

Circulating tumor cells in gastrointestinal malignancies

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Abstract: Circulating tumor cells (CTCs) are involved in the metastatic process and have been detected in malignancies of epithelial origin. Several isolation techniques have been developed for the detection of CTC. Herein, we review the current literature regarding cytometric and nucleic acid based CTC detection in different stages of gastrointestinal cancers, including colorectal, pancreatic, hepatobiliary, esophageal, gastric, and small intestine malignancies, along with their potential role in prognosis and management.

Key Words: Esophagus; stomach; gastric; pancreas; colorectal; hepatocellular; liver; biliary; cholangiocarcinoma; small intestine; CellSearch; circulating tumor cell (CTC)



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Introduction

Metastatic disease causes over 90% of cancer mortality (1). The first report of tumor cells found in the circulation of a patient with metastatic cancer was published nearly 150 years ago (2). These circulating tumor cells (CTCs) are thought to have potential metastatic activity or even originate from cancerous stem cells (3). Due to the prognostic and predictive potential, CTCs possess several techniques, cytometric (capturing individual cells) and nucleic-acid based [targeting messenger Ribonucleic Acid (mRNA) expression], have been developed in recent years to detect, isolate, enumerate, and/or characterize CTC in solid organ malignancies. These methods need to be sensitive, specific, reproducible, and overcome challenges of detecting rare tumor cells out of hundreds of thousands of peripheral blood cells. During the last two decades immunocytochemistry, flow cytometry, and reverse transcription- polymerase chain reaction (RT-PCR) have been used for these purposes (4,5).

During the past decade several new cytometric techniques have emerged, which rely on the different morphological and molecular characteristics of epithelial tumor cells. Common techniques for CTC enrichment (isolation) and detection (identification) include: (I) cellular

density gradient separation of CTC and mononuclear cells (MN) [OncoQuick® (Greiner, Frickenhausen, Germany)] followed by immunolabeling; (II) Isolation by size of epithelial tumor cells (ISET) that involves direct filtration (mechanical separation through polycarbonate membrane with 8 µm pores) and immunolabeling; and (III) Immunomagnetic separation (IMS). The latter is the most commonly performed enrichment technique, using either classic separation methods: MACS® [Magnetic Activated Cell Separation (Miltenyi Biotec, Auburn, CA, USA)] followed by immunolabeling and genetic or molecular characterization by fluorescence in situ hybridization (FISH) or RT-PCR; Dynabeads® (DynaL Biotech, Invitrogen) with anti-EpCAM/Epithelial Specific Antigen (anti-BerEP4) antibodies (Abs) [IgG1 monoclonal Ab to Epithelial Cell Adhesion Molecule (EpCAM)]; or the semi-automated CellSearch® (Veridex, Raritan, NJ, USA) system that utilizes immunomagnetic beads coated with EpCAM Abs (capturing EpCAM-expressing epithelial tumor cells), followed by immunostaining for 4',6-diamidino-2-phenylindole (DAPI) (nucleated cell) and CK8+/-18+/-19 presence (epithelial cell) along with CD45 (leukocyte marker) absence (5-7). New innovative detection methods have been investigated, such as the CTC-chip (8), based on

microfluidic platforms.

The prognostic implications of CTC detection and monitoring have been a matter of great controversy partially due to small patient populations and diverse detection techniques, protocols, and assays. Although the US Food and Drug Administration (FDA) approved the CellSearch® system in metastatic breast, prostate, and colorectal cancers (9) based on its prognostic value (10-12), use of the system has not been incorporated into clinical guidelines in metastatic colorectal cancer (mCRC) in terms of serial monitoring or clinical management.

Our objective is to review the usefulness, sensitivity, and specificity, as well as prognostic value, of CTC detection in both early and metastatic stages of the various gastrointestinal malignancies. The article outlines CTC status in colorectal, pancreatic, hepatobiliary, esophageal, gastric, and small intestine cancers, as well as neuroendocrine tumors.

Colorectal cancer

Multiple studies, mainly in the perioperative setting, examined CTC status in mCRC and non-metastatic colorectal cancer (nmCRC) via molecular and cytometric techniques.

