Sentinel lymph nodes of colorectal carcinoma: reappraisal of 123 cases

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SUMMARY

Aims — Results concerning the usefulness of the sentinel lymph node (SLN) in colorectal carcinoma have been discordant. The SLN technique may be used to guide surgical resection (lymph mapping), restrict the lymph node analysis solely to the SLN (accuracy) and upgrade tumor staging when micrometastases are specifically detected in the SLN.

Methods — The blue dye injection technique was used. Serial sections of the SLNs were analyzed after hematoxylin-eosin (HES) staining.

Results — The SLN technique was tested in 123 patients, successfully in 112/118 (feasibility 95%) (five intraoperative exclusions). On average, twenty lymph nodes (range: 5-74) and two SLNs (range: 1-5) were identified. Lymph mapping was used in 11% of patients to guide surgical resection; the SLN was negative in 14 of 36 N+ patients (39% false-negatives). HES staining enabled detection of micrometastases in 8 of 84 initially N0 patients (10% secondary upgrading to N+).

Conclusion — Limiting node analysis to the SLN cannot replace a complete pathology examination of all resected lymph nodes. Careful examination of serial sections of the SLN can however affect therapeutic decision making since staging may be upgraded in up to 10% of initially N0 patients.

Introduction

Colorectal cancer (CRC) is the most common gastrointestinal cancer in France; 36,257 cases were recorded in 2000 [1]. Lymph node involvement is one of the major prognosis factors. The expected five-year survival in stage III patients with lymph node involvement (N+) is about five years, much less than stage I/II patients free of nodal involvement (N-). Adjuvant chemotherapy is indicated in the event of nodal involvement, leading to significantly improved survival [2, 3]. Although the indication remains controversial in rectal cancer, many teams, including ours, have adopted the same therapeutic attitude for rectal tumors.

Nearby 30% of patients with early-stage disease at diagnosis (stage I/II) develop systemic recurrence [2, 3]. Most likely a certain percentage of these patients were under-staged histologically. Gross inspection of the operative specimen is not always sufficient to analyze all of the resected nodes. Small-sized nodes (<5 mm) are particularly difficult to detect, but are nevertheless known to harbor metastases more often [4]. Inversely, large-sized nodes may not be analyzed entirely [en bloc study of each node] unless serial sections of the entire specimen are examined, an option which is not particularly realistic for routine practice.

Node dissection procedures are still a subject of study in CRC [5], but it has been demonstrated that tumor staging is more accurate and reliable when more nodes are resected [6, 7]. At the present time, the TNM classification recommends the pathological examination of 12 nodes per operative specimen [8]. Minute examination of the operative specimen and/or use of lipodissolution techniques have demonstrated efficacy for improving tumor staging in CRC [6, 9]. These methods are however quite resource-intensive and time consuming.

In 1977, Cabanas introduced the concept of the sentinel lymph node (SLN) for the treatment of cancer of the penis. The

RéSUMÉ

Le ganglion sentinel dans les cancers colorectaux : étude de réévaluation sur 123 cas

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Objectifs — L’intérêt du ganglion sentinel (GS) est non établi pour les cancers colorectaux. Il pourrait : réorienter le curage en peropératoire (cartographie lymphatique), éviter une analyse de toute la pièce avec le seul GS (exactitude) et isoler des micrométastases permettant de modifier le stade histopronostique.

 Méthodes — La technique a été l’injection de bleu patenté. Les GS ont été analysées par coupes séries en HES.

Résultats — Un GS a été envisagé chez 123 malades, réélisé avec succès pour 112/118 cas (faisabilité 95%). Le nombre médian de ganglions et de GS a été respectivement de 20 (5-74) et de 2 (1-5). i) la cartographie lymphatique peropératoire du GS a été utilisée pour adapter le geste chirurgical dans 11 % des cas. ii) pour 36 malades classés N+, dans 14 cas le GS était négatif (39 % de faux négatif). iii) 84 malades ont été classés N0 en première lecture, puis 8 ont été up-gradés N+ (10%), après découverte de micrométastases en HES dans le GS.

Conclusions — L’analyse du GS ne peut remplacer l’analyse de tous les ganglions. Les coupes multiples en HES permettent de modifier le stade 10 % des N0 et modifie leur prise en charge.
goal was to adapt node dissection to tumor spread [10]. According to this concept, the SLN is the first node draining the tumor and as such the node with the greatest risk of metastatic involvement. In 1990, Morton reintroduced this concept, applying the SLN technique to the staging procedure for malignant cutaneous melanoma [11]. Later in 1994, Giuliano et al. reported results using the technique in patients with breast cancer [12].

