmark), which has a well-established sensitivity/specificity in detecting epithelial cells, was used for the ITC immunohistochemical assessment.[53-55]

All IHC reactions (including both negative [normal tonsil] and positive [CRC specimen] controls) were obtained automatically by using a standardized protocol implemented on the Ventana BenchMark IT immunostainer (Ventana Medical Systems, Milan, Italy) under the supervision of specialists (C. Lanza and V. Lazzarin).

ITCs were defined as phenotypically malignant, unequivocally MNF116-positive, single cells dispersed in sinusoidal/extrasinusoidal spaces.[1,56-58] Clusters of ITCs never exceeded 0.02 cm in the widest diameter, and they never showed signs of metastatic activity (ie, proliferation or stromal reaction).[1] Because occasional weak cytokeratin staining has been reported in interfollicular stellate cells, only phenotypically malignant cells were considered ITCs (Fig 1). Two pathologists (C.M. and L.A.) with no knowledge of the cancer's pTNM stage or clinical outcome examined all immunostained sections. Instances when they disagreed were reconsidered jointly by involving a third pathologist (M.R.).

### Statistical Analysis

The statistical analysis was performed by using Pearson's $\chi^2$ analysis for a $2 \times 2$ and $2 \times n$ contingency tables. A logistic regression model was used for binary outcomes, as appropriate. Odds ratios (ORs) and 95% CIs were also calculated from the logistic model and directly from the $2 \times 2$ tables with exact

**Table 1.** p Stage, Location, and Histology of 312 Consecutive pN0 CRCs

| p Stage | No. of Patients | % | No. of Men | No. of Women | Age (years) Mean | Age (years) Range | No. of Cancer Sites: Ascending Colon | Transverse Colon | Descending Colon | Rectum | Histology (No. of Adenocarcinomas): Low-Grade G1 | G2 | Mucinous Cancers | Tumor Necrosis (%): Absent | Tumor Necrosis (%): Present | Intramural Vascular Invasion (%) | Perineural Invasion (%) | Total LNs (No.) | No. of LNs per Case (mean) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 129 | 41 | 75 | 54 | 70 | 38-87 | 30 | 5 | 57 | 35 | 117 | 6 | 6 | 61 | 39 | 21 | 2 | 1,853 | 14 |
| IIA | 170 | 55 | 96 | 74 | 68 | 34-91 | 59 | 18 | 60 | 31 | 140 | 16 | 14 | 26 | 74 | 39 | 10 | 3,233 | 19 |
| IIB | 9 | 3 | 4 | 5 | 73 | 49-89 | 6 | 1 | 2 | 0 | 8 | 0 | 1 | 2 | 80 | 89 | 30 | 153 | 17 |
| IIC | 4 | 1 | 2 | 2 | 70 | 53-83 | 3 | 0 | 0 | 1 | 1 | 0 | 3 | 0 | 100 | 25 | 0 | 74 | 18.5 |
| Total | 312 | | 177 | 135 | 69 | 34-91 | 98 | 24 | 119 | 67 | 266 | 22 | 24 | 40 | 60 | 33 | 8 | 5,313 | 17 |

NOTE. The prevalence of intramural vascular invasion is also listed. In four patients, the cancer site was not known (two patients had p stage I CRC, and 2 patients had p stage II CRC).
Abbreviations: CRC, colorectal cancer; LN, lymph node; p, pathologic.

  