Detection rates of CTC in mCRC are between 18.7% and 75% (6,13-22). Patients without detectable CTC post chemotherapy (regardless of their pre-chemotherapy status) survived longer (17). The two larger studies in mCRC showed that CTC status was an independent predictor of progression free survival (PFS) and overall survival (OS) among 430 (13) and 467 (14) patients. Those who became seronegative during treatment [after 3-5 weeks (10) versus 1-3 (14) weeks, respectively] had improved PFS and OS than those who remained positive. The discrepancy between the two studies was attributed to the use of bevacizumab in the latter study, yet it remains unclear when a repeat CTC count needs to be completed. Additional studies, with fewer patients, reported comparable results, indicating the importance of CTC detection. The CellSearch® system received the FDA approval in 2007 for mCRC monitoring with positive cutoff of ≥ 3 CTC/7.5 mL (10). Postoperative positive CTC are associated with decreased PFS and OS (22). CTC detection increases during intraoperative liver manipulation (22) and also after colonoscopy (23), without clear clinical significance. In any stage colorectal cancer (CRC) the cytometric detection rate was 32% (of 93 patients) (24) and associated with advanced

disease (19,20) and peritoneal dissemination (21). Reduced CTCs counts following therapy correlated with response (in mCRC) (18) or remission (24), whereas elevated levels were associated with recurrence or disease progression (24), as well as reduced PFS and OS (18).

Wong *et al.* (9) used refined CK20-Dynabeads® protocol and detected CTC in 57% of 101 nmCRC patients pre-operatively, with correlation to disease stage and early recurrence. Gazzaniga *et al.* (25) reported decrease in CTC counts from median number of 2.7 to 0 post 3-4 months of chemotherapy and bevacizumab in 89% of 20 mCRC patients, yet 56% of them, developed disease progression. In the control group, treated with chemotherapy and cetuximab, 30% remained CTC positive (≥ 3) with disease progression in all of them. 93% of the remainder 14 patients with decreased CTC counts [0-2] were found to have stable disease or partial response. This phenomenon led the researchers to conclude that bevacizumab was likely associated with biological changes of CTC, perhaps endothelial mesenchymal transformation (EMT), thus undetectable by CellSearch®.

Detection of CTC was shown to depend on the IMS prior to CK20 RT-PCR, with intra-patient heterogeneity based on the beads-coated- EpCAM Abs used (26). A study that compared four different non-automated enrichment techniques in 38 patients with mCRC showed superiority of MACS® HEA MicroBeads over RosetteSep® (StemCell Technologies, Vancouver, Canada), OncoQuick®, and OncoQuick® Plus. Detectable CTC was associated with reduced PFS but similar OS (27).

Coumans *et al.* (28) explored 428 mCRC patients and predicted that 99% of patients will have at least 1 CTC in 5 L of blood at baseline and one month following chemotherapy, consistent with the low treatment success rates in metastatic cancer. They calculated survival decreased by 6.6 months for each 10-fold increase in CTC in metastatic cancer.

Several novel cytometric detection methods have been developed and studied in recent years. Fabbri *et al.* (29) used DEPArray (a di-electrophoresis-based platform), to detect CTC in 52.5% of 40 mCRC patients. Zhang *et al.* (30) developed an electrospun TiO₂ nanofibers (TiNFs) platform and detected CTCs in 2 out of 3 patients with CRC. Sheng *et al.* (31) developed an aptamer-mediated, micropillar-based microfluidic device (as an alternative to Abs) for capturing CTC from whole blood. The device was able to isolate as few as 10 colorectal

CTC from 1 mL of unprocessed whole blood, with about 93% captured cell viability. Pecot *et al.* (32) developed a microfluidic-based platform that is able to capture CTC in their post-EMT stage. The system includes a combination of Abs against epithelial cell surface antigens [i.e., EpCAM, HER2, MUC1, Epidermal growth factor receptor (EGFR)] and mesenchymal cell antigens (c-MET, N-cadherin, CD318, and mesenchymal stem cell antigen) and was shown to be more sensitive for CTC enumeration than CellSearch®. Desitter *et al.* (33) report the development of the ScreenCell®-single-use device which can filter, isolate, and sort CTC by size in a costly fashion and allows further biological analysis. Du *et al.* (34) tested a novel microfluidic capturing chip and detected CTC in 67.7% of 68 CRC patients: 22.2% of 9 patients with Dukes A, 41.6% of 24 with Dukes B, 95.5% of 22 with Dukes C, and in 100% of 13 with Dukes D (metastatic disease). CTC status correlated with clinical stage. Marrinucci *et al.* (35) used immunofluorescent staining with fiber-optic array scanning method to detect CTC in mCRC, and found significant intrapatient pleomorphism, yet with CTC correlation to the primary or secondary tumor samples. Yen *et al.* (36) developed KRAS membrane array and showed that mutation status in detected CTC can predict response to chemotherapy plus cetuximab in 76 mCRC patients and affect PFS and OS. The group (37) improved the gene expression array and developed weighted chemiluminescent membrane array for the detection of CTC harboring KRAS mutations. They reported increased sensitivity, specificity, and accuracy rates of 90.2%, 94.9%, and 93.5%, respectively.

