Utility of Cell-free Tumour DNA for Post-surgical Follow-up of Colorectal Cancer Patients

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Abstract. Background: While efficient surgical treatment is the key to prolonged survival of patients with colorectal cancer, post-surgical follow-up is important for the early detection of relapsing disease or of disease progression. Current dispensarization, typically based on imaging CT, PET, MR, is frequently supported by the observation of tumour markers (CEA, CA19-9). Due to their limited sensitivity and selectivity, better tools for monitoring of the disease are desirable. Tumour cell-free DNA (cfDNA) has been recently demonstrated as a new promising molecular marker for observation and early detection of disease progression. Patients and Methods: We present results of post-surgical monitoring tumour cfDNA in the cases of seven patients suffering from advanced forms of CRC. We applied a mutation-based approach in which the total cfDNA was screened for a specific somatic mutation present in the primary tumour. We screened a panel of the most frequent somatic mutations covering the genes APC, KRAS, TP53, PIK3CA and BRAF. All patients were tested positive for tumour cfDNA prior to surgery. cfDNA was then evaluated within a week after surgery and subsequently in monthly intervals. Results: We present typical cases of colorectal cancer patients who underwent surgical treatment at different levels of radicality with or without adjuvant chemo/biotherapy. The tumour cfDNA status was found to be always closely correlated with the actual clinical status of the patient. Conclusion: The cfDNA appears to be a viable tool for the monitoring of the clinical progression of CRC in patients with cfDNA positivity prior to surgery.

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the remaining non-tumour cfDNA, naturally present in the plasma (18). The most common approach to the examination of tumour cfDNA is based on tumour-specific mutations. A panel of somatic mutations is first evaluated in the primary tumour. The presence of tumour cfDNA is then detected upon occurrence of the tumour-specific mutations in the plasma (19, 20).

It was a primary objective of the current study to investigate the presence of tumour cfDNA and its correlation to the clinical course of the disease. We applied a routine mutation detection in order to monitor tumour cfDNA levels during post-surgical follow-up. We present several clinical cases, in which tumour cfDNA monitoring may become an important part of the clinical management of the disease.

Patients and Methods

The group of patients consisted of four females and three males 54 to 76 years of age, with clinically confirmed colorectal cancer and detectable tumour cfDNA prior to surgery. There were three rectal, two rectosigmoidal, one sigmoidal and one splenic flexure tumour in stages III or IV. All patients were directed to either curative or palliative surgical treatment. For each patient, multiple samples were acquired including an initial plasma sample obtained one day prior to surgery, tumour tissue sample collected during the surgery and up to three more plasma samples, acquired during the subsequent follow-up.

The plasma was separated from the blood cells by centrifugation, immediately after blood collection, and was then frozen at −20°C before transport to the laboratory. cfDNA was isolated from the plasma by NucleoSpin Plasma XS (Macherey-Nagel, Dueren, Germany). The detection of tumour cfDNA was based on a mutation-specific approach in which the plasma is screened for the presence of specific somatic mutations previously found in the tumour tissue. Thus firstly the presence of any of the most frequent, somatic mutations was examined in the tumor tissue collected during the surgery. The mutation panel included APC (Adenomatous polyposis coli, mutation cluster region), KRAS (Kirsten rat sarcoma viral oncogene homolog, exon 1), TP53 (tumor protein 53, exons 5-8), PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide, exon 9 and exon 20) and BRAF (v-Raf murine sarcoma viral oncogene homolog B1, exon 15). For each patient, only mutations detected in the tumour tissue were evaluated in the plasma samples.

All mutation detections were performed by a denaturing capillary electrophoresis technique (DCE) using Genoscan mutation detection kit (Genomac International, Prague, Czech Republic) in which a fluorescently labelled PCR, amplified regions of up to 140 bp fragments and separated them in forms of homo- and heteroduplexes at a precise given temperature on a standard DNA sequencer (ABI PRISM 3100, Applied Biosystems, Foster City, California) (21, 22). The technique provides the relative fraction of mutated alleles and has previously been applied for the detection of mutants in various tumour tissues (23-25).