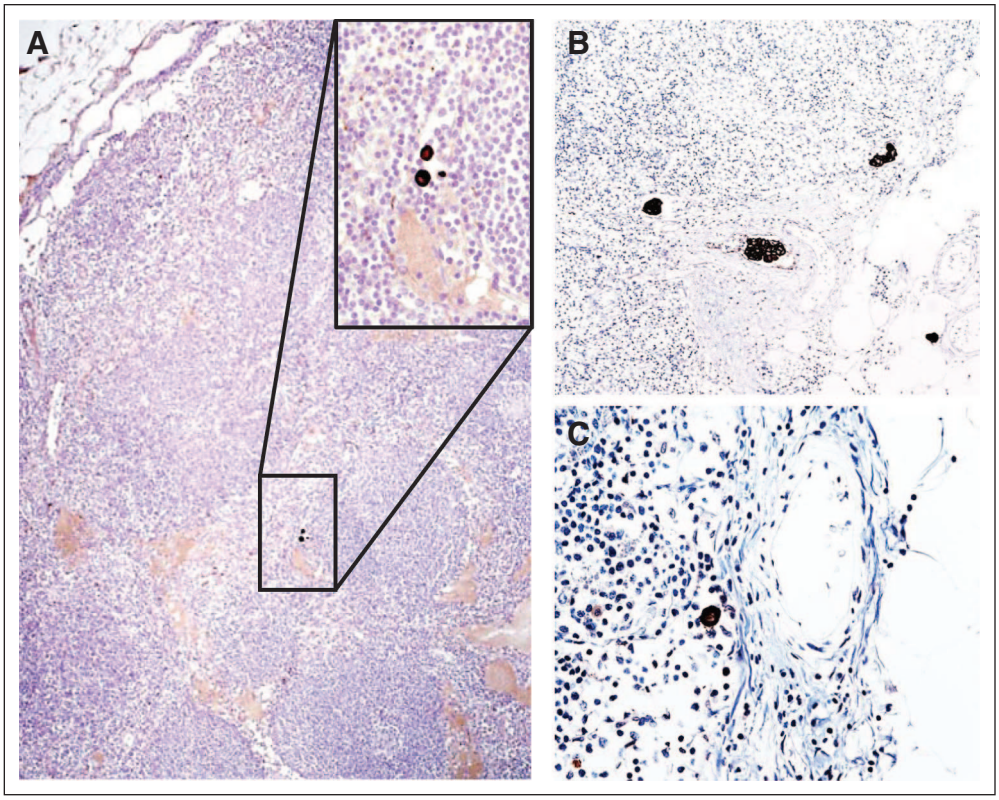


**Fig 1.** (A to C) Three different cases representative of isolated tumor cells in a node regional to colorectal cancer. The immunohistochemical stain (MNF116 monoclonal antibody) showed neoplastic epithelia located in the (A) medullary area or (B and C) within the marginal sinus of the LNs. In all photographs, ITCs (stained brown) consisted of isolated cells or small clusters of epithelia that never exceeded 10 to 20 cells (original magnification: A, ×20; B, ×50; C, ×75).

*P* values. Where required, the *z* test for proportions, the CI method for differences between means, and the *t* test (two sided) were applied.

The Kaplan-Meier product-limit method with the Mantel-Cox and Breslow tests and Cox model were used to perform the survival analysis.[59] A function on the basis of the variance inflation factor was used to check for the collinearity of variables. The stepwise backward elimination approach was applied to derive the final model. $P < 0.05$ was considered significant. The *k* coefficient for pairs of observers was interpreted according to benchmarks of Landis and Koch.[60] STATA software (Statistics Data Analysis, release 8.1, http//stata.com; STATA, College Station, TX; Computing Resource Center, Santa Monica, CA) was used for all calculations.

## RESULTS

The pathology profile of the primary cancer (pTNM stage, cancer location, histotype, and necrosis, intramural vascular invasion, and perineural invasion) is shown in Table 1. Vascular invasion (in blood and/or lymphatics) was demonstrated histologically in 103 of 312 patients (33%). The prevalence of vascular invasion was significantly higher when the higher the pT of the cancer (Pearson's $\chi^2$, $P < .001$; OR, 2.81; 95% CI, 1.71 to4.61; $P < .001$) and p stage (Pearson's $\chi^2$, $P < .001$; OR, 2.84; 95% CI, 1.72 to 4.69; $P < .001$; Table 1).

A total of 5,313 LNs, which ranged from 0.1 to 2.6 cm in the widest diameter, were harvested from 312 patients. Irrespective of the cancer stage or site, a mean of 17 LNs were obtained per patient (standard deviation, 11.42 LNs; median, 15 LNs; range, five to 107 LNs). No differences emerged in the number of LNs obtained from the gross surgical specimens collected at the different surgery units involved in the study (Pearson's $\chi^2$, $P = .8$).

### *ITC Status on Presentation*

Overall, ITCs were documented immunohistochemically in 185 of 312 patients (59%). The prevalence of ITCs was 61% (149 of 244 patients) and 53% (36 of 68 patients) for colon and rectal cancer, respectively (two-sample test of proportions, $P = .22$). The interobserver agreement in distinguishing ITC-positive from ITC-negative LNs was tested in a randomly selected population of 75 consecutive patients (1,237 LNs in all) by two pathologists (C.M. and L.A.); 1,186 of the 1,237 LNs tested (96%) were consistently judged to be ITC-positive (188 LNs) or ITC-negative (998 LNs) LNs, and the interobserver consistency was ranked excellent (*k* coefficient = 0.86; 95% CI, $0.785 < \kappa < 0.927$).[60]

ITC status by cancer pT value and p stage are listed in Table 2. ITCs were documented in six of 38 CRCs classified as pT1 (16%), 47 of 91 CRCs classified as pT2 (52%), 121 of 170 CRCs classified as pT3 (71%), nine of nine CRCs classified as pT4a (100%), and two of four CRCs classified as pT4b (50%). A significant correlation was detected between ITC status and pT value after collapsing the stages into patients with pT1/pT2 CRCs versus patients with pT3/pT4 CRCs (Pearson's $\chi^2$, $P < .001$; OR, 3.71; 95% CI, 2.24 to 6.15).

