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Circulating tumour cells during laparoscopic and open surgery for primary colonic cancer in portal and peripheral blood

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DJ Richel
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WA Bemelman

Submitted
Abstract

Introduction
The objective of this study was to detect and quantify circulating tumour cells (CTC) in peripheral and portal blood of patients who had open or laparoscopic surgery for primary colonic cancer.

Methods
Patients in the laparoscopic group were operated on in a medial to lateral approach (“vessels first”), in the open group a lateral to medial approach was applied. The enumeration of CTC was performed with the CellSearch System. Intra-operative samples were taken paired-wise (from peripheral and portal circulation) directly after entering the abdominal cavity (T1), after mobilisation of the tumour baring segment (T2), and after tumour resection (T3). Ploidy of both the CTC and tissue of the primary tumour was determined for chromosome 1, 7, 8 and 17.

Results
Thirty-one patients were included; 18 patients had open surgery, 13 patients were operated on laparoscopically. The percentage of samples with CTC at T1 was 7% in peripheral blood and 54% in portal blood (p=0.002). At T2, 4% and 31% respectively (p=0.031). And at T3, 4% and 26% respectively (p=0.125). The cumulative percentage of samples with CTC was significantly higher during open surgery as compared to the laparoscopic approach. Both the CTC and tissue of the primary tumour were diploid for chromosome 1, 7, 8 and 17.

Conclusion
The detection rate and quantity of CTC is significantly increased intra-operatively and is significantly higher in portal blood compared to peripheral blood. Significantly less CTC were detected during laparoscopic surgery probably as result of the medial to lateral approach.
Introduction

It is of great importance that survival and the most likely sites of recurrence can be predicted after surgery with curative intent. With respect to long-term outcome haematogenous and lymphatic spread are the pathways of metastasis and are therefore the most important factors associated with prognosis.1-5 Patients with unfavourable prognostic factors may benefit from adjuvant chemotherapy. However, pathologic staging systems (TNM) fails to adequately identify patients with stage II and III disease who will benefit from adjuvant chemotherapy.2-5 Detection of circulating tumour cells (CTC) during and after resection might be helpful to identify those patients who will benefit from adjuvant chemotherapy and expand our knowledge about the biology of metastasis and potential role of surgery in this process. CTC were first detected in patients with colorectal cancer more than 50 years ago.6;7 The introduction of the CellSearch™ system has provided a standardised method for the enumeration and characterisation of CTC that permitted the evaluation of potential clinical utilities of CTC through multi-centre prospective clinical trials.8 Prospective multi-centre studies in metastatic breast, colorectal and prostate cancer showed that the presence of CTC were associated with poor outcome and were an independent predictor of progression free survival and overall survival.9-11 In primary colorectal cancer little is known about the role of CTC although a relation with stage has been shown. The specificity of CTC detection is lower at low frequencies making the data interpretation more difficult.12 In colorectal cancer surgery the principle of early lymphovascular ligation before manipulation of the tumour has been termed the “no-touch isolation” technique.13;14 This technique was proposed to reduce intra-operative dissemination.15 In laparoscopic colorectal surgery the no-touch principles are represented in the so-called medial to lateral approach where the supplying vessels are ligated early in the procedure. Several randomised trials comparing laparoscopic with open segmental colectomy have indicated that laparoscopic surgery can be applied safely for both benign and malignant diseases.16-21 Furthermore, it has been shown that short term cancer related outcomes such as cancer free resection margins and the number of harvested lymph nodes, as well as long term cancer related outcomes such as disease-free survival are comparable between laparoscopic and open surgery.16 However, a potential oncological benefit of laparoscopic surgery in which a median to lateral approach is applied is still debated.

The objective of this study was to determine whether CTC could be detected in peripheral and portal blood during laparoscopic and open surgery for colonic cancer. Furthermore, differences in the amount of CTC between peripheral and portal blood were assessed. Finally, the effect of the surgical approach (i.e. open versus laparoscopic) on the presence of CTC during surgery was assessed.
Methods

Study population
The current study was performed on patients with primary colonic cancer (rectal cancers were excluded) who underwent surgery with curative intent at the Academic Medical Centre, Amsterdam between August 2005 and August 2007. All patients underwent abdominal ultrasonography and chest radiography, to identify metastatic disease preoperatively. Patients with distant metastases (stage IV) were excluded. Furthermore, patients with prior abdominal surgery, emergency surgery, age over 85 years, and American Society of Anaesthesiology (ASA) score above three were excluded. Surgery was performed or supervised by a colorectal surgeon. Operations were performed with curative intent, either via midline laparotomy or via laparoscopy. All laparoscopic operations were performed according to the “no-touch isolation” technique, i.e. a medial to lateral approach with early vessel ligation. A lateral to medial approach was performed during open resections. In laparoscopic procedures pneumoperitoneum was established with CO₂ gas with an intra-abdominal pressure of 15 mmHg or less.