The impact of detecting the SLN on decision-making in CRC patients remains a subject of debate [13, 14]. In 1999, Joosten et al. [15] reported the first study evaluating SLN detection in CRC. These authors concluded that the SLN technique is not applicable to CRC because of the low rate of successful identification and the high rate of false negatives (60%). In 2000, Saha et al. presented the first encouraging results and affirmed the feasibility and validity of the SLN technique for CRC [16]. At the present time, it is generally accepted that the technique is feasible, but the debate remains open concerning its usefulness in CRC. The problem lies in the fact that there is no consensus on what information the SLN technique is expected to provide.

Theoretically, three types of information could be expected:

1. Intraoperative lymph roadmapping could be useful for guiding (modifying) tumor resection. This is in line with results obtained with SNL detection in melanoma patients. Hypothetically, for colon cancer, lymph mapping would be relevant for 0 to 10% of colectomy procedures. One multicentric study including 203 patients reported a rate of 5% [17-19].

2. Sufficient pathological accuracy might be obtained by examination of the SLN alone, making the examination of the entire operative specimen redundant, similar to techniques used for breast surgery [20, 21]. The crucial element here is the proportion of false negative cases. One group has regularly reported the feasibility of this option, but generally when used in combination with immunohistochemistry techniques [17, 18, 22-24].

3. Identification of micrometastases in the SLN by examining serial sections could enable histological upgrading to stage III, as initially suggested by North American teams [17, 18] and specifically demonstrated in 5-20% of patients [17, 25-27]. Serial sections of the entire SLN with HES staining enables a more complete examination of the SLN in routine practice. Conversely, the potential impact of identifying occult metastases (tumor cells identified by non-routine techniques such as immunohistochemistry or molecular biology methods) or of circulating tumor cells (CTC) remains a controversial issue. Very few studies (including the most recent reviews) have retained these methods as providing useful information [28-30].

Many studies have examined the use of the SLN technique for CRC, but have reported contradictory results concerning its feasibility [31]. Furthermore, most of the reports to date have involved a limited number of patients (<50 patients). The purpose of this study was to assess the feasibility of the SLN technique and evaluate its contribution to these three types of information.

Material and methods

Patients

From February 2001 through June 2004, patients undergoing surgery for adenocarcinoma and/or severe dysplasia involving the colon or rectum were included in a protocol for intraoperative detection of the SLN. All data were collected prospectively. All patients included in the study provided their written consent for the specific analysis of their tumor. Exclusion criteria were: stage IV recognized preoperatively, massive lymph node involvement including spread to the lumboaortic area, emergency surgery. Prior gastrointestinal surgery, preoperative radiotherapy and/or the presence of more than one tumor were not considered as exclusion criteria. Patients with nodal involvement recognized at inspection, peritoneal carcinomatosis and/or hepatic metastasis noted at laparotomy were excluded secondarily.

Techniques

Theoretical resection and node dissection were determined after exploration of the abdominal cavity. Search for the SLN was undertaken if all exclusion criteria were ruled out. For each surgeon participating in this study, a learning curve of five procedures was considered necessary before including a patient in the study.

The in vivo technique

The in vivo technique was used for colonic tumors. After releasing the tumor and exposing its meso, 2 cc of blue dye V (Guebert, Aulnay-sous-bois, France) were injected with a 22-gauge needle under the serous at four diametrically opposed points around the tumor [19]. One to five minutes later, without manipulating the tumor, a blue cord appeared in the mesocolon wall, mapping the lymph drainage to the SLN(s). The first nodes mapped were landmarked with a thread before the dye diffused into more distal nodes. Final resection was guided by any atypical lymph drainage pattern and could be different from the dissection planned preoperatively. After resection, the SLN(s) was(were) isolated to ensure separate pathological examination(s).

The ex vivo technique

The ex vivo technique was used for rectal tumors and for tumors involving the rectosigmoid junction which cannot be mobilized easily. Search for the SLN was performed in the operating theatre on the operative specimen [25].