ITC-positive status correlated significantly with cancer necrosis (absent *v* present; Pearson's $\chi^2$, $P = .001$), perineural invasion (absent *v* present; Pearson's $\chi^2$, $P = .004$), and the number of LNs harvested (mean number of LNs in ITC-negative patients of 14.5 *v* mean number of LNs in ITC-positive patients of 18; *t* test, $P < .001$). ITCs were identified in 53 of 129 patients (41%) in p stage I and 132 of 183 patients (72%) in p stage II (a + b + c); a significant association emerged between p stage and ITC status (Pearson's $\chi^2$, $P < .001$; OR, 3.61; 95% CI, 2.12 to 5.82). No significant association came to light

**Table 2.** Prevalence of ITC-Positive LNs in 312 Patients With pN0 CRC

| | | LNs | | | | | | | | | | Cancer Relapse | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cancer Stage | pT Value | No. of ITC-Positive Patients | % | Total LNs Considered (No.) | No. of LNs per Patient (mean) | No. of ITC-Positive LNs < 3 cm | % | No. of ITC-Positive LNs > 3 cm | % | Total ITC-Positive LNs (No.) | % | Among ITC-Positive Patients | % | Among ITC-Negative Patients | % |
| I | 1 | 6 of 38 | 16 | 495 | 13 | 8 of 495 | 1.6 | 1 of 495 | 0.2 | 9 of 495 | 1.8 | 0 of 6 | 0 | 0 of 32 | 0 |
| | 2 | 47 of 91 | 52 | 1,358 | 15 | 125 of 1,358 | 9 | 26 of 1,358 | 2 | 151 of 1,358 | 11 | 5 of 47 | 11 | 3 of 44 | 7 |
| IIA | 3 | 121 of 170 | 71 | 3,233 | 19 | 469 of 3,233 | 14 | 65 of 3,233 | 2 | 534 of 3,233 | 16 | 20 of 121 | 16 | 3 of 49 | 6 |
| IIB | 4a | 9 of 9 | 100 | 153 | 17 | 24 of 153 | 16 | 2 of 153 | 1 | 26 of 153 | 17 | 0 of 9 | 0 | 0 of 0 | 0 |
| IIC | 4b | 2 of 4 | 50 | 74 | 18.5 | 2 of 74 | 3 | 2 of 74 | 3 | 4 of 74 | 6 | 0 of 2 | 0 | 0 of 2 | 0 |
| Total | | 185 of 312 | 59 | 5,313 | 17 | 628 of 5,313 | 12 | 96 of 5,313 | 1.8 | 724 of 5,313 | 14 | 25 of 185 | 13 | 6 of 127 | 5 |

NOTE. Also shown are the total number of LNs examined in the whole series (by stage) and the number of LNs in which ITCs were detected immunohistochemically. For each stage and pT value, LNs were also distinguished according to their distance from the neoplasia. The number of relapsing CRCs is shown by ITC status.

Abbreviations: CRC, colorectal cancer; ITC, isolated tumor cell; LN, lymph node.

between ITC status and the sex or age, cancer site, histotype, tumor grade, or vascular invasion(as assessed at the primary tumor site) of patients. In all, 5,313 LNs obtained from the 312 patients were considered, and cytokeratin-positive cells were detected in one or both sections obtained from 726 LNs (14%).

Among the 185 ITC-positive patients, 128 patients (69%) revealed ITCs only in LNs within 3 cm of the neoplasia, and eight patients (4%) only had ITCs in LNs more than 3 cm away from the primary tumor. In 48 patients (26%), MNF116-positive cells were detected in nodes both more and less than 3 cm away from the cancer.

To test the consistency of ITC detection in the same LN, two serial histologic sections were considered from each of the 5,313 LNs (ie, 10,626 histology sections in all). At the individual patient level, the following situations emerged: (1) in 127 of 312 patients (41%), neither of the two sections obtained from the same LN revealed MNF116-positive cells (ie, ITC-negative status); and (2) 185 of 312 patients (59%) were recorded with ITC-positive status as follows: in 73 of 185 patients (39%), both sections from the same LN showed MNF116-positive cells (ie, concordant ITC-positive status), in 112 of 185 patients (61%), MNF116-positive cells were found in only one of the two sections (ie, discordant ITC-positive status).

