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ORIGINAL ARTICLE

Sentinel Lymph Node Biopsy in Colon Cancer: an Institutional Experience

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Abstract
Lymph node staging is a major prognostic factor in colorectal cancer and remains to be the most important criterion for selecting patients for adjuvant therapy. The standard approach for lymph node evaluation is based on manual dissection and histological evaluation of HE-stained slides. For stage III disease (node positive), adjuvant chemotherapy increases the survival rate, while in node-negative stage II disease, in most cases, the chemotherapy is contraindicated due to increased morbidity without real benefit. Up to 30% of patients with node-negative colon cancer staged by standard pathologic techniques ultimately suffer disease recurrence and tumour-related mortality following potentially curative primary resection. Variations in outcome among patients with node-negative early-stage disease may reflect inadequate nodal resection and inaccuracies of pathologic staging. Hence, an accurate pN stage becomes essential. It is seen that classic pathological exam sometimes fails to identify lymph node micrometastases or isolated tumour cells, which might explain local or distant relapses in stage II patients. Sentinel lymph node study has the potential to detect micrometastases and lead to upstaging the disease which is crucial for planning adjuvant therapy and follow-up in these patients. In our study, we carried out SLNB in 40 clinically stage II patients operated for colon cancer. We used peritumoural injection of dye at the time of surgery to detect SLN(s) and analysed them using both microsectioning and immunohistochemical (IHC) staining. Our results show that SLNB can improve the accuracy of pTNM staging.

Keywords Colon cancer · Sentinel lymph node biopsy · Immunohistochemistry (IHC)

Introduction

The presence and extent of regional nodal metastasis predict the outcome in patients with colon cancer and the most prognostic factor for predicting survival in colon cancer is its stage at initial diagnosis.

While surgery alone is considered curative in most cases when the disease is confined within the bowel wall (AJCC stage I/II), the survival decreases significantly by about 25–30%, once the disease spreads beyond the bowel wall draining lymph nodes (AJCC stage III).

Therefore, any diagnostic accuracy of nodal metastasis remains critical for the proper prediction of survival and appropriate therapeutic planning.

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Up to 30% of patients with node-negative colon cancer staged by standard pathologic techniques ultimately suffer disease recurrence and tumour-related mortality following potentially curative primary resection [1].

Standard pathologic evaluation may overlook low-volume nodal metastasis, thereby failing to identify nodes imperative to accurate staging.

Nodal step sectioning may improve staging accuracy; however, this technique cannot be applied to all harvested nodes, as processing time, human resource requirement and cost would be prohibitive.

Directed and detailed examination of a limited number of nodes at the highest likelihood of metastases would be a practical way to enhance staging accuracy.

The recognition that there exists an orderly, sequential and predictable dissemination of epithelial cancer cells from the site of primary disease, through regional lymphatic channels, to the principal or 'sentinel' first draining node(s) can be used to enable focused, detailed pathologic assessment of a few nodes most predictive of the status of

the regional nodal basin without compromising diagnostic accuracy [2].

The aim of the present study included the feasibility of detection of sentinel lymph node(s) in colon cancer and delineate the subset of pN0 (I+), i.e. malignant cells in the regional lymph node(s) no greater than 0.2 mm, detected by H&E or IHC (including isolated tumour cells).

Material and Methods

The study was conducted from October 2014 and concluded in December 2017 in a tertiary care hospital. This study was cleared by the Ethics Committee, and patient consent obtained in all cases.

Patients with stage II (cN0) colon cancer, i.e. with tumours located > 15 cm from the anal verge, were registered in a prospective database and included in the present study. The study was conducted on 40 consecutive operable colon cancer patients who were staged and operated at this hospital.

Inclusion Criteria

Patients with endoscopic and histopathological diagnosis of adenocarcinoma of the colon planned for operation with curative intent.

Exclusion Criteria

Presence of synchronous lesions in the large intestine, malignant or inflammatory in nature, prior colorectal resection, metastatic disease, rectal cancer, advanced disease with the invasion of adjacent structures and distant metastatic disease.

Method of Data Collection

All patients underwent the standard surgical resection with adequate lymphadenectomy.