Heitzer *et al.* (15) performed genomic profiling of CTC and showed mutation correlation between CTC, the primary tumor, and metastases, thus creating new possibilities for peripheral biopsy via CTC analysis. Fabbri *et al.* (29) showed KRAS mutational concordance between CTC and the primary tumor in 50% of mCRC patients. However, Gasch *et al.* (16) showed considerable intra- and interpatient molecular characterization heterogeneity in EGFR, KRAS, and PIK3CA gene mutations, as well as EGFR expression. CTC-genes in mCRC patients are associated with cell movement, adhesion, signaling, death and proliferation, and their expression correlates with clinical and prognostic factors (38).

On the other hand, cytometric methods produced very low detection rates in nmCRC (39). Using the CellSearch® system, CTC detection rate in nmCRC is between 0% and 25.7% (16,19-21,39).

Studies that explored CTC detection via molecular techniques, mainly RT-PCR, examined mostly carcinoembryonic Antigen (CEA) (40-47), CK19 (40,42,43), CK20 (40-45,48-51), and survivin (45,52) mRNA. No single method was proven to be superior to others. Molecular CTC detection with RT-PCR was reported in a meta-analysis that included seven studies in 4% to 57% of 1,260 patients with nmCRC (53) and is associated with reduced OS (48) and PFS (43,48,53,54). Uen *et al.* (55) explored post-operative CTC detection with a multipanel marker (CEA/hTERT/CK19/CK20 mRNA) in 194 patients with stage II CRC. They found 27-fold hazard ratio for relapse in patients with positive CTC status. A membrane array with the latter panel was tested in 157 patients with nmCRC showed reduced PFS and OS (56).

Multimarker CEA/CK/CD133 mRNA assay was not prognostic in 735 patients post curative surgery with Dukes A or B with predetermined favorable features (42). However, in Dukes B and C it is associated with reduced OS (40,42,57) and reduced PFS (40,42), suggesting the need for adjuvant therapy post curative surgery (42,54).

A recent meta-analysis (58) looked at CTC and disseminated tumor cells (DTC) in CRC with resectable liver metastases or widespread metastases and analyzed twelve studies (representing 1,329 patients). Positive CTC status had a 2.5-fold increased chance of death and a twofold increased chance of disease progression or recurrence (yet only PFS was statistically significant). In 2010, a meta-analysis (59) pooling 3,094 nmCRC patients from 36 studies, utilized different molecular and cytometric methods and showed reduced PFS and OS.

A PCR-based method, the transcription-reverse transcription concerted reaction (TRC) technique, was found to be comparable with the CellSearch® system in terms of CTC detection and correlation with OS in mCRC patients, yet the former was a quicker and cheaper alternative (60). Gervasoni *et al.* (61) compared the detection rates of CTC in CRC patients using three different methods. The detection rates were 75% in a multimarker RT-PCR assay, 20% in the CellSearch® system, and 14.3% in a dHPLC-based gene mutation analysis. None of the negative RT-PCR samples was found to be positive by the other two methods, indicating it provided the best CTC detection rate.

Bessa *et al.* found no significance to perioperative CTC detection via CEA mRNA RT-PCR in nmCRC (62,63). Positive CTC status was associated with disease recurrence (49) and reduced survival in colorectal liver

metastasis patients undergoing radical liver resection, suggesting the need for adjuvant therapy (52). In any CRC stage, positive CTC is associated with clinical stage (44,45,50), reduced OS (44,64), and reduced PFS (50).

In conclusion, there is need to standardize and improve the sensitivity and specificity of the available CTC detection methods (65). Randomized trials are required to compare the standard monitoring versus CTC-guided management of patients with CRC. Currently only the CellSearch® system is FDA-approved. Prospective studies with tailored therapy based on CTC status are warranted to improve outcomes in subgroup of patients who require adjuvant therapy.