Therefore, ITC status was concordant in the two histologic sections in 200 of 312 patients (64%), 73 of whom had ITC-positive status and 127 of whom had ITC-negative status. At the single LN level (ie, looking at the consistency of the ITC status found in the two histology sections considered), a concordant ITC picture emerged in 5,069 or 5,313 LNs (95%; ie, 4,590 concordant ITC-negative LN sections and 479 concordant ITC-positive LN sections).

### *Long-Term Follow-Up*

CRC recurred in 31 of 312 (10%) patients, 25 patients of whom were ITC-positive patients (81%). Cancer recurred locally in 12 patients and at distant sites in 19 patients.

There were no significant differences in the length of follow-up when patients that relapsed were compared with those that did not (relapsers: mean, 70 months; median, 73 months, range, 35 to 89 months; nonrelapsers: mean, 67 months, median, 63 months, range, 8 to 102 months; difference between means, 3.07 months; 95% CI = −3.94 to 10.08, not significant).

The prevalence of recurrent CRC among ITC-positive and ITC-negative patients was 14% versus 4.7%, respectively. In the univariate analysis (life tables), only ITC status was a significant predictor of cancer recurrence (hazard ratio, 3.00; 95% CI, 1.23 to 7.32; $P = 0.013$; ]Table 3). No correlation emerged for any of the other clinicopathologic variables considered (ie, sex, age, pT, p stage, histotype, cancer grade, cancer necrosis, vascular/perineural invasion, or total number of nodes considered; Table 3).

On the basis of Kaplan-Meier survival estimates, cumulative estimated recurrence curves for ITC-positive versus ITC-negative patients (Mantel-Cox test, $P = .011$; Breslow test, $P = .007$) are shown in Figure 2.

Sex, age, p stage, pT value, histotype, cancer grade, cancer necrosis, vascular invasion, perineural invasion, number of LNs considered, and ITCs were all included in the Cox multivariate analysis to test the relationship between the variables considered and cancer recurrence: the analysis identified only ITC status as being significantly associated with recurrent cancer (Cox model; hazard ratio: 3.00; 95% CI, 1.23 to 7.32; $P = .013$).

**Table 3.** Univariate Analysis: Clinicopathologic Variables *v* Disease-Free Survival

| Variables | Hazard Ratio | 95% CI | *P* |
|---|---|---|---|
| Sex | 0.92 | 0.45 to 1.88 | .83 |
| Age | 0.99 | 0.90 to 1.02 | .42 |
| Stage | 2.01 | 0.90 to 4.50 | .08 |
| pT | 1.42 | 0.91 to 2.21 | .64 |
| Histotype | 0.40 | 0.04 to 3.72 | .42 |
| Cancer grade | 1.27 | 0.70 to 2.33 | .80 |
| Cancer necrosis | 1.45 | 0.96 to 2.19 | .07 |
| Vascular invasion | 1.00 | 0.47 to 2.14 | .98 |
| Perineural invasion | 1.76 | 0.61 to 5.06 | .28 |
| LNs, total No. | 0.97 | 0.99 to 1.01 | .26 |
| ITC status | 3.00 | 1.23 to 7.32 | .013 |

Abbreviations: ITC, isolated tumor cell; LN, lymph node.

  

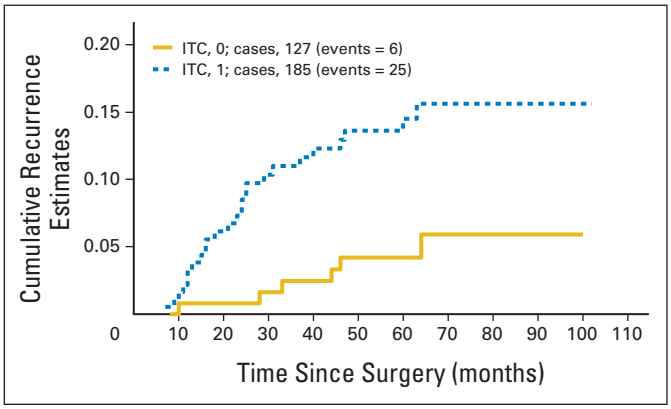


**Fig 2.** Cumulative recurrence estimates showing a significant difference in disease-free interval between the isolated tumor cell (ITC) –positive (ITC, 1) *v* ITC-negative (ITC, 0) groups (Mantel-Cox test, $P = .011$; Breslow test, $P = .007$).

