Circulating tumor cells in patients undergoing surgery for hepatic metastases from colorectal cancer

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Circulating tumor cells (CTCs) have been detected in patients with a variety of metastatic cancers, including colorectal, and may be a significant prognostic variable in patients with liver metastases. This prospective study involved 20 patients (13 men and 7 women) undergoing surgical excision or ablation of liver metastases from a colon or rectal primary tumor. Four 7.5-mL vials of peripheral blood were drawn preoperatively, 2 weeks postoperatively, and during mobilization of the liver or at the beginning of radiofrequency ablation. The samples were centrifuged, the sera combined to a final volume of 7.5 mL, and the CellSearch system used to identify circulating epithelial cells. A CTC count >2 was defined as clinically significant. Preoperative CTC levels averaged 3.9 (range, 0–56) and were significant in 2 patients (10%). Postoperative CTC levels averaged 1.0 (in 18 patients; range, 0–9) and were significant in 1 patient (5%). Intraoperative CTC levels averaged 28.2 (range, 0–315) and were significant in 10 patients (50%). At a median follow-up of 11.5 months (range, 5–25), 6 patients (30%) were dead of disease, 6 patients (30%) showed no evidence of disease, and 8 patients (40%) were alive with disease. Statistical analysis suggested a correlation between the presence of postoperative CTCs and survival ($P = 0.036$), as well as with disease-free survival ($P = 0.036$). Thus, CTCs are present and quantifiable in many patients with colorectal hepatic metastases, and peripheral CTCs are present in greater quantity during intraoperative liver manipulation. This preliminary study suggests a relationship between the presence of postoperative CTCs and outcome. Further accrual and follow-up of this group is needed to confirm these findings.

The liver is often the only site of metastatic disease in patients with colorectal cancer, and such patients are considered for local therapies such as liver resection or tumor ablation. The 5-year survival rate for surgically treated patients is approximately 30% (1). Disease recurrence may be related to intraoperative factors such as the shedding of tumor cells or may be a manifestation of clinically occult disease.

Circulating tumor cells (CTCs) in patients with hepatic metastases from colorectal cancer have been identified preoperatively and postoperatively, and intraoperative levels have been shown to correlate with tumor relapse (2, 3). These studies have used the polymerase chain reaction (PCR) detection method of cytokeratin-20 (CK-20)–positive cells to detect CTCs. However, this method is not commercially available.

A commercially available assay (CellSearch, Veridex, LLC, Warren, NJ) has recently been approved by the US Food and Drug Administration for the detection of CTCs in metastatic breast cancer. This method has been used to show that pretreatment CTC levels independently predict progression-free and overall survival in metastatic breast cancer (4).

The objective of this study was to identify the frequency of CTCs preoperatively, intraoperatively, and postoperatively in patients undergoing surgery for colorectal hepatic metastases using the commercially available CellSearch assay from whole blood. In addition, we examined the effect of operative technique on the presence of CTCs and the correlation of CTC counts with other indicators of recurrence and survival.

METHODS

Patients

Patients with a prior diagnosis of adenocarcinoma of the colon or rectum who had documented hepatic metastases treatable with liver resection or tumor ablation were enrolled in this prospective study at Baylor University Medical Center in Dallas, Texas.

Patients were preoperatively assessed with a computed tomography (CT) scan of the chest, abdomen, and pelvis and a positron emission tomography (PET) scan to evaluate the extent of disease. Magnetic resonance imaging (MRI) was used selectively. Patients with an intact primary tumor undergoing a combined resection were eligible for the study, as were patients with limited extrahepatic disease, provided that it was believed to be surgically resectable. Data points evaluated included the Fong prognostic criteria for colorectal liver metastases as well as intraoperative tumor and resection status (1). The Fong criteria, which have been described in detail, provide a score from 0 to 5, with higher scores associated with increased risk of recurrence. In general, complete

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Table 1. Circulating tumor cell counts and clinical data for 20 patients with colorectal cancer and liver metastases

Patient	Age	Fong score	Complete resection?	Ablation, resection, or both	Circulating tumor cells			Disease Status	Follow-up (months)	Disease-free survival (months)
					Preop	Intraop	Postop			
1	46	2	No	Both	1	3	9	DOD	7	3
2	77	0	No	Both	1	0	ND	DOD	22	6
3	53	3	Yes	Ablation	1	51	0	DOD	12	0
4	44	0	No	Both	2	1	1	DOD	5	5
5	51	0	Yes	Resection	2	10	1	AWD	23	4
6	51	2	Yes	Both	2	7	2	AWD	20	20
7	57	2	Yes	Resection	0	0	LF	AWD	11	10
8	57	0	Yes	Ablation	0	3	0	NED	18	18
9	59	1	No	Ablation	1	33	0	AWD	18	18
10	76	3	Yes	Resection	2	6	1	AWD	10	5
11	54	1	Yes	Ablation	0	46	2	NED	7	7
12	67	1	Yes	Ablation	0	2	0	NED	17	17
13	70	0	Yes	Both	1	315	0	DOD	6	6
14	58	1	Yes	Resection	0	0	0	NED	15	15
15	39	3	Yes	Resection	0	0	2	AWD	8	8
16	45	5	No	Both	56	115	2	AWD	12	5
17	54	0	Yes	Resection	0	2	0	NED	10	10
18	41	0	Yes	Both	1	2	0	DOD	8	1
19	81	1	Yes	Both	1	1	0	AWD	8	5
20	52	1	Yes	Resection	3	1	0	NED	25	25