Overall, seven samples from primary tumour tissue and 35 plasma samples were examined, resulting in a total of 119 mutation analysis tests performed. In addition to tumour cfDNA, the levels of standard tumour markers CEA and CA19-9 were also assessed.

Results

Data regarging the monitored patients are summarized in Table I. The levels of tumour cfDNA were characterized according to the estimated fraction of mutated alleles present in the plasma cfDNA sample as follows: (−) no mutated alleles found; (+) ca. 5% of mutated alleles; (+++) ca. 5% –30% of mutated alleles and (++++) more than 30% of mutated alleles. There were three cases (two in Stage III, one in Stage IV) of curable R0 resections (including one total pelvic exenteration) with subsequent long-lasting periods without detectable tumour cfDNA and no signs of disease progression. In addition there was one case of stage IV disease with a curable R0 resection without detectable tumour cfDNA after surgery, but a re-appearance of tumour cfDNA and detection of disease progression seven months after the surgery. Finally, levels of tumour cfDNA fluctuated in one patient with stage IV disease, in palliative chemotherapy following an R2 pelvic exenteration and during palliative biological therapy of inoperable stage IV tumour. The detailed descriptions of the clinical courses of the disease for individually monitored patients follow.

Patients after R0 resection with no signs of relapse.

A) Female, 62 years old, operated on for sigmoidal colon cancer invading the small bowel (T4N0M0), in whom an en bloc R0 resection was performed. No neoadjuvant chemotherapy was indicated, and preoperative tumour marker levels were 1.3 μg/l CEA and 14.4 kU/l CA19-9. Tumour cfDNA was identified in the plasma prior to surgery. Adjuvant chemotherapy was administered. Neither in the clinical postoperative course, nor during cfDNA detection were there any signs of relapse.

B) Female, 76 years old, operated on for synchronous splenic flexure adenocarcinoma and segment VI liver metastasis. No neoadjuvant chemotherapy was indicated and a simultaneous left colectomy with liver resection was performed (R0). Preoperative staging was cT3NXM1hep with tumour markers of 3.4 μg/l CEA and 14.1 kU/l CA19-9. The postoperative course was uneventfull and adjuvant chemotherapy was administered. Tumour cfDNA, detected prior to surgery, disappeared after surgery and remained undetected in consecutive postsurgical blood samples. Twenty seven months after surgery, the patient remains without clinical signs of relapse, as shown by PET/CT, colonoscopy and tumour markers.

C) Male, 68 years old, operated on for obstructive ileus, rectosigmoidal cancer invading the small bowels and the bladder, with rectovesical fistula. R2 enterooanastomosis and sigmoido stomy was performed. Radiotherapy and chemotherapy were contraindicated due to the malnutrition and the poor general condition. With 35.3 μg/l CEA and 12.4 kU/l CA19-9, radical surgery was indicated and a supraleaver total pelvic exenteration was performed. The operation included
triple small bowel resection, including resection of internal iliac vessels. No adjuvant oncological therapy was indicated due to sacral bed sore. Histology confirmed massive tumour infiltration from the rectosigmoideum to the bladder, pT4N0M0, and poorly differentiated mucinous tubular carcinoma. Tumour cfDNA was only detected prior to pelvic exenteration, but remained undetected in sampling following surgery. Twenty nine months after the surgery, the patient has fully recovered with no clinical, CT, colonoscopy or marker signs of relapse.

Patients with subsequent disease progression after R0 resection.

