Decreased incidence of isolated tumor cells in lymph nodes after laparoscopic resection for colorectal cancer

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Abstract

Background Laparoscopic surgery has potential for less tumor cell spread because of the no-touch technique. We assessed the effect of the surgical approach (open versus no-touch laparoscopic) on the presence of tumor cells in sentinel lymph nodes (SN) of patients with stage I and II colorectal cancer.

Methods A single-center consecutive prospective series of patients operated on for colorectal cancer was analyzed. After conventional hematoxylin and eosin (H&E) staining, 107 patients without lymphatic metastases were included; 59 patients had open surgery, and 48 patients underwent laparoscopic resection. Patients in the laparoscopic group underwent a no-touch medial to lateral approach, whereas the conventional lateral to medial approach was applied in open surgery. A SN procedure was performed in all patients. The SNs were immunohistochemically analyzed for presence of occult tumor cells (OTC). According to the American Joint Committee on Cancer (AJCC) these tumor cells were divided into micrometastases (0.2–2 mm) or isolated tumor cells (ITC, < 0.2 mm).

Results In ten patients micrometastases were found, equally distributed between the two groups. However, ITC were more often found after open surgery (18 versus 5 patients, \( p = 0.03 \)). Presence of OTC was related to depth of tumor invasion and tumor diameter > 3.5 cm. Logistic regression analysis identified lymphovascular invasion as a predictor for micrometastases [odds ratio (OR) 18.4], whereas open resection was predictive for presence of ITC (OR 3.3).

Conclusions No-touch medial to lateral laparoscopic surgery results in less isolated tumor cells in lymph nodes compared with open lateral to medial surgery in patients with stage I and II colorectal cancer.

Keywords Occult tumor cells · SN procedure · No-touch technique · Laparoscopic surgery

During intraoperative manipulation of colorectal carcinomas, levels of circulating tumor cells (CTC) can be demonstrated in peripheral and portal blood [1]. Recently, it has been demonstrated that the cumulative percentage of CTC in peripheral and portal blood was significantly lower after laparoscopic resection in which no-touch technique was used [2]. This term refers to the principal of early lymphovascular ligation before manipulation of the tumor. In 1966, Turnbull et al. showed that early ligation increased survival rates [3]. A randomized trial suggested favorable patient-free survival and overall survival rate, however without significant difference [4]. Today, the no-touch technique is not considered the standard surgical approach in open surgery. However in laparoscopic colorectal surgery, no-touch principles can optimally be represented with the preferable medial to lateral approach where the supplying vessels are ligated before the tumor is manipulated.

If fewer CTC are detected in blood during laparoscopic surgery, it can be hypothesized that the same holds true for
lymphatic flow with occult tumor cells (OTC) passing the lymphatic sinus. Based on the sentinel lymph node (SN) concept in which the lymphatic drainage from the primary tumor follows a specific order, we showed that OTC are preferentially found in the SN of patients with colorectal cancer [5].

The objective of this study is to assess the effect of the surgical approach (i.e., open lateral to medial versus laparoscopic no-touch medial to lateral approach) on levels of occult tumor cells in sentinel lymph nodes of patients with stage I and II colorectal cancer.

**Patients and methods**

**Study population**

A prospective consecutive series of patients operated on between November 2006 and June 2009 were analyzed. Only patients undergoing potentially curative resection for biopsy-proven colorectal cancer were eligible. Patients with solid organ metastases detectable by preoperative radiological staging or intraoperative visualization were excluded, as were patients with macrometastases on conventional H&E slides. In total, 107 patients with stage I and II colorectal cancer were included (Fig. 1).

In 87 patients the choice of open or laparoscopic approach depended on the surgeon to whom the patient was referred. Twenty patients also participated in a randomized trial comparing open and laparoscopic surgery (ISRCTN: 79588422) [6]. All procedures were performed by experienced colorectal surgeons performing more than 20 procedures each year. All laparoscopic procedures were performed by one surgeon expert at laparoscopic surgery. All these resections were performed according to the no-touch isolation technique (i.e., medial to lateral approach with early vessel ligation). Lateral to medial approach was performed during open resection. Patients who had early conversion, e.g., because of dense adhesions, were analyzed in the open group if the vascular trunk was not ligated before mobilization of the bowel and the lateral to medial approach was used.

The study was done in accordance with the guidelines of the local ethics committee.

**Sentinel node procedure**

A SN procedure was performed in all patients. Ex vivo sentinel lymph node mapping was used, as described previously in detail [7]. After resection, 0.5–2 ml patent blue (depending on the volume of the tumor) was injected below the subserosa, around the tumor, with the colonic specimen left intact. The first one to four blue lymph nodes were identified as sentinel nodes and either dissected or marked with a suture.