Pancreatic cancer

Several studies have evaluated the use of the CellSearch® system. In the setting of advanced or metastatic disease, detection rates of 40% to 80.5% has been reported in various studies using the CellSearch® system or other IMS methods followed by RT-PCR (66-68). ISET (69) had a CTC detection rate of 90% *vs.* 40% using the CellSearch® system in patients with inoperable or metastatic pancreatic cancer. High definition CTC assay (HD-CTC) is a new technique using different immunofluorescence staining and high resolution cell imaging. It detected more CTCs when compared to the CellSearch® system (5/15 *vs.* 4/15) in non-gastrointestinal cancers (70). CTC detection has been shown to negatively predict prognosis and response to chemotherapy (67). In a recent study (68) using the CellSearch® system and immunologic marker for MUC1 and EGFR in patients with metastatic pancreatic cancer treated with first line chemotherapy (n=40); ≥ 1 CTC per 7.5 mL peripheral blood were detected in 50%, 39% and 28% at baseline, 7 days and 6-10 weeks, respectively. Patients who have a positive CTC status at baseline had worse OS and PFS compared to CTC negative patients (125 *vs.* 298 days, $P=0.12$). De Albuquerque *et al.* (71) compared CTC detection between pancreatic cancer patients (n=34) and healthy controls using IMS with MUC1 and EpCAM Abs followed by RT-PCR. CTC was detected in approximately half of the cancer patients and none of the control group. PFS was 66 days for CTC positive patients compared to 138 days for CTC negative patients ($P=0.01$) showing that CTC detection is associated with poor prognosis.

CTC detection rates in non-metastatic resectable pancreatic cancer are currently limited. Intra-operative CTC detection by CEA mRNA via RT-PCR correlated with hematogenous metastasis after surgery (37.5% in CTC

positive group *vs.* 11.4% in the negative group). The OS among stage I, II, and III patients was worse in the CTC positive group ($P=0.03$) (72).

CTC detection is shown to correlate with lower OS and PFS in pancreatic cancer. Further research is needed to increase the accuracy of CTC testing, thereby facilitating its use in early stage cancer, detection of micrometastasis and monitoring residual disease.

Hepatobiliary cancer

Studies utilizing cytometric methods for CTC detection in hepatocellular carcinoma (HCC) are scarce. Vona *et al.* (73) evaluated the clinical significance of ISET for CTC detection in patients with non-metastatic HCC compared to a control group comprising of chronic hepatitis, cirrhosis and healthy subjects. CTC was detected in 23 out of 44 patients with HCC and none in the control groups. CTC detection correlated with tumor invasion, portal tumor thrombosis, and reduced survival. Sabile *et al.* (74) mixed blood of human volunteers with HepG2 liver tumor cells and analyzed the efficiency of various isolation techniques. They showed that density gradient separation and ber-Ep4 immuno-capture are the most sensitive techniques for capturing liver CTC *in vivo*. When Ber Ep4 immunobeads are used after density gradient centrifugation followed by RT-PCR, ≥ 10 HCC CTC/5 mL peripheral blood were detected (75). Waguri *et al.* (76) used telomerase reverse transcriptase (hTERT) mRNA to detect 10^{0-1} CTC in 2 mL blood of HCC patients after IMS. Fifty-three percent of cases were CTC positive compared to none in the control group. Zee *et al.* (77) evaluated the CellSearch® system for CTC detection in 20 patients with locally advanced or metastatic HCC patients. Nine patients had detectable CTC (≥ 2 CTC per 7.5 mL of blood). Schulze *et al.* (78) studied the CellSearch® system in 59 HCC patients and 19 control patients (with cirrhosis and benign hepatic lesions). Detection of ≥ 1 CTC was found in 18 HCC patient versus a single control patient ($P=0.026$). Identification of CTC correlated with worse OS (460 *vs.* 746 days, $P=0.017$). Further studies are needed to validate the system in HCC.