D) Male, 59 years old, indicated for laparoscopic sigmoidostomy and insertion of port catheter due to stenosing rectal cancer with multiple liver metastases before neoadjuvant chemotherapy (T3N0M1hep). Initial marker levels were 8.4 μg/l CEA and 79.1 kU/l CA19-9. After downsizing of the tumour (2.2 μg/l CEA, 31.7 kU/l CA 19-9), radical R0 surgery was performed with simultaneous low anterior resection, with 11 non-anatomical resections for the liver metastases. The postoperative course was without complications and the patient continued chemotherapy. Tumour cfDNA was detected preoperatively as shown in Figure 1A, and was undetected at the days following the surgery (Figure 1B). Six months after the surgery tumour cfDNA was again detected and the CT scan revealed multiple lung metastases, as shown in Figure 1C, also confirmed by histology exams. The patient is continuing chemotherapy with good performance 17 months after the original surgical treatment.

Table I. Comprehensive overview of all patients showing cfDNA positivity prior to surgery and their subsequent monitoring after surgery.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, gender</th>
<th>Treatment</th>
<th>Marker</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>Clinical status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tumor site</td>
<td>stage(+)</td>
<td></td>
<td>(&lt;1 week)</td>
<td>&lt;1 month</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>62y female</td>
<td>sigmoid colon</td>
<td>en bloc R0 resection</td>
<td>cfDNA +</td>
<td>–</td>
<td>No signs of progression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T4N0M0</td>
<td>adjuvant chemo</td>
<td>CA19-9 –</td>
<td>–</td>
<td>18 months after surgery</td>
</tr>
<tr>
<td>B</td>
<td>76y female</td>
<td>Primary + liver mt splenic flexure</td>
<td>R0 resection</td>
<td>cfDNA ++ –</td>
<td>–</td>
<td>No signs of progression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3N1M1hep</td>
<td>adjuvant chemo</td>
<td>CA19-9 –</td>
<td>–</td>
<td>27 months after surgery</td>
</tr>
<tr>
<td>C</td>
<td>68y male</td>
<td>rectosigmoid</td>
<td>R0 pelvic exenteration</td>
<td>cfDNA ++ –</td>
<td>–</td>
<td>Full recovery with no signs of relapse 29 months after surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T4N0M0</td>
<td>–</td>
<td>CA19-9 –</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>59y male</td>
<td>Primary + liver mt rectum</td>
<td>R0 resection</td>
<td>cfDNA ++ –</td>
<td>–</td>
<td>Lung metastases detected 7 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T3N0M1hep</td>
<td>adjuvant chemo</td>
<td>CA19-9 –</td>
<td>–</td>
<td>after surgery, alive on chemotherapy</td>
</tr>
<tr>
<td>E</td>
<td>54y female</td>
<td>rectosigmoid</td>
<td>R2h resection</td>
<td>cfDNA ++ –</td>
<td>–</td>
<td>12 months from surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T4N1M1hep</td>
<td>–</td>
<td>CA19-9 –</td>
<td>+</td>
<td>Adjuvant chemotherapy and biological therapy with slow progression,</td>
</tr>
<tr>
<td>F</td>
<td>62y male</td>
<td>Inoperable rectum</td>
<td>palliative chemotherapy</td>
<td>cfDNA ++ –</td>
<td>–</td>
<td>death 24 months after surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T4N2M1hep</td>
<td>–</td>
<td>CA19-9 –</td>
<td>+</td>
<td>Palliative biological therapy with slow progression,</td>
</tr>
<tr>
<td>G</td>
<td>71y female</td>
<td>Neoadjuvant biological rectum</td>
<td>R2 pelvic exenteration</td>
<td>cfDNA ++ –</td>
<td>+</td>
<td>death 25 months after surgery</td>
</tr>
<tr>
<td></td>
<td></td>
<td>T4N2M1hep</td>
<td>–</td>
<td>CA19-9 –</td>
<td>+</td>
<td>Palliative chemotherapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>with steady progression, death</td>
</tr>
</tbody>
</table>

cfDNA –) undetectable –) <5 μg/L –) <40 U/mL
+ ) ca. 5% mutated alleles +) 5-10 μg/L +) 40-1000 U/mL
++ ) ca. 30% mutated alleles ++) >10 μg/L ++) >1000 U/mL
+++ >30% mutated alleles +++ >100 μg/L.