For tumours located at the splenic flexure and left colon, resection of lymph nodes along the left branch of the middle colic, left colic and first sigmoidal pedicles was done. Lymphadenectomy for sigmoid colon lesions was carried out along the inferior mesenteric artery and the pedicle ligated distal to the left colic artery at the level of the superior haemorrhoidal artery. Routine high ligation of inferior mesenteric artery at the level of aorta was not done in our centre as various meta-analyses have not proven survival benefit [3].

For the right-sided lesions, the right colic and ileocolic vessels were taken at their origins to ensure adequate lymph node harvest.

The steps in the identification of SLN used were as follows:

(i). Isosulfan blue dye was used for lymphatic mapping intraoperatively.
(ii). After laparotomy and mobilisation of the tumour-bearing part of the colon, 1–3 ml of 1% isosulfan blue dye was injected into the subserosal layer immediately adjacent to the tumour in 4 portions. Care was taken not to inject into the lumen of the colon or rectum bowel (Fig. 1a and b).
(iii). The first to fourth blue-staining nodes within 10 min of injection were tagged the sentinel nodes. Subsequent resection of the tumour was performed as a standard radical hemicolectomy (left or right) (Fig. 2a and b)
(iv). After resection of the specimen, the tagged lymph node(s) were excised and separately placed as sentinel lymph nodes (SLN).
(v). Thereafter, as many non-SLN lymph nodes as could be identified were dissected from the specimen (aiming at a minimum of 12 lymph nodes as recommended by the UICC/AJCC).

The non-sentinel nodes were studied conventionally using a single section and haematoxylin–eosin staining.

If tumour deposits were identified in any non-sentinel node(s), the appropriate *N* staging was made and sentinel node(s) examined similarly for validation, but not subjected to any detailed examination.

If tumour deposits were not seen in the node(s) by routine sectioning, study of the sentinel node was then carried out.

If the sentinel node was negative by conventional H&E staining, it was further subjected to evaluation by microsectioning and cytokeratin immunohistochemistry (IHC) searching for occult micrometastasis.

Exhaustive Study of the Sentinel Lymph Node

Two-mm-thick sections were prepared, and a single section was prepared for lymph nodes under 5 mm.

After fixation in 4% buffered formalin for 24 h, six 4-μm sections were prepared. Haematoxylin–eosin and immunohistochemistry (pancytokeratin monoclonal antibody CAM 5.2) staining techniques were sequentially applied so that three sections were studied with each technique.

Interpretation of the Histopathology Findings

According to the AJCC classification, we observed the following definitions:

- *Metastasis* for a deposit greater than 2 mm
- *Micrometastases* for deposits 2 to 0.2 mm

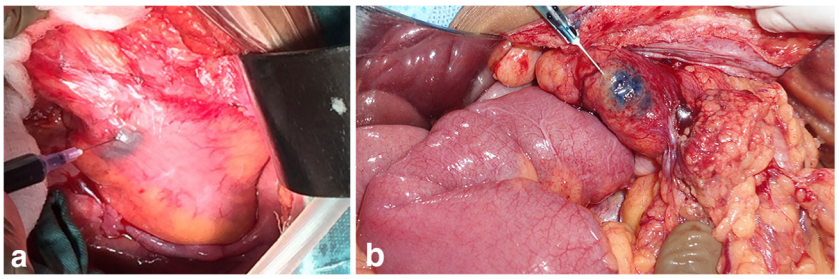


Fig. 1 **a** Peritumoural dye infiltration. **b** Peritumoural dye infiltration

- *Tumour groups of colonies and isolated cells* for deposits equal to or less than 0.2 mm.

The presence of metastases and micrometastases modified staging, as pN1 and pN1mi, was considered, respectively.

Lesions 0.2 mm or smaller did not change staging and were considered pN0 (i+).

The remaining nodes were conventionally studied using single section and haematoxylin–eosin staining.

If the node was negative by conventional H&E staining, it was further subjected to evaluation by microsectioning and cytokeratin immunohistochemistry (IHC) searching for occult micrometastasis.

Calculation formulas used were as follows:

SLN detection rate:

$$\frac{\text{Number of patients with successfully retrieved SLN}}{\text{Number of patients enrolled}} \times 100$$

Upstaging rate (%):

$$\frac{\text{Number of patients positive for malignancy after SLN study}}{\text{Number of patients classified pN0 by routine lymph node study}} \times 100$$

Results

This is a prospective clinical study of 40 cases of colon cancer that underwent open surgery.

The age and sex distribution are shown in Chart 1.