The majority of the patients (24/31) also participated in a randomised trial comparing open and laparoscopic surgery (ISRCTN: 79588422). Tumour stage and grading were classified according to the sixth edition of the TNM classification of the International Union Against Cancer. Informed consent was obtained from all patients. The study protocol was approved by the local medical ethics committee.

Blood collection
Peripheral whole blood samples were collected through an intravenous cannula. In left sided colectomies, portal whole blood samples were collected by insertion of a 15 centimetres long 5.5 French catheter (Arrow-Howes™ Paediatric Multi-Lumen Central Venous Line) into the inferior mesenteric vein which was advanced about 10-15 centimetres to secure position at portal vein level. In laparoscopic procedures a 30 centimetres long catheter was used which was positioned intra-abdominally through a large infusion needle. Subsequently, a small hole was created in the inferior mesenteric vein with a pair of scissors followed by insertion of the catheter laparoscopically. The catheter was advanced 10-15 centimetres to secure position at portal vein level and fixed with a purse-string suture. In case of right sided colectomy a similar procedure was performed using a venous branch of the right branch of the middle colonic vein to insert the catheter.

Blood samples were collected at four different time points which are shown in Figure 1. The first sample was collected before surgery (only peripheral, T0). Subsequently samples were taken paired-wise (peripheral and portal) after entry into the abdominal cavity and before mobilisation of the tumour bearing segment (T1), after mobilisation of the tumour bearing segment (T2), and after tumour resection (T3).
Figure 1. Time line which shows the different time points at which blood was collected. The first sample was collected before surgery (only peripheral, T0) and subsequently paired-wise (peripheral and portal) after entry into the abdominal cavity and before mobilisation of the tumour bearing segment (T1), after mobilisation of the tumour bearing segment (T2), and after tumour resection (T3).

Enumeration of CTC

Blood samples were drawn into 10 ml tubes which contained a cell preservative (CellSave Preservative Tubes, Immunicon, Huntingdon Valley, USA). Samples were maintained at room temperature and processed within 72 hours after collection. The CellSearch™ Circulating Tumour Cell Test (Veridex LLC, Warren, USA) was used for the isolation and enumeration of circulating epithelial cells. The cell detection system consists of a sample preparation and cell analysis platform that have been described elsewhere in detail. In brief, ferro-fluids coated with epithelial cell-specific EpCAM antibodies were used to immuno-magnetically enrich epithelial cells from 7.5 ml of blood. The enriched samples were stained with phycoerythrin conjugated antibodies directed against cytokeratins 8, 18, and 19, an allophycocyanin conjugated antibody to CD45 and the nuclear dye DAPI. The isolated and stained CTC were transferred to a CellTracks® Cartridge and analysed on the CellTracks® Analyser II, a four colour semi-automated fluorescence microscope (Veridex). Image frames covering the entire surface of the cartridge for four different fluorescence filter cubes were captured. From the captured images, a gallery of objects meeting pre-determined criteria was automatically presented in a web-enabled browser for interpretation by a trained operator who made the final selection of cells. The criteria for an object to be defined as a CTC included round to oval morphology, a visible nucleus (DAPI positive), positive staining for cytokeratin, and negative staining for CD45. Results of cell enumeration were expressed as the number of cells per 7.5 ml of blood. The performance of the assay system is described in detail elsewhere. In this study the presence of one or more CTC in the sample was considered CTC positive.