After opening the operative specimen via an incision in the anterior aspect, 2 cc of blue dye V were injected in the submucosa at four diametrically opposed points. Five minutes later, blue node(s) were identified and resected (taking care to avoid opening the mesorectum facing the tumor) for subsequent separate pathological examination.

Pathology

A specific protocol was used to examine the surgically harvested SLN. The SLN were fixed separately in alcohol-formol-acetic acid for 24 hours. SLNs measuring more than 3 mm were bisected entirely. SLNs measuring less than 3 mm were bisected along the greater diameter, each half of the same node being fixed separately. Three 5 µm slices cut at a 150 µm interval were stained with hematoxilin eosin saffron (HES). The semi-serial technique being applied, all serial slices situated between the three stained slices were preserved in a paraffin ribbon. These slices were used later for complementary immunohistochemistry studies.

By definition, tumor metastases visible after HES staining measuring 0.2 to 2 mm along their greatest diameter were considered as “authentic” micrometastases and thus classified pN1. “Occult” micrometastases were defined as the presence of isolated tumor cells or tumor cell clusters detected either with HES staining and measuring less than 0.2 mm along the greatest diameter, or with special techniques such as immunohistochemistry or molecular biology methodologies; these tumors were classified pN0 in accordance with current recommendations [32].

If the SLN was negative at the standard examination of the three HES-stained slices, it was reappraised using anti-cytokeratin 20 (Dako®, Trappes, France) and anti-pan-cytokeratin AE1/AE3/PCK26 (Ventana®, Illkirch, France) for immunohistochemistry analysis of the slices between the HES-stained slices which were retained in the paraffin ribbon.

Results

One hundred twenty-three patients were eligible for inclusion in our study. Five patients were excluded intraoperatively due to the discovery of peritoneal carcinomatosis (N=1), hepatic metastases (N=2) and/or gross node involvement (N=3). The SLN technique was thus performed for 118 patients, 59 males and 59 females. Mean age was 65 years (range 37-89, median 66 years) (table I).
Tumors sites were: cecum (N=12), right colon (N=12), right flexure (N=2), transverse colon (N=11), left flexure (N=3), left colon (N=4), sigmoid (N=10), rectosigmoid junction (N=17), rectum (N=47). The histological diagnosis was adenocarcinoma in 112 patients; four patients had a villous tumor and two patients with an adenocarcinoma presented an associated neuroendocrine component.

Feasibility

One or more SLN were successfully detected in 112 of 118 patients (95%). The six failures concerned four cases where no lymphatic structure could be identified at the pathology examination (ex vivo technique) which mainly revealed fatty aggregates, and two cases where a technical error prevented correct diffusion of the blue dye.

A total of 2620 lymph nodes were dissected, median 20 nodes per operative specimen (range 5-74). Among the 2620 nodes dissected, there were 217 blue SLN. A median two SLNs were obtained per operative specimen (range 0-5).

Contribution to the three hypothetical information items was analyzed by the different techniques used (Table II).

LYMPH NODE ROADMAPPING AND SURGICAL PROCEDURE

Lymph node mapping using the in vivo technique demonstrated a particular lymphatic pattern in 7/60 (11.6%) patients (two right flexure tumors, one left flexure tumor four transverse colon tumors). The drainage pattern thus influenced the intraoperative decision for the dissection procedure and was noted as such in the operative report. For four patients (two right flexure tumors and two transverse tumors) dissection was extended to the colica media area which included the SLN. The pathology examination of the SLNs dissected in these four patients was negative. For three (one left flexure tumor and two transverse tumors), the colica media area (flexure tumor) and the flexure vascular areas (transverse tumors), which were devoid of SLN, were preserved. For two patients, SLN-guided dissection yielded a positive specimen after the final histological analysis (one N1 and one N2). These two patients are alive without recurrence at more than three years follow-up despite the fact that the colica media area was preserved by the SLN-guided, but limited dissection.

ACCUAACY

Without considering the SLN analysis, 84 (75%) patients were staged pN0. After more detailed analysis of the SLN, the staging was pN1 for 14 of these patients (12.5%) and pN2 for 14 others (12.5%).