The youngest patient was 33 years old and the oldest was 71 years old.

The highest incidence was noted in the age group of 51–60 years, accounting for 38% of the patients.

The mean age of presentation was 56 years.

The male to female ratio was 5:2.

The most common site of involvement was found to be sigmoid colon ($n = 16$) followed by ascending colon ($n = 9$).

The histopathology of the tumours is shown in Chart 2.

The colectomies were performed by 2 surgical oncologists and all were open procedures.

The median number of nodes harvested was 16 (14–22).

Sentinel Lymph Node Mapping

Sentinel Lymph node mapping was successfully done in 37 of the cases.

The mean number of sentinel lymph nodes being 2 (0–4).

In three cases, the sentinel lymph node could not be mapped even after 20 min of injecting the dye. Two of these cases had splenic flexure tumours. One case pertained to a sigmoid colon growth.

These three cases were not included in the study.

The SLN detection rate:

$$\frac{\text{Number of patients with successfully retrieved SLN}}{\text{Number of patients enrolled}} \times 100$$

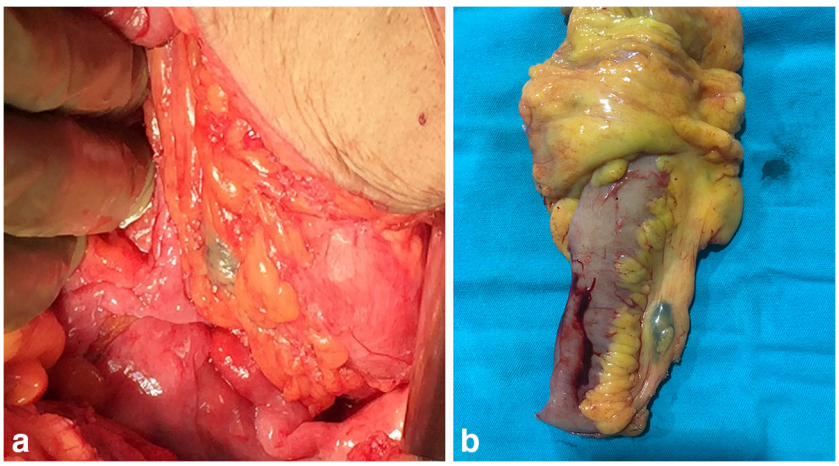
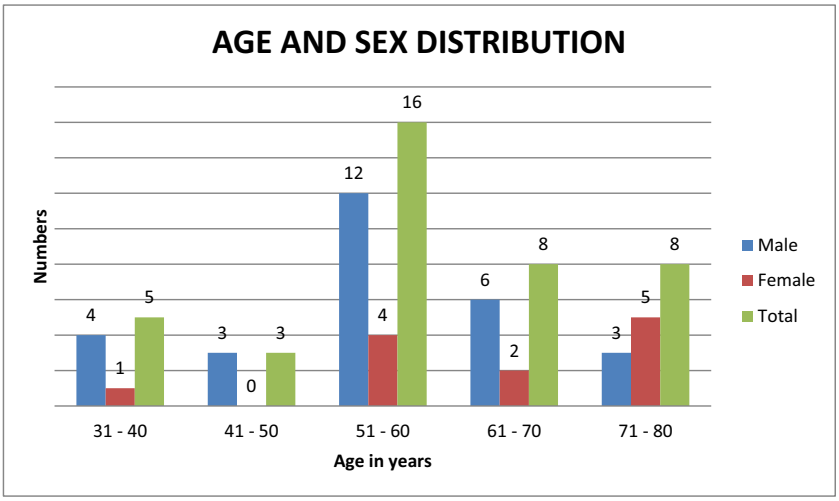


Fig. 2 **a** SLNs identified by uptake of the blue dye within the first 10–20 min of injection. **b** Specimen with sentinel node

Chart 1 Age and sex distribution



= 37 × 100/40 = 92.5%

This is comparable to several published studies that report SLN identification in ranges between 58 and 100%, and the greatest percentage is reached in case series with the largest number of patients [4].

Examination of Lymph Nodes

In this study, firstly all the non-sentinel lymph nodes were subject to examination by conventional sectioning and staining technique.

The non-sentinel lymph nodes were positive for tumour deposits in 28 of the patients and negative for tumour deposits in 12 of the patients.