Fluorescence in Situ Hybridization on CTC and primary tumour tissue

To determine cytogenetics changes of the CTC and as an additional identifier, Fluorescence In Situ Hybridization (FISH) was applied to CTC containing cartridges. After the CTC were isolated and identified as described in the previous section, the CTC were labelled with fluorescent FISH probes against the centromeric region of chromosomes 1, 7, 8, and 17. The location of the previous identified tumour cells was maintained during FISH analysis.
which allows revisiting of the same cell. Fluorescence images of the FISH signals were acquired and the number of FISH spots for each of the four chromosomes was determined in each CTC. For the patients with the highest number of CTC the ploidy, on basis of the number of copies of the chromosomes 1, 7, 8, and 17, of the CTC was compared with the ploidy of the primary tumour tissue. Paraffin tissues of the primary tumour were treated and hybridized and the number of FISH spots for chromosome 1, 7, 8, and 17 were determined.

Statistics
Statistical calculations were performed using SPSS® version 12.0.2 (Statistical Package for the Social Sciences, Chicago, IL, USA). To compare categorical data the Chi-square was used. The Mann-Whitney test was used to compare continuous variables. The McNemar and Wilcoxon tests were used to test for differences between the detection rate in portal and peripheral blood with respect to the timing of blood collection. An unpaired non-parametric test was used to assess the influence of open and laparoscopic surgery on the detection rate of CTC.

Results
Study population
During the study period 38 patients were included. Subsequently, seven patients were excluded; in five patients the cannulation of the inferior mesenteric vein was not successful and a further two patients were excluded when histological evaluation of the resected specimen revealed adenomas containing no invasive carcinoma. Thirty-one patients remained for final analysis; 19 male and 12 female patients with a mean age of 67 (±12) years. Eighteen patients had a laparotomy (open-group); the remaining 13 patients were operated on laparoscopically (laparoscopic-group). There were 16 right-sided colectomies, seven left-sided colectomies, seven (recto-) sigmoid resections, and one subtotal colectomy (Table 1).
### Clinicopathologic characteristics

The clinicopathologic characteristics of the overall study population (n=31), the open-group (n=18), and the laparoscopic-group (n=13) are summarised in Table 1. Operating times were significantly longer in the laparoscopic-group compared to the open-group (153 vs. 224 minutes, *p*=0.001). Furthermore, there was a higher T-stage and more lymphovascular invasion in the open-group as compared to the laparoscopic-group, although these differences were not significant. In the laparoscopic group there was slightly more N2 disease. Finally, there were more right sided resections in the open group than in the

<table>
<thead>
<tr>
<th></th>
<th>all patients</th>
<th>open-group</th>
<th>laparoscopic-group</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age* (yrs)</td>
<td>67 (12)</td>
<td>70 (10)</td>
<td>63 (13)</td>
<td>0.21</td>
</tr>
<tr>
<td>Male:female‡</td>
<td>19 (61):12 (39)</td>
<td>1 (61):7 (39)</td>
<td>8 (62):5 (38)</td>
<td>0.98</td>
</tr>
<tr>
<td>Preoperative CEA level*</td>
<td>2.1 (2.5)</td>
<td>1.7 (1.8)</td>
<td>2.6 (3.4)</td>
<td>0.44</td>
</tr>
<tr>
<td>Operative procedure‡</td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>- right hemicolecotmy</td>
<td>16 (51)</td>
<td>12 (67)</td>
<td>4 (31)</td>
<td></td>
</tr>
<tr>
<td>- left hemicolecotmy</td>
<td>7 (23)</td>
<td>2 (11)</td>
<td>5 (38)</td>
<td></td>
</tr>
<tr>
<td>- (recto)sigmoidectomy</td>
<td>7 (23)</td>
<td>3 (17)</td>
<td>4 (31)</td>
<td></td>
</tr>
<tr>
<td>- subtotal colectomy</td>
<td>1 (3)</td>
<td>1 (5)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Operating time* (min)</td>
<td>178 (55)</td>
<td>153 (39)</td>
<td>224 (50)</td>
<td>0.001</td>
</tr>
<tr>
<td>pT stage‡</td>
<td></td>
<td></td>
<td></td>
<td>0.06</td>
</tr>
<tr>
<td>- T 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- T 2</td>
<td>5 (16)</td>
<td>1 (5)</td>
<td>4 (31)</td>
<td></td>
</tr>
<tr>
<td>- T 3</td>
<td>26 (84)</td>
<td>17 (95)</td>
<td>9 (69)</td>
<td></td>
</tr>
<tr>
<td>- T 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumour diameter* (cm)</td>
<td>4.6 (2.0)</td>
<td>5.0 (2.3)</td>
<td>4.1 (1.4)</td>
<td>0.27</td>
</tr>
<tr>
<td>pN stage‡</td>
<td></td>
<td></td>
<td></td>
<td>0.46</td>
</tr>
<tr>
<td>- N 0</td>
<td>21 (68)</td>
<td>12 (67)</td>
<td>9 (69)</td>
<td></td>
</tr>
<tr>
<td>- N 1</td>
<td>5 (16)</td>
<td>4 (22)</td>
<td>1 (8)</td>
<td></td>
</tr>
<tr>
<td>- N 2</td>
<td>5 (16)</td>
<td>2 (11)</td>
<td>3 (23)</td>
<td></td>
</tr>
<tr>
<td>Differentiation‡</td>
<td></td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>- good</td>
<td>1 (3)</td>
<td>1 (5)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>- moderate</td>
<td>24 (77)</td>
<td>12 (67)</td>
<td>12 (92)</td>
<td></td>
</tr>
<tr>
<td>- poor</td>
<td>6 (19)</td>
<td>5 (28)</td>
<td>1 (8)</td>
<td></td>
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<tr>
<td>Lymphovascular invasion‡</td>
<td></td>
<td></td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>- no</td>
<td>24 (77)</td>
<td>12 (67)</td>
<td>12 (92)</td>
<td></td>
</tr>
<tr>
<td>- yes</td>
<td>7 (23)</td>
<td>6 (33)</td>
<td>1 (8)</td>
<td></td>
</tr>
</tbody>
</table>