**Pathological examination**

The surgical resection specimens were analyzed immediately at the department of pathology using a standardized protocol. Tumor stage and grading were classified according to the sixth edition of the AJCC tumor–node–metastasis (TNM) classification [8]. All lymph nodes (SNs and non-SNs) were stained with hematoxylin and eosin and evaluated for tumor involvement.

For histologically proven N0 patients, three serial sections at 500-μm intervals were immunohistochemically stained with three different monoclonal antibodies to reveal OTC. The anti-epithelial cell antibody Ber-EP4, directed against membrane glycoproteins (DAKO, The Netherlands), was combined with two anticytokeratin antibodies: CK20 with its expression limited to gastrointestinal epithelial cells (Euro Diagnostica, Arnhem, The Netherlands) and Cam5.2.
directed against cytokeratin 7 and 8 expressed in all epithelial cells (Becton and Dickinson, Alphen aan den Rijn, The Netherlands).

Immunohistochemically detected cells with any of the three antibodies were considered as OTC only when they showed unequivocal morphological features of cancer cells. These tumor cells within lymph nodes were subdivided into two categories according to the AJCC revised guidelines: tumor cell deposits between 0.2 mm and 2.0 mm were referred to as micrometastases, and those smaller than 0.2 mm as isolated tumor cells (ITC) [9].

Statistical analysis

Statistical calculations were performed using the Statistical Software Package version 14.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were compared using the chi-square test, while the t-test was used for continuous data with normal distribution. Univariate logistic regression analysis was used to identify factors predictive for detection of OTC in histologically proven N0 patients. Method of resection, T-stage, differentiation grade, and tumor diameter were selected a priori as important cofactors in identification of CTC. Since the limited number of events (from a statistical point of view) meant that only a restricted number of possible predictors could be included [10], variables with multiple categories were recoded into dichotomous variables by combining categories with comparable prognosis (T-stage: I and II versus III and IV; differentiation grade: well and moderate versus poor). For tumor diameter, a cutoff of 35 mm was decided upon, as suggested in a study of a specifically constructed receiver-operating curve [11]. All tests were performed two sided, and \( p < 0.05 \) was considered statistically significant. To determine the clinicopathological characteristics predictive for presence of OTC, logistic regression was performed.

Results

Characteristics of the study population

During the study period, 107 patients with stage I and II colorectal cancer were included (Table 1). Sixty-two patients were analyzed in the open group, whereas 45 patients were analyzed in the laparoscopic group (Fig. 1). There were 48 right-sided colectomies, 5 left-sided colectomies, 53 (recto)sigmoid resections, and 1 subtotal colectomy.

The patient and tumor characteristics were not different between the two patient groups, except for tumor size, which was slightly larger in the open group (Table 1).

Immunohistochemical detection of occult tumor cells

A total of 208 SNs were analyzed. Ten patients had true micrometastases of more than 0.2 mm in one or more SNs (9.3%, pN1mi+), whereas ITC were found in 23 patients (21.5%, pN0itc+). Presence of micrometastases was equally distributed between the open and laparoscopic groups \( (n = 5 \text{ in both groups}) \) (Table 2).

In contrast, isolated tumor cells were more frequently found in the conventional open resection group when compared with the no-touch laparoscopic group. Eighteen patients (29%) in the open group had ITC, compared with five patients (11%) in the laparoscopic group. This results in an absolute risk reduction for incidence of ITC of 18% and a number needed to treat of six in favor of the laparoscopic group (Table 2). One of the five patients in the laparoscopic group with ITC had a larger cluster of 0.18 mm with evident signs of proliferation and stromal reaction, which could therefore be considered as a micrometastasis, although we adhered to the quantitative characteristics of the AJCC. ITC were most frequently found in the sinuses of the lymph nodes, as would be expected when these cells are considered as transiently shed cells with limited lifespan.

Predictive factors for presence of occult tumor cells

A relation between presence of OTC and larger tumors (both infiltration depth and tumor diameter) was found. The occurrence of true micrometastases in the SN was strongly related to lymphovascular invasion, irrespective of surgical approach. However, this could not be demonstrated for occurrence of isolated tumor cells. Presence of these cells was only related to method of resection, with odds ratio of 3.3 (1.1–9.6) for open lateral to medial resection (Table 3).