Considering the 36 N+ patients, 8 (22%) had one positive SLN. Some were positive after examining the HES-stained slices without considering the serial slices which were nevertheless systematically examined in the SLN protocol. One node was positive in all of these patients, the other node or nodes being negative. For 14 patients, the SLN was positive as were other nodes. But nearly 40% of the 36 N+ patients (14/36) had a negative SLN while other nodes were positive. Among the patients with a negative SLN, 12 were classified pN1, including 9 with only one positive non-sentinel node; two were pN2. The in vivo technique was used in nine patients and the ex vivo technique in five. The false-negative rate was thus 39% (determined for N+ patients) or 12% (determined for all patients including the N0).

UPGRADING

Among the 84 patients free of nodal involvement (N0), standard examination of the SLN upgraded 8 patients (9.5%). Among these, 5 had SLN with micrometastases which could only be detected on serial sections (Figure 1). Only the SLN was positive in all of these cases.

For these 84 patients, 1849 nodes were analyzed; only 8 (0.4%) were uninformative. Also for these 84 patients, analysis of 185 SLNs yielded 8 (4.3%) which were uninformative. For five patients (6%) circulating cells were detected on the serial sections or immunohistochemistry tests were positive.

Discussion

Intraoperative detection of the SLN was feasible in the large majority of our patients (>95%). This is in agreement with earlier

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Table I. – Tumor characteristics in the included patients. 
Caractéristiques tumorales des patients.

<table>
<thead>
<tr>
<th>Gender (male/female)</th>
<th>59/59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feasibility of the SLN technique (Number of cases)</td>
<td></td>
</tr>
<tr>
<td>success</td>
<td>112</td>
</tr>
<tr>
<td>failure</td>
<td>6</td>
</tr>
<tr>
<td>Causes of failure</td>
<td></td>
</tr>
<tr>
<td>No SLN at histology</td>
<td>5</td>
</tr>
<tr>
<td>Technique</td>
<td>1</td>
</tr>
<tr>
<td>Technique used and feasibility by technique</td>
<td></td>
</tr>
<tr>
<td>Ex vivo</td>
<td>58</td>
</tr>
<tr>
<td>Success rate (%)</td>
<td>52/58 (90%)</td>
</tr>
<tr>
<td>In vivo</td>
<td>60</td>
</tr>
<tr>
<td>Success rate (%)</td>
<td>60/60 (100%)</td>
</tr>
<tr>
<td>Neoadjuvant treatment</td>
<td>38</td>
</tr>
<tr>
<td>Radio-chemotherapy (RC)</td>
<td>20</td>
</tr>
<tr>
<td>Radiotherapy (RT)</td>
<td>14</td>
</tr>
<tr>
<td>RC or RT for other pelvic tumor</td>
<td>4</td>
</tr>
</tbody>
</table>

Table II. – Contribution of sentinel lymph node detection according to the problematic.
Apports de la technique de détection du ganglion sentinelle selon la problématique.

<table>
<thead>
<tr>
<th>Problematic</th>
<th>N</th>
<th>%</th>
<th>where information was relevant</th>
<th>Relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph map</td>
<td>60</td>
<td>11%</td>
<td>information used intraoperatively to decide on type of resection and dissection</td>
<td>+</td>
</tr>
<tr>
<td>Accuracy of N+ staging</td>
<td>36</td>
<td>67%</td>
<td>Or about 40% false negative for these patients</td>
<td>No</td>
</tr>
<tr>
<td>Upgrading to grade III</td>
<td>84</td>
<td>9.5%</td>
<td>Including five cases with micrometastases detected on HES serial sections</td>
<td>+ + +</td>
</tr>
</tbody>
</table>
results in the literature [14-27, 33, 34]. Failures to detect the SLN reported in the literature have either concerned technical errors (dye injected intraluminally) [5, 32], insufficient surgical experience [15, 21, 35], or use of an insufficient amount of dye [36]. The rate of failure may also be higher after preoperative radiotherapy for cancer of the rectum [37].

The purpose of our study was to reappraise in a large number of patients (over one hundred) the possible contribution of the SLN technique to the resolution of three distinct issues. This broad goal and an already extensive body of literature led to a complex comparative analysis of the findings.