†Chi-square test, Mann-Whitney U test applied when appropriate; *Values are mean (SD); ‡Values are absolute numbers (%)

Table 1. Clinicopathologic characteristics of the included patients

The clinicopathologic characteristics of the overall study population (n=31), the open-group (n=18), and the laparoscopic-group (n=13) are summarised in Table 1. Operating times were significantly longer in the laparoscopic-group compared to the open-group (153 vs. 224 minutes, *p*=0.001). Furthermore, there was a higher T-stage and more lymphovascular invasion in the open-group as compared to the laparoscopic-group, although these differences were not significant. In the laparoscopic group there was slightly more N2 disease. Finally, there were more right sided resections in the open group than in the
laparoscopic group, although this difference was not significant. Regarding the other clinicopathologic characteristics the groups were comparable.

**Enumeration of CTC**

In *Figure 2* the average number of CTC in patients who underwent laparoscopic or open surgery at the subsequent time points is shown. Although the standard deviations were large, the number of CTC detected in portal blood was higher at all subsequent time points. In the samples taken at T1, CTC were identified in 7% of the peripheral samples and in 45% of the portal samples (*p*=0.002). With respect to the samples taken at T2, CTC were identified in 4% and in 31% respectively (*p*=0.031). Finally, at T3 the detection rate was 4% and 26% respectively (*p*=0.125). Subsequently, to assess the overall difference between peripheral and portal blood the dichotomous results on the three intra-operative time points were summed, resulting in a score ranging from zero (all samples negative) to three (all samples positive). The cumulative score was significantly higher in portal blood compared to peripheral blood (*p*<0.001, *Table 2*).

**Table 2.** Cumulative result of the enumeration of CTC in peripheral and portal blood on the three intra-operative time points (*p*< 0.001)

<table>
<thead>
<tr>
<th></th>
<th>peripheral blood</th>
<th>portal blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples negative</td>
<td>83%</td>
<td>28%</td>
</tr>
<tr>
<td>One sample positive</td>
<td>17%</td>
<td>45%</td>
</tr>
<tr>
<td>Two samples positive</td>
<td>-</td>
<td>27%</td>
</tr>
<tr>
<td>All samples positive</td>
<td>-</td>
<td>7%</td>
</tr>
</tbody>
</table>

The number of CTC in portal blood of patients who underwent open surgery was higher compared to patients who underwent laparoscopic surgery. According to open vs. laparoscopic surgery the percentage of samples with CTC at T1 was 0% vs. 18% (*p*=0.076) in peripheral blood and 65% vs. 36% (*p*=0.142) in portal blood respectively. At T2, 0% vs. 8% (*p*=0.288) in peripheral blood and 44% vs. 9% (*p*=0.046) in portal blood respectively. Finally, at T3, 8% vs. 0% (*p*=0.347) in peripheral blood and 29% vs. 20% (*p*=0.590) in portal blood respectively. To assess the overall difference between open and laparoscopic surgery the dichotomous results in portal blood (as this was more sensitive compared to peripheral blood) on the three intra-operative time points were added up, resulting in a score ranging from zero (all samples negative) to three (all samples positive). The cumulative score was significantly higher in the open-group compared to the laparoscopic-group (*p*=0.039, *Table 3*).