## DISCUSSION

LN involvement is the most important prognostic variable in patients who have undergone radical surgery for CRC, and LN metastases carry a high risk of recurrent disease.[22,31,53,56-58,60,61] On the partial Basis of these grounds, sentinel LN-testing procedures have been recommended, particularly for patients with CRC in whom cancer upstaging would mean that they meet the criteria for adjuvant treatments.[63]

Apart from the expected patient-related variability in the number of regional LNs (eg, different segments of the large bowel, age, and obesity),[4,5,63] differences in surgical procedures (extent of lymphadenectomy) and the handling of surgical specimens (accuracy of LN collection) are considered the main reasons for inconsistencies in the retrieval of LNs after surgery and the subsequent assessment of metastatic nodal disease.[63-65] In this monoinstitutional study, the mean or median number of LNs harvested was consistently higher than that required by the international literature and did not vary between the surgical teams involved.

In 1999, the College of American Pathologists stated that "12 to 15 negative LNs predict for regional node negativity."[6] In 2005, a meta-analysis on the adequacy of pN staging in 116,995 CRCs demonstrated that "most patients with CRC did not receive adequate LN evaluation."[5] In this study, a mean of 17 LNs (median, 15 LNs) were harvested per patient, which made our results consistent concerning node staging and, especially, ITC status. Significantly more LNs were retrieved from the peri-intestinal fat within 3 cm of the neoplasia than further away, irrespective of the cancer site.[5]

Nodal micrometastases can be confidently considered the earliest step in any nodal metastatic implant.[66] Nodal micrometastases differ in biology and dimensions from ITCs in LNs. In terms of their dimensions, only single cells or foci of cytokeratin-positive cells (< 0.02 cm) are defined as ITCs and, on the basis of pTNM, ITC-positive status should be identified as "pN0(i+)."[1] Consistency with such a definition is crucial to any evaluation of the clinical impact of pN0(i+) status.[7] In most of the available literature, the terms ITC and micrometastasis have been considered interchangeable,[8,11,13-15,18,27-31] and the use of other definitions (eg, minimicrometastases) adds to a confusion that is not only semantic.[36] The prevalence and prognostic value of immunohistochemically detected ITCs remain controversial, in part because data in the literature lack uniformity.[8-20] The variability seen in published studies stems from different numbers of histology sections, different numbers of LNs examined, different sensitivities/specificities of the antibodies applied, interobserver variability, and missing patient follow-up data.[17]

This study used IHC to retrospectively assess the long-term prognostic impact of ITC status (in terms of cancer recurrence) in a consecutive series of pN0 CRCs. Patients were prospectively collected according to a standardized preanalysis protocol for gross specimen fixing, node sampling, and cancer histology assessment. In particular, the definition of ITCs recommended by the International Union Against Cancer was strictly applied,[7] which resulting in an excellent *k* statistic for interobserver agreement.[67]

In this series, the prevalence of pN0(i+) patients was 59%, which, to our knowledge, is the highest percentage ever reported in the literature (in trials that involved 100 patients).[8,11,12,21,25,31] IHC testing was performed on two serial sections of each LN to explore the consistency of our ITC findings within the same node; the results entitled us to assume that the findings in one of the two sections were reliable.

As might be expected biologically, and consistent with experience gained from applying sentinel node testing procedures in early CRC, our study documented an elective location of ITCs in LNs closer to the primary cancer (< 3 cm away); this result showed that we should focus mainly on the LN stations closest to the CRC when assessing ITC status.[62,68]

Cox multivariate analysis consistently identified ITC-positive status as the only variable associated with cancer recurrence. The current guidelines of the American Society for Clinical Oncology suggest adjuvant chemotherapy for stage II CRCs with relapse risk factors such as "inadequately sampled nodes, T4 lesions, perforation, or poorly differentiated histology, vascular, lymphatic or neural invasion the discretion of the treating physician."[69] This study documented a high prevalence of ITCs in pN0 CRCs. The significant association between ITC-positive status and pN0 relapsing CRCs showed that pN0(i+) CRCs should be included among the risk factors for cancer recurrence. Our results indicate that ITC-positive patients should be considered among those patients who might benefit from adjuvant treatments.[70]

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Conception and design:** Claudia Mescoli, Laura Albertoni, Valentina M. Russo, Massimo Rugge
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**Administrative support:** Massimo Rugge
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**Manuscript writing:** All authors
**Final approval of manuscript:** All authors

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