In the 28 patients with positive non-sentinel lymph nodes, the sentinel node(s) were examined by routine steps to validate detection but were not subjected to further dedicated evaluation as it was considered unnecessary [Fig. 3].

In all these 28 cases, the blue coloured tagged sentinel nodes were also positive and did not confound our detection interpretation.

In 02 out of 28 cases in this group, blue coloured node(s) could not be identified and biopsy was not successful.

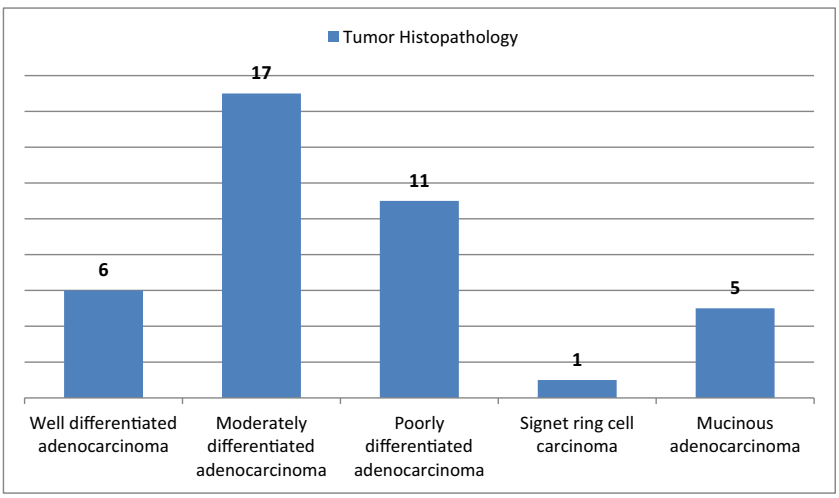
In 11 out of 12 patients with negative non-sentinel lymph nodes by routine H&E staining, the tagged sentinel lymph nodes were taken up for dedicated examination.

A blue (sentinel) node was not detected in 01 out of these 12 cases with negative non-sentinel lymph nodes, and hence, no further evaluation could be done.

The results of the detailed evaluation of the 11 cases are as follows.

Micrometastasis was detected in 4 additional cases upon examination under higher magnification (× 400) (Fig. 4a and b).

Chart 2 Tumour histopathology



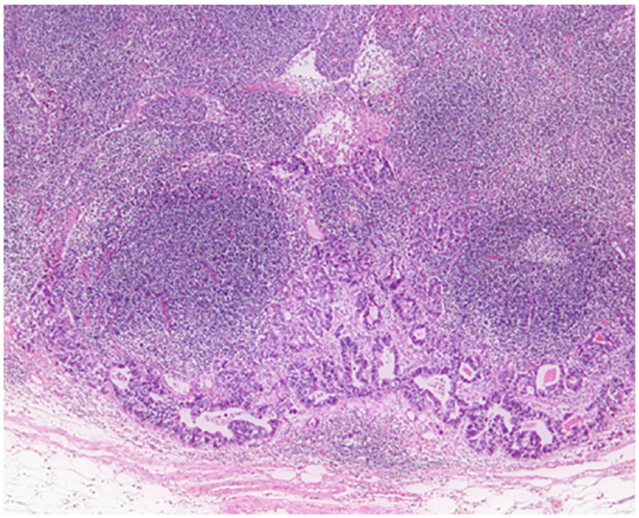


Fig. 3 Non-sentinel node showing tumour deposits

The sentinel lymph nodes which remained negative for metastasis by this method too were subjected to further evaluation by IHC staining.

This resulted in detection of isolated tumour cells in 02 cases (Fig. 5).

In 5 cases, there were no tumour deposits detected on focused microsectioning even under higher magnification (× 400) and IHC (Fig. 6).

Hence, the upstaging rate = (4 + 2) × 100/37 = 16.2%.

Thus, the use of sentinel lymph node examination in this study helped in detecting otherwise undetectable metastasis to lymph nodes leading to upstaging of the disease stage in six (16.2%) of our patients.

The results of the study are summarised in Fig. 7 and Chart 3.

These results are comparable to various similar published studies with the upstaging between rates reported 6 to 33% [5].