Molecular detection methods have utilized mainly alpha fetoprotein (AFP) mRNA (76,79-81), along with hTERT (82) and CK19 (83) mRNA. AFP mRNA based CTC detection ranges between 10% to 54% and can positively predict recurrence and extra-hepatic metastasis (4). Use of AFP mRNA as a marker for HCC is limited because of high false positive rates due to detection in patients with chronic liver

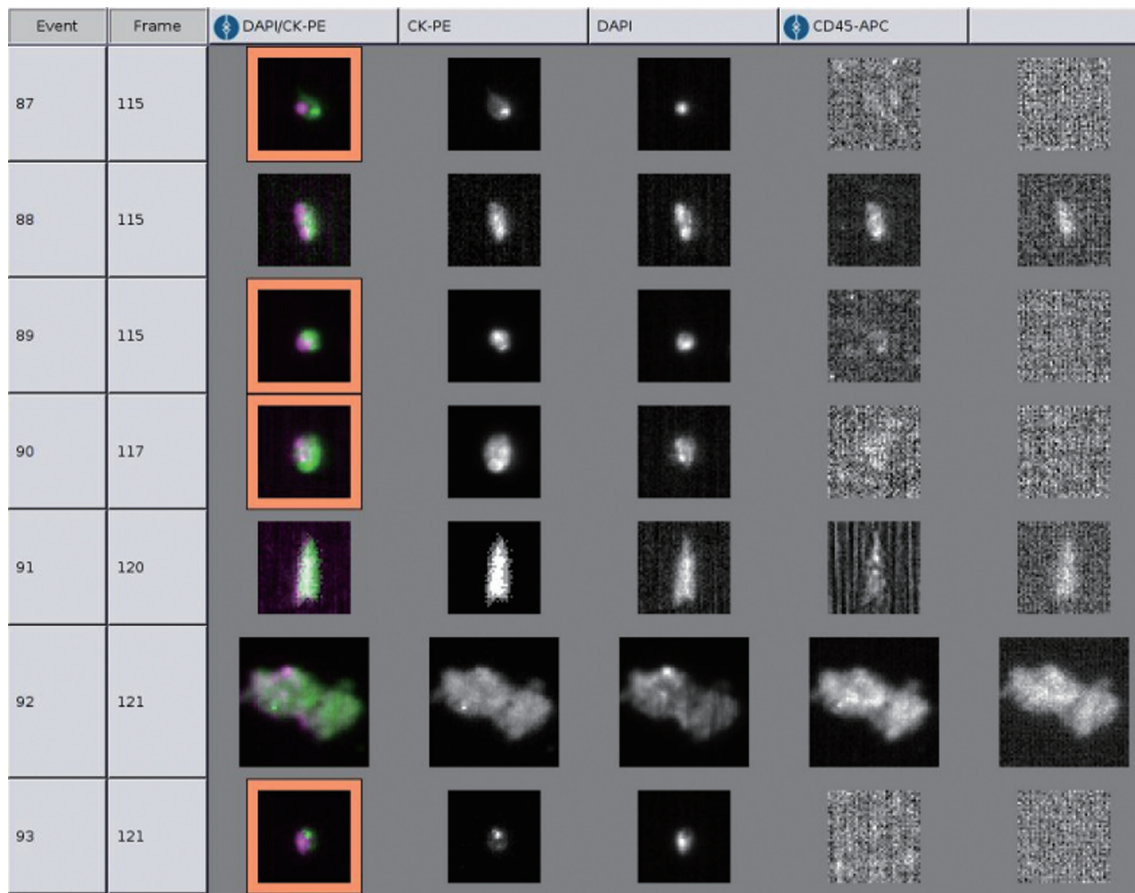


Figure 1 Selected representation of CTCs isolated from peripheral blood of one of our patients with biliary cancer by immunomagnetic capture and analyzed by fluorescent microscopy. CTCs are positive for CK and DAPI and negative for CD45

disease (12.5%) (84). In a recent study (82), pre-operative peripheral blood hTERT mRNA did not predict recurrence in patients with HCC following resection or transplant.

CTC could be a promising method for surveillance of HCC and assessing eligibility for liver transplantation. Specific hepatic CTC markers are currently lacking. Further research is warranted to find a marker that has good efficacy in detecting liver derived CTC.

Moreover, the data regarding CTC detection in biliary cancer are also minimal. We (85) recently reported the use of CellSearch® system to detect CTC from 16 patients with cholangiocarcinoma and gallbladder cancer (*Figure 1*). Using a cutoff of >1 CTC/7.5 mL blood, four patients were found to have positive CTC status, all in stage III or IV. At 12 months of follow up, 25% of patients with positive CTC compared to 50% of patients with negative CTC were alive. Although it was not a statistically significant result, it served as a proof of concept in this rare disease. Prospective

validation is currently ongoing for this matter.