ND indicates not drawn; LF, lab failure; DOD, dead of disease; AWD, alive with disease; NED, no evidence of disease.

resection of metastases was the surgical goal, and ablation was utilized as needed in patients with unresectable disease, significant medical comorbidities, or significant extrahepatic disease encountered during surgery. The resection was considered complete if all gross disease was resected or ablated. The resection was considered incomplete if residual disease was left in situ following the surgical procedure or if there was a positive resection margin.

Blood samples

Four 7.5-mL vials of peripheral blood, for a total volume of 30 mL, were drawn on three separate occasions: preoperatively, intraoperatively, and postoperatively. Preoperative levels were drawn from a peripheral vein before induction of anesthesia. Postoperative levels were drawn 14 days (± 2 days) after the procedure via the peripheral vein. Intraoperative levels were drawn peripherally during mobilization of the affected hepatic lobe if the patient was undergoing resection, or 1 minute after initiation of radiofrequency ablation (RFA) of the largest lesion. All samples were maintained at room temperature and processed within 72 hours. A 30-mL blood draw was used to enrich the population of positive samples for study. The technique for concentrating the 30-mL aliquot of blood has been previously described (5).

CTC identification

The CellSearch system was utilized to isolate and enumerate CTCs from the blood samples. This assay has previously been described and validated (6). A semiautomated system (CellPrep system) was used for the sample preparation. The sample was then enriched with magnetic beads, which were coated with antibodies for epithelial-cell adhesion molecule. Nuclear dye 4′,6-diamidino-2-phenylindole was used to fluorescently label cells. Fluorescently labeled monoclonal antibodies specific for epithelial cells (CK-20) and leukocytes (CD45) were used to isolate epithelial cells from leukocytes. The CellSpotter Analyzer was used to identify and enumerate CTCs by allowing a computer-generated reconstruction of the actual CTCs. CTCs were defined as nucleated cells lacking CD45 and expressing CK-20. In general, prior data suggest that patients without a malignancy are rarely found to have <2 CTCs based on these criteria (6). Data were expressed as an integer with >2 CTCs per 30-mL sample defined as significant.

Follow-up

Patients were followed with office examination and laboratory evaluation including carcinoembryonic antigen as well as CT scans every 3 months for 2 years or until recurrence. PET and MRI were used selectively. Patients were defined as having recurrent disease based on definitive imaging or biopsy.

Statistical analysis

Statistical analysis compared preoperative, intraoperative, and postoperative CTC levels to Fong score, disease status, survival time, and disease-free survival time. Fisher's exact test was used for Fong score comparison and disease status. Kaplan-Meier estimator was used for survival and disease-free survival analysis. All analyses were univariate. A type I error rate of 0.05 was set for the study.

Table 2. Relationship of preoperative, intraoperative, and postoperative circulating tumor cell counts to other variables in 20 patients with colorectal cancer and liver metastases

Covariate	Time of CTC	P value*
Fong score	Preop	0.368
	Intraop	0.582
	Postop	1.000
Disease status	Preop	1.000
	Intraop	0.297
	Postop	0.300
Liver procedure	Intraop	0.088
	Postop	0.131
Overall survival	Preop	0.119
	Intraop	0.798
	Postop	0.036
Disease-free survival	Preop	0.446
	Intraop	0.248
	Postop	0.036

*Fisher's exact test used for Fong score and disease status; Kaplan-Meier estimator used for liver procedure, overall survival, and disease-free survival.

RESULTS

Patients

Twenty patients were enrolled in the trial (13 men and 7 women), with a mean age of 57 years. Fourteen patients (70%) presented with synchronous disease, and 6 patients (30%) presented with metachronous disease. The mean Fong score of the 20 patients was 1.3 (range, 0–5). Seventeen patients (85%) were treated with chemotherapy before surgery, and 7 patients (35%) received treatment after surgery. Chemotherapy regimens used preoperatively included FOLFOX (n = 1), FOLFOX-Avastin (n = 12), FOLFIRI (n = 2), and 5-fluorouracil (5-FU)-leucovorin (n = 2). Preoperative assessment of tumor response to chemotherapy included progression in 6 patients (35%), partial response or stable disease in 8 patients (47%), and complete radiographic response in 3 patients (18%). Surgical treatment included hepatic resection (n = 7, 35%), RFA (n = 5, 25%), and both resection and RFA (n = 8, 40%). Fifteen patients (75%) were felt to have complete removal or ablation of all disease. Morbidity included pneumonia (n = 1), bleeding (n = 1), ileus (n = 2), acute renal failure (n = 1), and biloma (n = 1). There was no perioperative mortality.