Statistical Analysis

	Disease				
Test	Present	*n*	Absent	*N*	Total
Positive	True positive	$a = 6$	False positive	$c = 0$	$a + c = 6$
Negative	False negative	$b = 11$	True negative	$d = 0$	$b + d = 11$
Total		$a + b = 17$		$c + d = 0$	

The additional node detection sensitivity obtained is 35.29% (95% CI 14.21–61.67%).

The specificity cannot be determined as the number of 'true negatives' cannot be ever stated; it is limited by the modality/technology available.

Discussion

The current study included 40 patients, who were operated for colon cancer over the study period of 3 years at a tertiary care hospital.

Lymph node status is the most important predictor of outcome and dictates the use of adjuvant chemotherapy in these tumours [6].

The detection of node positivity in 28 out of 40 clinically N0 cases is not surprising as it is known that CT scan imaging has low accuracy in staging of colon cancer, both in respect of local invasion T staging (54.4–76.6%) and more so for nodal metastases *N* staging (45.4–60.3%) [7].

Despite the favourable prognosis of patients with localised colon cancer without regional lymph node metastasis, 20–30% of these patients will develop recurrent disease, after apparently curative resection [8].

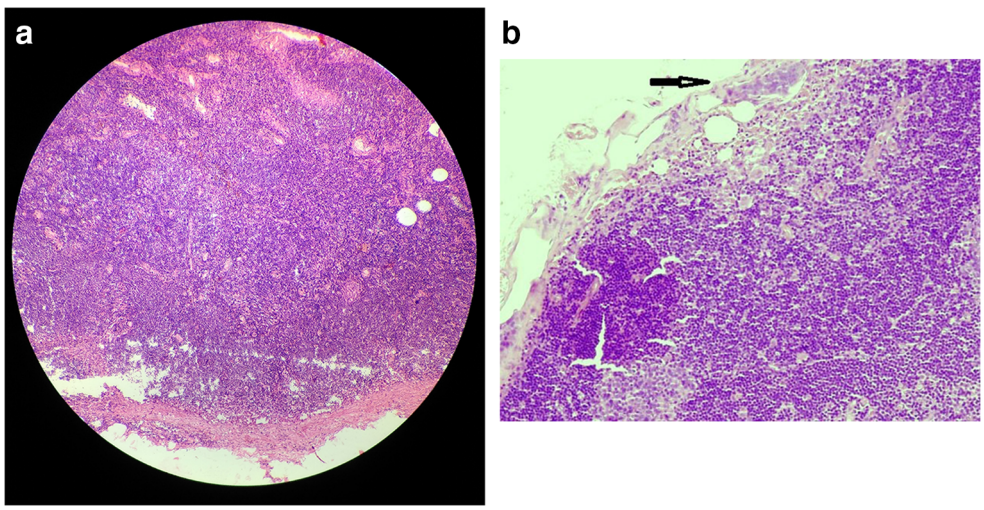


Fig. 4 **a** Sentinel node negative on routine examination. **b** Sentinel lymph node: micrometastasis detected by higher magnification (× 400)

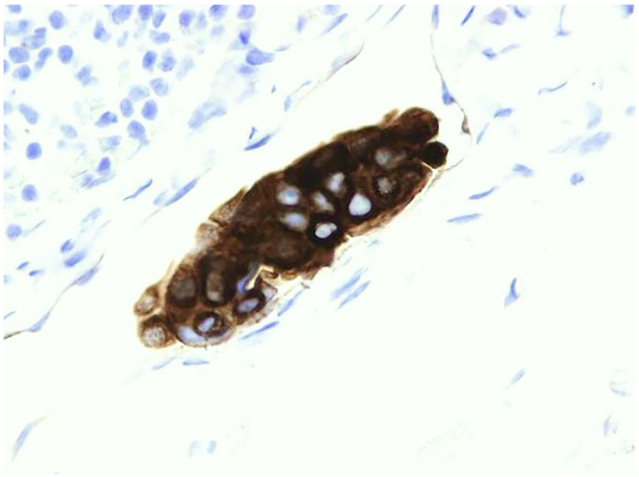


Fig. 5 Sentinel lymph node: tumour deposits on IHC (isolated tumour cells)

The yield of sentinel nodes can be increased with utilisation of radiotracer uptake methods. However, these require gamma camera probes for detection, and where such facility is not available such as in our centre, an effort to improve the staging accuracy in a clinically feasible setting can be made using the blue dye method.