On the other hand, molecular techniques utilizing CK19 or hTERT mRNA were used to detect CTC in 40 biliary cancer patients. CTCs were found in 45% of the patients and were associated with worse OS (86). A cohort of pancreaticobiliary cancer patients was studied after curative resection (n=53) using RT-PCR to detect CEA mRNA. Positive CTC status was found in 75% of relapsed patients but only in 5.4% in disease-free patients, suggesting that it might indicate early relapse (87). Using the same technique in biliary cancer patients, intraoperative detection of CEA mRNA was associated with recurrence and worse survival (72).

Esophageal and gastric cancer

Cytometric detection has been rarely reported in esophageal cancer (21,88). Hiraiwa *et al.* (21) detected CTC with the CellSearch® system in 38 patients. CTC detection

did not correlate with tumor stage, however, in metastatic disease, it correlated with dissemination and decreased survival. De Albuquerque *et al.* (89) used IMS with MUC1 and EpCAM Abs followed by RT-PCR analysis of a multi-marker gene panel (KRT19, MUC1, EpCAM, CEACAM5, BIRCS, SCGB2A2, and ERBB2). Nakamora *et al.* (88) enriched blood samples of 47 squamous cell carcinoma (SCC) patients via Dynabeads® following immunostaining with anti-CK Abs. Eighteen patients (38%) were CTC positive. Four of 7 (57%) positive patients who underwent surgery and 2 out of 26 (7.7%) negative patients suffered from recurrence. Those who remained positive post chemotherapy (with or without radiation) had decreased survival rates than those who became CTC negative.

Most esophageal CTC detection studies utilized molecular techniques, mainly RT-PCR for the detection of tumor-specific mRNA. Researchers often examined CEA (47,90-97), but also SCC (90,98), cytokeratin (CK)7 (91), CK19, CK20, and survivin mRNA (92,99,100). CTC detection was also reported in primary malignant melanoma of the esophagus by RT-PCR (101). CEA or SCC mRNA were detected in 13.9% of 244 patients prior to esophagectomy and 16.8% post-surgery, possibly due to cell dissemination during surgery. CTC status post-surgery was an independent prognostic factor of PFS and was a predictor for local and systemic recurrence (90). CEA, CK19 and Survivin mRNA were detected in 54.2% and 38.9% of 72 patients with SCC by nested RT-PCR prior and post radiotherapy, respectively. Positive CTC post-radiotherapy was an independent poor prognostic factor (92). Detection rates are heterogeneous between studies, even for identical markers. CEA mRNA was reported in 61.6% (of 125 SCC perioperative patients) (95); 28.3%, 60.4%, and 42.9% (before, immediately after, and three days following surgery in 53 patients) (94); 25% (of 28 patients) (96); and 57.4% (of 54 SCC patients) (97). SCC mRNA was reported in 33% and 45.8% of 70 patients with SCC prior to curative esophagectomy and intraoperative, respectively (98). Nearly all of these studies correlated positive CTC status with poor prognosis, including reduced OS (99,100), post adjuvant therapy relapse (100), advanced disease (93,95,97), recurrence and shortened DFS (95).

Cytometric detection again has been rarely reported in gastric cancer (21,102). Hiraiwa *et al.* (21) demonstrated with the CellSearch® system that positive CTC status correlated with advanced stage, peritoneal dissemination, and decreased survival. The group also showed that CTC counts were higher in metastatic disease (55%) versus non-metastatic disease (14%). Matsusaka *et al.* (102)

prospectively examined CTC with the CellSearch® system in 52 patients with advanced cancer prior to and following chemotherapy, showing decreased OS and PFS in CTC positive patients. De Albuquerque *et al.* (89) used IMS with MUC1 and EpCAM Abs followed by RT-PCR of a multi-marker gene panel, showing detection rate of 62.2%. Hosokawa *et al.* (103) developed a size-selective microcavity array followed by image-based immunophenotypic analysis using a fluorescence microscope, with high detection efficiency of over 80%. The system was found beneficial for detection of EpCAM-negative CTC. As mentioned (under CRC), Zhang *et al.* (30) developed an electrospun TiO₂ (2) nanofibers (TiNFs) platform with anti-EpCAM assay and detected CTC in 7 out of 7 patients.