Discussion

This study demonstrates that the incidence of isolated tumor cells in lymph nodes of patients with stage I and II colorectal cancer is lower after no-touch laparoscopic resection when compared with lateral to medial open resection. These results are in line with other studies demonstrating reduced levels of CTC in peripheral and portal blood during laparoscopic resection [2, 12]. This is the first study analyzing tumor cells in lymph nodes using immunohistochemical techniques, thereby overcoming the historically controversial issues regarding reliability and reproducibility of CTC measurement in blood [13].

An advantage of analyzing OTC in lymph nodes is that histology can be preserved, discriminating between OTC and
falsely positive stained cells. Most studies sampling periphera-
ral or portal blood for CTC during surgery use reverse-trans-
criptase polymerase chain reaction (RT-PCR) or quantitative
real-time RT-PCR. However, histological confirmation is lost
in these techniques, which harbors the danger of identifying
shed debris or hematopoietic cells as tumor cells.

The clinical relevance of our finding is hard to assess.
Although the prognostic role of OTC seems to be estab-
lished in metastasized breast, colon, and prostate cancer
patients [14–16], the clinical relevance of peroperatively
detected cells is still debated. So far, these cells are
generally considered to be transiently shed cells without
prognostic significance. The metastatic process is extre-
mely inefficient, involving survival in circulation or
lymphatics, arresting at a distant target organ, extravasation
into surrounding tissue, and survival in the foreign micro-
environment, followed by proliferation and induction of
angiogenesis while evading apoptotic death or immuno-
logical response [17]. Tumors can shed millions of cells
into the bloodstream daily, but it has been demonstrated
that only a small percentage of tumor cells (0.05%) can
survive and initiate a metastatic focus [17].

| Table 1 Clinicopathological characteristics of the included patients |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | All patients \( (n = 107) \) | Open group \( (n = 62) \) | Laparoscopic group \( (n = 45) \) | \( p \) Value |
| Age (years)\(^a\)            | 70 ± 11                      | 70 ± 10                     | 69 ± 12                     | 0.8                        |
| Gender                      |                             |                             |                             |                            |
| Male\(^b\)                  | 48                          | 28                          | 20                          | 0.9                        |
| Female\(^b\)                | 59                          | 34                          | 25                          |                            |
| Body mass index (kg/m\(^2\))\(^c\) | 26 (18–31)                  | 24 (18–28)                  | 26 (22–31)                  | 0.7                        |
| Preoperative CEA level\(^c\) | 2.2 (0.5–10)                | 2.4 (0.9–10)                | 2.2 (0.5–8.4)               | 0.2                        |
| Tumor location \(^b\)       |                             |                             |                             | 0.2                        |
| Colon                       | 87                          | 53                          | 34                          |                            |
| Rectum                      | 20                          | 9                           | 11                          |                            |
| Tumor diameter (cm)\(^a\)   | 5.1 ± 2                     | 4.2 ± 2                     | 4.2 ± 2                     | 0.04                       |
| Number of resected lymph nodes\(^a\) | 15.5 ± 7                  | 15.5 ± 7                    | 15.6 ± 7                    | 1.0                        |
| Number of identified SN \(^a\) | 2.0 ± 2                    | 1.9 ± 2.1                   | 2.1 ± 2.4                   | 0.7                        |
| Depth of invasion\(^b\)     |                             |                             |                             | 0.7                        |
| pT1                         | 8                           | 3                           | 5                           |                            |
| pT2                         | 29                          | 17                          | 12                          |                            |
| pT3                         | 68                          | 41                          | 27                          |                            |
| pT4                         | 2                           | 1                           | 1                           |                            |
| Differentiation grade\(^b\) |                             |                             |                             | 0.2                        |
| Well                        | 9                           | 5                           | 4                           |                            |
| Moderate                    | 80                          | 43                          | 37                          |                            |
| Poor                        | 18                          | 14                          | 4                           |                            |
| Lymphovascular invasion\(^b\) |                             |                             |                             | 0.1                        |
| Absent                      | 97                          | 54                          | 43                          |                            |
| Present                     | 10                          | 8                           | 2                           |                            |

\(^a\) Mean (SD)  
\(^b\) Absolute numbers  
\(^c\) Median (min.–max.)