**Table 3.** Cumulative result of the enumeration of CTC in portal blood according open or laparoscopic surgery on the three intra-operative time points (*p*=0.039)

<table>
<thead>
<tr>
<th></th>
<th>open-group</th>
<th>laparoscopic-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples negative</td>
<td>17%</td>
<td>45%</td>
</tr>
<tr>
<td>One sample positive</td>
<td>44%</td>
<td>45%</td>
</tr>
<tr>
<td>Two samples positive</td>
<td>28%</td>
<td>9%</td>
</tr>
<tr>
<td>All samples positive</td>
<td>11%</td>
<td>-</td>
</tr>
</tbody>
</table>
CTC in relation to other clinicopathologic characteristics
To assess potential associations between the presence or absence of CTC and certain clinicopathologic characteristics all the available intra-operative samples, both peripheral and portal, and the cumulative score (see above) of both peripheral and portal samples, were tested in an univariate analysis. The following clinicopathologic characteristics were analysed; age, gender, operative procedure, tumour diameter, tumour differentiation grade, lymphovascular invasion, presence of lymph node metastasis per se, N-stage, and T-stage (T1-T2 vs. T3-T4). Besides a non-significant trend towards a higher number of samples with CTC during right-sided resections compared to left-sided resections (71% vs. 39%, p=0.085) there were no associations.

Morphology of the detected CTC
The tumour cells detected in peripheral blood had a morphology that was similar to the morphology in other carcinoma’s described elsewhere. Different morphology was observed for CTC detected in portal blood at T1 and T2. Cells present in these samples looked frayed and appeared mostly as sheets and clumps of cellular material in which the
individual cells were difficult to discriminate. No difference in morphology between the laparoscopic and open group was observed.

**FISH**

As the morphology of the CTC in portal blood at T1 and T2 deviated, the ploidy of these cells was determined as an additional tumour cell identifier, using chromosome 1, 7, 8 and 17. The CTC all contained two copies for each of the chromosomes. Subsequently, the primary tumour tissue ploidy using chromosome 1, 7, 8 and 17, was compared to that of the CTC. For each of the tested chromosomes the tumour tissue cells displayed also two copies which was equal to the detected CTC. So, ploidy of the CTC and tissue of the primary tumour did match, however both were diploid.

**Discussion**

The present study shows that the presence of CTC was significantly increased intra-operatively and was significantly higher in portal blood (26-45%) as compared to peripheral blood (4-7%). With respect to the surgical approach, the percentage of CTC containing blood samples was significantly lower in the portal blood sample after mobilisation of the tumour bearing segment during laparoscopic surgery compared to open surgery (9% vs. 44%, p=0.046). Others that assessed the presence of CTC intra-operatively, also found significant higher detection rates in portal blood (50-65%) compared to peripheral blood (11-49%).

Theoretically, tumour cells must pass through the liver, lungs and the microcirculation of other tissues before they pass into the systemic venous circulation. Animal studies have demonstrated that 80-100% of injected human colon carcinoma cells were trapped in the capillary bed of the first organ encountered. Furthermore, a higher prevalence in portal blood might be explained by the fact that there is some dilution in the larger blood volume of the peripheral circulation. Yamaguchi et al. found a PCR-positive (CEA, cytokeratin 20) detection rate of 31% in mesenteric blood and 15% in peripheral blood (p=0.033). The presence of CEA and cytokeratin 20 mRNA in peripheral blood was not correlated with survival. However, with respect to portal blood uni- and multivariate analysis indicated that it was the only independent prognostic factor (hazard ratio 13.6, p=0.028). Various techniques have been described in the literature to study CTC and the large differences in the reported results bring in question whether the same events are being studied. We choose to use the CellSearch system for CTC evaluation as it has been validated and used in prospective multi-centre trials. In this study the presence of one or more CTC in the sample was considered a CTC positive sample. This low threshold was chosen because of the significant relationship between the presence of one or more CTC and poor outcome in patients with metastatic disease as shown by others. The specificity of the CellSearch system is limited by the users ability to assign an event as a tumour cell. We therefore tried to improve the specificity by investigating the ploidy of
the detected tumour cells. In the cases examined, however, both CTC and primary tumour tissue were diploid for chromosome 1, 7, 8, and 17 thus not providing a definite answer whether or not the detected CTC were true tumour cells or normal epithelial cells shed into the portal blood through surgical trauma.