Again, most CTC detection studies utilized molecular techniques, mainly RT-PCR for mRNA expression. Most researchers examined CEA (47,104-114), CK7 (114), CK18 (110), CK19 (108-111,113-115), CK20 (51,109,110), hTERT (108,109,116), MUC1 (108,116,117), and survivin (111,118) mRNA, without significant evidence to support one method over the other. CEA mRNA was reported in 24.1% at diagnosis and 34.4% at 10.6 months follow-up in 29 patients with gastric adenocarcinoma (104). It has been reported in 18.8% of 16 resectable patients and in 100% of 10 metastatic patients (47). Other studies reported a range of detection between 7.7% and 78.6% (105-107,109,112-114). CTC detection has been associated with outcome and poor survival (108,110,112,118,119). Positive CTC status is associated with tumor invasion depth (105,108,117), major vascular invasion (113), metastatic disease (105,114), recurrence (107,110,112,120), advanced disease (112,117-119), and post-operative metastases (108,109,117). Conversion to a positive CTC status is associated with recurrence post curative resection (107). A multi-marker panel was an independent predictor for postoperative metastasis or recurrence with a sensitivity and specificity of 89.1% and 91.3%, respectively (108). Positive status was shown to correlate with shorter survival among chemotherapy-non-responders (115). In a blinded prospective study in 810 Japanese gastric cancer patients, positive CTC status along with increased VEGFR-1 expression were associated with metastatic disease (114).

Gastroenteropancreatic neuroendocrine tumors

This subset of neuroendocrine tumors (29) is a diverse group of cancers that secrete various amines and peptides, has heterogeneous clinical manifestations, and strongly

Table 1 Comparison between the two methods for detection of CTC

Variable	Cytometric assays	Nucleic-acid based assays
Specificity	+++	+/-
Sensitivity	++	+++
Automated	Semi-automated	-
Stability for further analysis	++	-
Reproducibility	++	-

expresses EpCAM. Khan *et al.* (121) utilized the CellSearch® system to explore CTC status in 42 midgut neuroendocrine tumors (NET) (including 26 ileal and 7 appendiceal), 3 gastric, and 19 pancreatic NET patients, divided into progressive and non-progressive disease per imaging studies. CTC was positive in 43% and 21% of metastatic midgut and pancreatic NET, respectively. CTCs was found to be associated with disease progression. Also, an increase in more than 33% in the number of CTC following one month of therapy was associated with poor prognosis (122). Later the group conducted a single-center prospective study and reported prevalence of CTC (≥ 1) in 60.3% of 146 patients with metastatic NET (85.7% of 42 pancreatic NET, 50.5% of 101 midgut NET, and 33.3% of 3 hindgut NET). Detection of CTC is associated with advanced stage, high serum chromogranin A level, high tumor burden, as well as worse OS and PFS (123).

Small intestine malignancy

The data on the use of CTC in small intestinal carcinoma are limited. De Albuquerque *et al.* (89) used IMS with MUC1 and EpCAM Abs followed by RT-PCR analysis of the abovementioned multi-marker panel and showed detection rate of 33.3% of small intestine adenocarcinomas.

Limitations

The field of CTCs is rapidly evolving yet still faces many challenges on the path to finding the optimal method of detection for prognostication purposes (*Table 1*). Some of the challenges arise from the heterogeneity of different tumors biology, therefore different detection cutoff definition has been used even for a single technique (94). Another topic that was discussed above is the notion of EMT, where some CTCs do not express epithelial markers such as EpCAM and consequently could not be recognized by the current methods (21). This could be surpassed by using improved techniques or targeting different antigens

other than CK or EpCAM (32). These issues in addition to differences in sample handling and processing has led to inconsistent research results. Also, the current methods are able to detect a “cancer cell” yet current studies are still ongoing to phenotypically or genetically characterize those CTCs (124). Each of the available methods has advantages and disadvantages. Where cellular methods such as CellSearch® provide high specificity in semi-automated fashion, RT-PCR molecular techniques provide better rates of detection, yet lacking the reproducibility or FDA approval and the ability to further test the identified cells (53).

In conclusion, detection of CTCs may provide prognostic and predictive tool in gastrointestinal malignancies. Large clinical trials are warranted similarly to those conducted in CRC to clearly define the utility of these techniques in clinical practice.

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