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Patients with subsequent disease progression after R2 resection.

F) Male, 62 years old at the time of surgery, was admitted to the surgical department for rectal bleeding; rectal cancer was confirmed by biopsy. CEA was 26.9 μg/l and CA19-9 was 1668 kU/l. PET/CT described multiple liver and pulmonary metastases. The primary tumour had invaded the prostate (cT4NXM1hep, pulm). During surgery, multiple diffuse inoperable liver metastases were confirmed and palliative sigmoidostomy was performed (R2). Tumour cfDNA was positive before as well as after surgery. The postoperative course was uneventful (CEA=16.1 μg/l, CA19-9=2989 kU/l). Palliative chemotherapy was administered and the tumour markers decreased (CEA to 4.4 μg/l, CA19-9 to 78.4 kU/l), accompanied by tumour cfDNA temporary disappearance. Despite this, CT demonstrated a gradual slow progression of both liver and lung secondary tumours. Tumour cfDNA reappeared one year after the surgery and the mutated allele fraction steadily increased over the subsequent year. The patient died 25 months after the operation date.

G) Female, 71 years old, was diagnosed with synchronous rectal cancer invading the rectovaginal septum with three liver metastases and a histologically confirmed duplicity of bilateral ductal breast cancer (cT4NXM1hep). A rectal adenocarcinoma with poor differentiation was diagnosed; biopsy from the liver secondaries proved their rectal origin, CEA was 26.9 μg/l and CA19-9 2.8 kU/l. Neoadjuvant therapy was indicated with concomitant chemoradiotherapy and bevacizumab biological therapy. When the tumour progressed and became symptomatic, surgery was indicated. Subsequently, abdominoperineal resection with en bloc hysterectomy and resection of the part of the infiltrated vagina was performed.
Due to the extent of the disease, simultaneous liver resection was not indicated. Histology confirmed pT4, pN2 (13 positive nodes), pM1. The postoperative course was complicated with deep ileofemoral thrombosis. Even after the change of the chemotherapy regimen, permanent progression of the disease was confirmed clinically and by CT (CEA=1740 μg/l, CA 19-9=313 kU/l). The liver metastases doubled in diameter and number within two months and pulmonary metastases also appeared. The patient died 18 months after diagnosis. Tumour cfDNA was detected preoperatively and during the postoperative chemotherapy.

Discussion

A high level of free circulating DNA is generally considered to be a negative prognostic factor. In patients who underwent curative resection of liver metastases from colorectal cancer, a high level of free circulating DNA was reported to be a predictive factor of recurrence (26). In our study, during the follow-up, tumour cfDNA typically disappeared after curative R0 surgery, while in patients with non curative treatment (R2 surgery or palliative surgery) tumour cfDNA remained detectable immediately after operation. In the case of concurrent resection of the primary tumour and of hepatic metastases (Patient D), the tumour cfDNA was undetectable one week after surgery, but reappeared in a sample acquired seven month after the surgery. The patient was subsequently examined by CT and a progression of the disease, through new liver metastases, was confirmed.

Although the actual levels of mutated allele fractions in plasma cfDNA do not correlate with the tumour stage among different patients, they seem to closely follow the actual development of the disease in each individual. As seen in Table 1, a progression of the disease was typically translated into an increasing fraction of mutated cfDNA alleles in plasma (patients D and E), (Figure 1). At the same time, the outcome of anticancer therapy can be observed as a decrease in the tumour cfDNA levels, for positive response to biological palliative therapy, up to six months after the surgery. In contrast, no response to palliative chemotherapy is evident from the steadily increasing levels of mutated cfDNA (Patient G).

When comparing to the levels of CEA and CA19-9, the tumour cfDNA appears to be a more universal marker for monitoring of the clinical course of the disease, correlating to either of the two tumour markers. Indeed, such a notion needs to be confirmed by the study of additional patients.

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References