In comparison with the in vivo technique, the ex vivo technique offers the advantage of avoiding longer operative time as well as the risk of exceptional, but real, allergic reactions [38]. For this reason, and also because with the in vivo technique the colon has to be mobilized before making the vascular ligatures, many teams have preferred the ex vivo option [20] despite the fact that in vivo dye injection is the only way to obtain a real roadmap of the lymphatic system. Lymph mapping was thus used for slightly over 10% of the patients in our series, which does not mean that 10% of patients had an abnormal or unusual drainage pattern. The lymph roadmap was one of the elements contributing to intraoperative decision making. The limited dissection sparing the colica media chosen in two N+ patients was later validated as appropriate since the patients remained recurrence-free at three years follow-up. If lymphatic spread had involved the colica media, recurrence would have been noted. More recently, and as described by Japanese authors [39, 40], we have used endoscopic injections for lymph mapping to guide surgical resection, for small lesions since only larger tumors would have several lymph drainage pathways. Similarly, most reports on breast cancer show that the limit size for the SLN technique would be 2 cm, much smaller than the average size of the colonic tumors reported here.

It is known that a given area of a colonic cancer can be drained by several lymph pathways. Saha et al. promoted the SLN method, but noted that the SLN was unique in only 42% of patients [16]. Several SLN can thus be observed on distinct pathways, raising the risk of false negatives. Theoretically one pathway could be free of invasion, with a negative SLN but with a positive dissection because of the invasion of another pathway, as was observed in 40% of our patients (false negatives). The accuracy of the nodal extension predicted from examination of a unique SLN was insufficient, irrespective of the way the rate of false negatives was determined (all or N+ patients). These findings are in agreement with most published series [26, 33] but very different from the 4% false negative rate published by two North American teams who regularly publish in this field [16-18, 22-28, 27]. Use of other information collected from the SLN (immunohistochemistry or molecular biology) [22-24] can reduce rate of false negatives, but artificially in our opinion.

Irrespective of the type of analysis applied, limiting lymph node examinations to the SLN, with the objective of cost containment, is unacceptable [14, 21]. The SLN technique cannot replace standard pathology examination of the colectomy specimen, but the goal is probably different for the use of the SLN for colorectal cancer [19, 25, 41]. The SLN technique would be contributive if it could provide a more accurate assessment of lymphatic spread in N0 patients. Theoretically, some N0 patients could be upgraded using the information provided by HES staining of the SLN applied with a validated standardized technique. Some authors have suggested that the proportion of patients with micrometastases discovered by examining serial sections of all nodes in the operative specimen would be the same as that demonstrated by examination of the SLN alone. The pathology team at the Saint-Antoine hospital investigated this question by applying the SLN serial section technique to all non-sentinel nodes, but was unable to demonstrate the presence of micrometastases [20]. A German team also attempted to elucidate this point using multiple sections and immunohistochemistry techniques on non-sentinel nodes. They found isolated tumor cells in only 4% of the 1011 nodes analyzed [26]. This would validate in part the notion that SLN analysis is sufficient for upgrading purposes.

The proportion of tumors upgraded after HES staining of serial sections of the SLN has varied from 7 to 15% in different studies [16-19, 22-27, 35]. Our figure falls in this range. The serial-section technique is clinically pertinent since upgrading in the UICC classification would change the postoperative strategy by adding complementary chemotherapy.

The proportion of upgraded patients increases from 7% to 31% if immunohistochemistry results are taken into consideration and up to 30 or 40% if molecular biology techniques (RT-PCR) are applied [22-24]. The therapeutic impact and the prognostic value depend on the technique used to obtain such results. Indeed, there remains a certain amount of controversy concerning the prognostic value of occult metastases detected by immunohistochemistry or RT-PCR in colorectal cancer. Applying the UICC classification, the tumor stage is not modified [32]. This issue has led to a vast body of literature but there is no decisive conclusion [33].

Eight patients in our series were given adjuvant chemotherapy after the discovery of metastases (3 patients) or micrometastases (5 patients) in the SLN. The real impact on staging was probably less than 10% since the metastases would probably have been detected, at least in part, by the standard technique for SLN examination.

Our study enabled a certain clarification of the difficulties and potential contributions of detecting SLNs in colorectal cancer patients. Few teams apply this technique in routine practice despite that fact that it is a simple low-cost method which is not particularly resource-intensive both in terms of the pathologist’s time and material needs if the technique is limited to multiple sections, which would be reasonable since other examination techniques are not validated for clinical practice. The technique could be used to guide surgical resection in tumors involving the transverse colon or the flexures. About 10% of patients initially staged grade I or II can be upgraded to stage III and subsequently be proposed for postoperative chemotherapy. For the standard pathology examination, care must be taken to examine the entire operative specimen to avoid an excessive rate of false negatives.

REFERENCES