Intensive pathologic examination of lymph nodes by IHC for cytokeratin or reverse transcriptase-polymerase chain reaction (RT-PCR) may reveal micrometastases that would be missed by routine haematoxylin & eosin (H&E) examination [9].

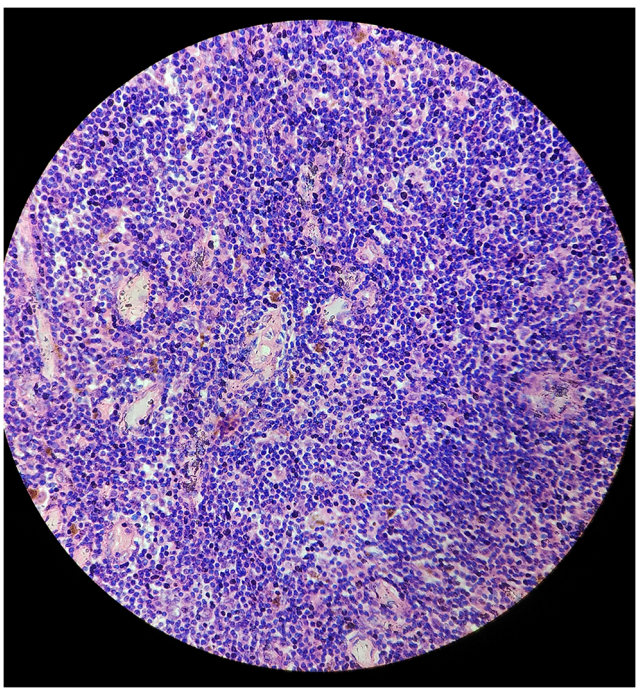


Fig. 6 Sentinel node negative after ultrastaging

In colon cancer, as the SLNs are likely to have the greatest potential to harbour metastatic disease when present, enabling focused examination with multilevel microsectioning of the SLN's can provide more efficient and cost-effective detection of micrometastases [10].

The technique of the sentinel node biopsy was first described and performed in 1977 by Cabanas in penile carcinoma [11].

Accurate detection of these micrometastases could identify the patients who are most likely to benefit from adjuvant therapy [12].

Cytokeratin antibodies (e.g. Cam 5.2, MNF 116, Pan Cytokeratin; AE1/AE3) have been used in many studies to aid the detection of metastases (macrometastases and micrometastases, ITCs), often in combination with step sectioning and on multiple levels in cases with negative H&E findings.

In 1992, the International Union against Cancer (UICC) recommended defining the micrometastasis as metastatic single or groups of tumour cells not larger than 2 mm in diameter.

Natsugoe et al. considered lymph nodes micrometastases to be metastatic single or groups of tumour cells not larger than 0.5 mm [13].

Adell et al. described lymph nodes micrometastases to be single cells or groups of tumour cells not more than 100 μm [14].

Those micrometastases measuring larger than 1.0 mm more accurately represented 'missed' or 'overlooked' lesions.

So far, there has been no uniform standard for definition and detection of lymph node micrometastases.

The various methods employed are as follows:

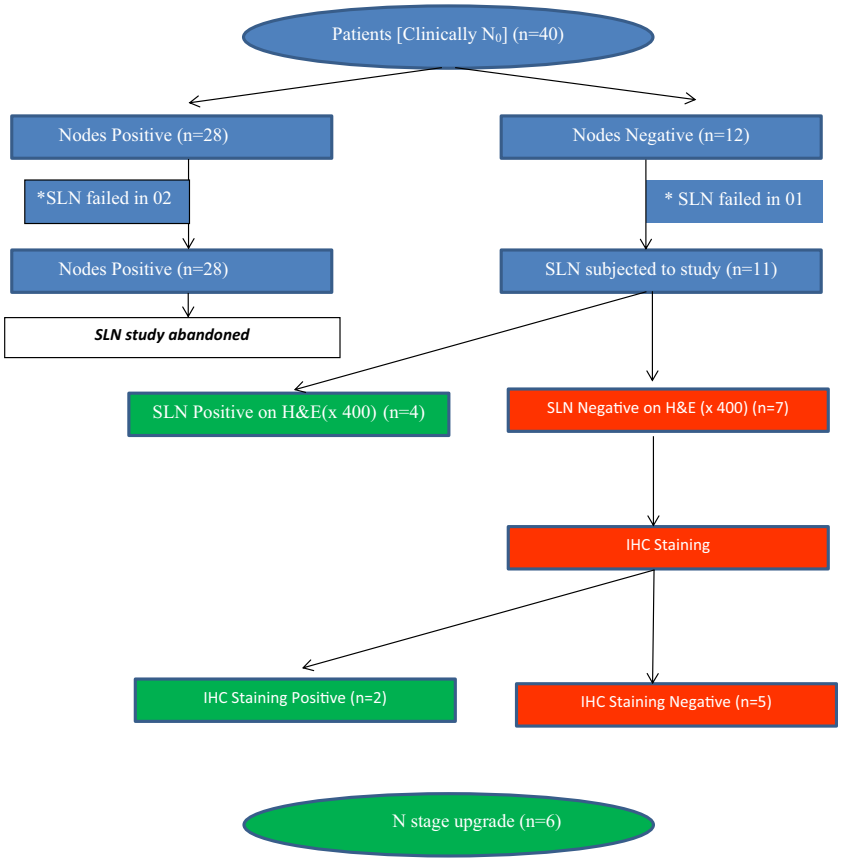
(i) Serial sectioning technique: This method can improve the detection of the positive lymph nodes. Gusterson reported that up to 20% of node-negative breast cancer patients assessed by routine single-section examination could be found to contain micrometastases after serial sections [15].

This procedure is, however, time-consuming and not feasible routinely.

(ii) Immunological techniques: These include flow cytometer, radioimmunoassay and immunohistochemistry, using various antibodies, and are more sensitive than conventional histological techniques. Recent techniques for exposing CK19 (one-step nucleic acid amplification) showed that CK19 is present in the colorectal metastatic cancer cells in a significant proportion [16].

(iii) Molecular biological techniques: The reverse transcription-polymerase chain reaction (RT-PCR) is sensitive than other methods but not routinely available or employed [17].

Fig. 7 Summary of results

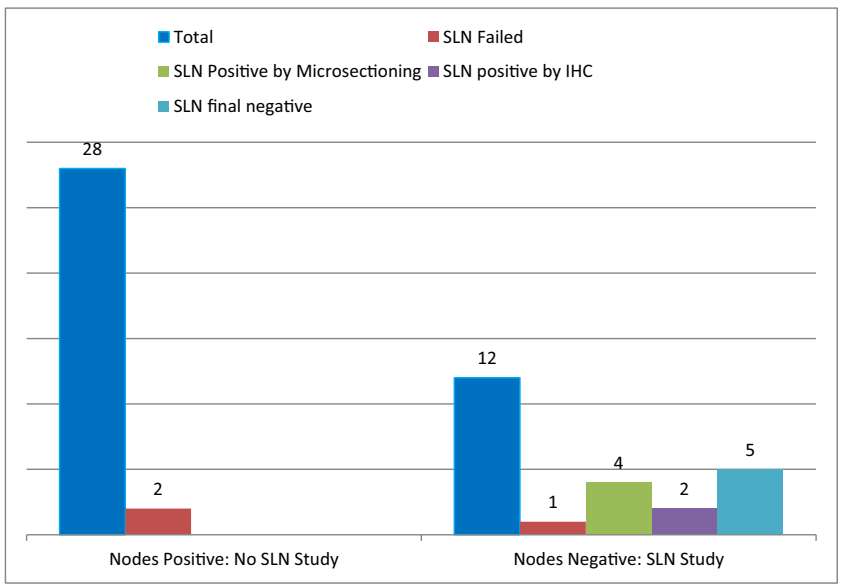


Conclusion

Lymph node staging is a major prognostic factor in colorectal cancer and remains to be the most important criterion for selecting patients for adjuvant therapy. The standard approach for lymph node evaluation is based on manual dissection and histological evaluation of HE-stained slides.

The blue dye method has the potential to detect sentinel lymph node(s) that may help detect micrometastases and lead to upstaging the disease which is crucial for planning adjuvant therapy and follow-up in these patients [18].

Chart 3 Study outcomes



Extending chemotherapy to these patients may improve disease-free survival.

However, more research is needed to establish whether SLN detected nodal micrometastases will change the *N* stage as per AJCC and/or direct definitive adjuvant chemotherapy.

Compliance with Ethical Standards

This study was cleared by the Ethics Committee, and patient consent obtained in all cases.

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