Since a considerable number of patients with resectable colorectal cancer subsequently develop metastatic disease, dissemination is likely to be an early event that may happen pre- or intra-operatively and is not detected by conventional staging techniques.\(^\text{35}\) In metastatic breast, colorectal and prostate cancer the presence of CTC was associated with a short progression free and overall survival and predicted treatment efficacy earlier and more effectively than imaging in breast cancer and PSA in prostate cancer.\(^\text{9,11,34}\) Nevertheless, the presence of CTC does not necessarily predict subsequent metastatic disease as this process is very inefficient. Fidler reported that less than 0.1% of tumour cells placed into the circulation survived to produce metastatic lesions in animals. However, activation of blood coagulation and relative immune suppression due to the surgical stress might enhance the metastatic potential of these intra-operatively spilled cells.\(^\text{35,36}\) So far, several studies have demonstrated a negative effect of CTC on prognosis.\(^\text{27,37-39}\) Furthermore, in some studies the presence of CTC was found as the only independent prognosticator.\(^\text{32,40}\) Nevertheless, others have found no association between CTC and survival.\(^\text{28,41}\) A major difficulty in interpreting these results is the variety of detection methods, the small sample sizes of the individual studies, and the relatively short follow-up.\(^\text{33}\)

In the present study, less CTC were found in the laparoscopically operated patients. Both the open-group and laparoscopic-group were comparable with respect to most clinicopathologic characteristics, with the exception of a non-significant trend towards more right-sided resections, a higher T-stage, and more lymphovascular invasion in the open group, and longer operating times and more N2 disease in the laparoscopic-group. Some have found an association between the presence of CTC and a higher T-stage, lymph node involvement, poorer differentiated tumours, and lymph- and blood vessel invasion. However, in these studies it was also demonstrated that in patients with early-staged cancers the detection rates were also considerable.\(^\text{27,28,35}\) Moreover, in the present study it was demonstrated that the only factor influencing the detection rate, besides the location of sampling (i.e. peripheral vs. portal), was the surgical approach (i.e. laparoscopic or open surgery) and that the other analysed factors were not associated with the presence or absence of CTC.

It could be hypothesised that laparoscopic surgery with a medial to lateral approach with early lymphovascular ligation is the ultimate form of no-touch surgery. In our open-group a lateral to medial approach was applied, therefore this group could be considered as ‘conventional surgery’. The results obtained in the present study therefore support the no-touch isolation technique rather than that the results suggest an oncological benefit of laparoscopic surgery itself. In a recent review on the no-touch technique the overall conversion rates (i.e. negative pre-operatively, positive intra-operatively) for studies employing the no-touch technique were 0–16%, compared to 0–80% for studies utilising conventional surgery.\(^\text{15}\) Comparable to the present study, Bessa et al. assessed neoplastic cell mobilisation in 50 patients who were randomly assigned to open or laparoscopic...
surgery. In contrast to the present study, the no-touch isolation technique was applied in both groups. In Bessa’s study, surgical neoplastic cell mobilisation was observed in only four patients, with no difference with respect to the surgical approach.\(^{42}\) Therefore, the detection rate of CTC might be unrelated to the surgical approach (i.e. laparoscopic or open surgery) but related to the moment of lymphovascular ligation as these data suggest that there is a trend towards reduced tumour cell dissemination for the no-touch isolation method. The benefit of this in terms of improved patient survival remains to be established.\(^{15}\) In conclusion, the detection rate and quantity of CTC is significantly increased intra-operatively and is significantly higher in portal blood compared to peripheral blood. Significantly less CTC were detected during laparoscopic surgery probably as result of the medial to lateral approach.

**Acknowledgement**

We would like to thank dr. J.B. Reitsma from the department of Epidemiology and Biostatistics for his statistical advise.

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Circulating tumour cells during laparoscopic and open surgery