CTC identification and enumeration

CTC counts and other clinical data for the 20 patients are presented in *Table 1*. Preoperatively, 2 patients (10%) had significant CTCs. The mean preoperative CTC count was 3.9 (range, 0–56). Postoperatively, 1 of 18 patients (6%) had significant CTCs. The mean postoperative CTC count was 1 (range, 0–9). There was one laboratory failure postoperatively, and one patient had no postoperative lab draw.

Intraoperatively, 10 patients had significant CTCs (50%). The mean intraoperative count was 28.2 (range, 0–315). Of the 7 patients who underwent resection only, 2 patients (29%) had significant CTC counts, with a mean count of 3 (range, 0–10). Of the 5 patients who underwent RFA alone, 4 (80%) had significant CTCs, with a mean count of 27 (range, 2–51). Of the 8 patients who underwent both hepatic resection and RFA, 4 (50%) were positive for CTCs, with a mean count of 55 (range, 0–315). Eight of the 13 patients (62%) who were treated with RFA or both RFA and resection were positive for CTCs intraoperatively.

Follow-up

Patients' median follow-up time was 11.5 months (range, 5–25). At the time of last follow-up, 6 patients (30%) were dead of disease, with a median survival of 7.5 months. Six patients (30%) were without evidence of disease, with a median survival of 16 months. Eight patients (40%) were alive with disease, with a median survival of 11.5 months (Table 1).

The correlation of CTCs to other variables is summarized in *Table 2*. The detection of CTCs during or after surgery could not be predicted by patients' preoperative clinical risk assessment. The detection of CTCs at any of the three time points was not predictive of disease status. The type of liver procedure, resection only versus a type of ablative technique, was not predictive of overall survival. Detection of CTCs preoperatively and intraoperatively was not predictive of disease-free or overall survival. However, the presence of CTCs postoperatively was predictive of both disease-free survival and overall survival in this group of patients.

DISCUSSION

Detection

CTCs in patients with hepatic metastases from colorectal cancer have successfully been identified in operative patients using reverse-transcription PCR (RT-PCR) (2, 3). These studies used RT-PCR for CK-20 from peripheral blood and bone marrow samples. Weitz et al showed that CTCs were detectable by this method preoperatively in 23.7% of patients and postoperatively in 28.9% of patients (2). RT-PCR for CK-20 is very sensitive and may suffer from false-negative readings due to a high level of detection of "background" CK-20 (7–9). The current study used an established, commercially available assay. The preoperative detection rate of CTCs with the CellSearch method is comparable to that with RT-PCR as described by Weitz (10% vs 23.7%).

The intraoperative presence of CTCs has been shown to correlate with tumor recurrence (3). It has been implicated that the dissemination of CTCs is the probable cause of recurrence. At this point in this small sample, this study did not show a correlation between intraoperative CTC levels and survival or disease-free survival. It is clear from these data that CTCs are detectable using the CellSearch assay perioperatively in patients with colorectal liver metastases.

Type of surgery

One variable evaluated in this study was the effect of tumor manipulation on CTC levels intraoperatively. In considering the

classic "no touch" or "Halsted" approach to cancer surgery, one might speculate that tumor cells are shed into the blood during liver manipulation or tumor ablation. Indeed, 50% of patients in this study had detectable CTCs intraoperatively compared with 10% preoperatively. In addition, 62% of the patients who were treated with RFA had significant CTCs intraoperatively. This raises the possibility that ablation leads to higher rates of intraoperative hematogenous tumor cell dissemination than resection. This was not borne out statistically; however, individual cases exhibited very high levels of hematogenous CTCs during the ablation procedure. Statistically, the type of liver surgery was not predictive of outcome in this small group. Prior studies have suggested that patients treated with liver ablation suffer recurrence more often than similar patients treated with resection (10).

The current study found a significant relationship between the postoperative presence of CTCs and disease-free and overall survival. This is very similar to the data demonstrated in breast cancer. Cristofanilli et al showed that the presence of CTCs using CellSearch in patients presenting for adjuvant chemotherapy following breast surgery predicted relapse in these patients (4). Similarly, the association between persistently elevated carcinoembryonic antigen following colorectal surgery and resection of colorectal liver metastases has also been well documented (11, 12). From a clinical standpoint, postoperative detectable CTCs may prove to be the most relevant value, as it may suggest clinically occult disease.

One limitation of the current study is the small sample size. Clearly, the association of CTCs and survival data, although intriguing in this preliminary subset, needs to be validated in a larger trial. These data were also generated using a pooled sample of 30 mL of peripheral blood as opposed to the 7.5 mL used in most studies validating the CellSearch assay. The number of CTCs and possibly the detectability of CTCs in this group may have differed if the 7.5 mL sample size were used. The median follow-up in this preliminary cohort was 11.5 months, and these patients will need continued surveillance to determine their overall outcome and the utility of the CellSearch assay.

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