| Table 2 Presence of occult tumor cells in SN in patients with colorectal cancer: comparison between open and laparoscopic resection |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | All patients \( (n = 107) \) | Open group \( (n = 62) \) | Laparoscopic group \( (n = 45) \) | \( p \) Value |
| Occult tumor cells         | 33                          | 23                          | 10                          | 0.6                        |
| Micrometastases            | 10                          | 5                           | 5                           |                            |
| 0.2–2.0 mm                 | 8%                          | 11%                         | –3% (–15 to 8%)             |                            |
| Isolated tumor cells       | 23                          | 18                          | 5                           | 0.03                       |
| <0.2 mm                    | 29%                         | 11%                         | 18% (3–33%)                 |                            |
In contrast, data on patients with breast cancer show that OTC in sentinel lymph nodes are related to reduced 5-year disease-free survival [18]. Cultured single cells from rib marrow of patients with esophagealogastric cancer were viable when inoculated subcutaneously in athymic nude mice [19]. In addition, in patients with colon cancer, improved 5-year survival rates were reported combined with reduced frequency of cancer cells in portal blood using the open no-touch isolation technique [3]. The only randomized trial on no-touch technique in patients with colon cancer did not show a significant increase in disease-free survival, but data demonstrated better results in all analyses for the no-touch patient group (time to recurrence, incidence of liver metastases, and disease-free survival) [4].

So far, several randomized trials comparing laparoscopic with open surgery with long-term follow-up did not show survival differences [20, 21]. This would be in line with our hypothesis that the decreased number of detected ITC is merely a mechanical process depending on the surgical approach, rather than being viewed as a prognostic factor. Better disease-free survival was demonstrated in one early trial comparing laparoscopic with open resection, but this improvement was only seen in stage III patients, and therefore these results cannot be compared with our findings in histologically N0 patients [22].

Previously, it has been suggested that the decreased detection rate of CTC could be attributed to early ligation of the lymphovascular trunk (medial to lateral approach) [2]. We have demonstrated in a previous study that OTC are preferentially found in peritumoral SNs [5]. Since lymphatic drainage to peritumoral lymph nodes is not disturbed by early ligation, it is unlikely that solely a medial to lateral approach would result in decreased numbers of ITC. Therefore, our results suggest that predominantly the no-touch technique of the laparoscopic approach explains the lower incidence of ITC when compared with conventional open resection. Even without demonstrated prognostic relevance, this would make laparoscopic no-touch resection the treatment of choice for patients with stage I and II colorectal cancer. The development of all metastases will start with a single cell, and so far there are no techniques available to predict the possible survival of ITC. Although most cells will not survive circulation and immunological responses, activation of blood coagulation and relative immune suppression due to surgical stress might enhance the metastatic potential of these intraoperatively spilled cells [23, 24].

A major drawback of our study is the nonrandomized comparison. However, the decrease in ITC is not likely to be attributed to patient selection since patient and tumor characteristics, carcinoembryonic antigen (CEA) levels, and number of resected lymph nodes were comparable in the two treatment groups. There was a difference in tumor diameter, with smaller tumors in the laparoscopic group. However, regression analysis showed no relation between presence of ITC and tumor diameter. The only parameter predictive for presence of ITC was method of resection, with OR of 3.3 for open lateral to medial surgery.

**Conclusions**

Laparoscopic no-touch surgery results in fewer isolated tumor cells in lymph nodes compared with open lateral to medial surgery in patients with stage I and II colorectal cancer. Although prospective survival analyses in these patients with ITC and randomized trials should be awaited, every attempt should be made to prevent worsening of the prognosis of patients during surgery. In that respect, laparoscopic resection with the no-touch technique may have benefits over open surgery in patients with stage I and II colorectal cancer.

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**Table 3** Logistic regression analyses of predictors for presence of occult tumor cells, micrometastases (0.2–2.0 mm), and isolated tumor cells (<0.2 mm) in histologically N0 patients with colorectal cancer

| Variable                        | Occult tumor cells | Isolated tumor cells |
|---------------------------------|--------------------|----------------------|
|                                 | OR\(^a\) (CI\(b\)) | p Value              |
| Method of resection             | Open versus laparoscopic | 0.5 (0.20–1.16) | 0.1 |
| Depth of invasion               | pT3/4 versus pT1/2  | 2.5 (0.99–6.6)      | 0.06 |
| Differentiation grade           | Poor versus well and moderate | 2.1 (0.75–5.78) | 0.2 |
| Tumor diameter                  | >3.5 cm versus < 3.5 cm | 2.7 (1.05–7.08) | 0.04 |

| Micrometastases | Isolated tumor cells |
|-----------------|----------------------|
| Lymphovascular invasion Present versus absent | 18.4 (4.0–85.1) | <0.001 |

\(^a\) Odds ratio
\(^b\) 95% CI
Disclosures  Authors van der Zaag, Buskens, Vlug, Peters, Bouma, and Bemelman have no conflicts of interest or financial ties to disclose.

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