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Review Article

Evaluating the Potential for Circulating Tumor Cells as a Diagnostic and Prognostic Biomarker in Various Cancer Types

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ABSTRACT

Circulating tumor cells (CTCs) are cells that can be found circulating in the peripheral blood of cancer patients. They originate from primary solid tumors and are thought to contribute to metastases and poor prognosis. Since cancer treatment is shifting toward greater personalization, a major goal in the field is the development of less invasive and more cost-effective measures in diagnosis, staging, treatment, prognostic implications, and surveillance of cancer. Utilizing CTCs as a biomarker from a "liquid biopsy" or sample of patients' blood would be transformative in accomplishing this goal. In this review, we aim to critically assess current pre-clinical and clinical literature over the past two decades implicating CTCs' potential for use as a predictive biomarker in various cancer types either in addition to or instead of current standards of care. We also are interested in understanding several aspects of CTCs including the role CTCs play in resistance to treatment, the immune system evasion properties of CTCs, the feasibility of using CTCs in clinical practice, and the utility of CTCs for predicting outcomes including patient survival. Furthermore, here we discuss gaps in the literature, limitations of CTCs, potential for their other uses, as well as the significance of CTC detection in patients following surgery.

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Introduction

Circulating tumor cells (CTCs) are cells found circulating in the peripheral blood of cancer patients that originate from primary solid tumors and are involved in metastatic spread following epithelial-mesenchymal transition (EMT) and shedding [1, 2]. The pathologist Thomas Ashworth was the first person to identify CTCs in a deceased patient in 1869, as well as proposed their origin from patients' own cancer masses [3]. CTCs are now widely accepted to be the origination of distant metastasis based on Steven Paget's 19th-century "seed and soil" theory of metastasis from 1889, which attempted to explain the complex interactions between tumor cells and their microenvironment in the human body [4-6]. Since that time, various theories have been investigated for the exact biological mechanisms that allow CTCs to produce metastatic disease, which are beyond the scope of this review [7].

The amount of CTCs detectable in human blood samples is very low, with variability in numbers across cancer subtypes [8]. Typically, they are very rare in the bloodstream and have a short half-life [9]. Thus, maximizing sensitivity and specificity of CTCs from patient samples is critical in drawing appropriate conclusions for their utilization. Remarkable improvements in detection and counting methods during the past two decades attest to the future feasibility and potential use of CTCs as multifunctional biomarkers from a "liquid biopsy," as simply drawing blood is more low-risk than conducting invasive tissue biopsies, which are the current standard of care in patient diagnosis and treatment [10].

Most importantly, CTC detection and quantification has significance as it can aid clinicians and surgeons in assessing diagnosis, staging,

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prognosis, treatment response, disease relapse, surveillance, and even guiding therapeutic intervention in various cancer types including breast, lung, colorectal, pancreas, and other cancer subtypes. Here in this review, we aim to provide a critical evaluation of various pre-clinical and clinical studies that have played a role in the detection and utilization of CTCs in the previously mentioned aspects of ongoing patient care. In addition, we aim to address gaps in the literature, the role of CTCs in treatment resistance and immune system evasion, their importance in predicting survival outcomes, cost-effectiveness, and their potential for future use either as a supplement to or as a replacement for invasive procedures in patient care.

A Comparison of Circulating Tumor Cells to Other Liquid Biopsy Biomarkers and Solid Tumor Biopsy

Currently, cancer diagnosis primarily incorporates methods such as patient history, physical exam, imaging studies, gross pathology, histological staining, and biomarker detection from either blood or histological patient samples, all of which vary based on tumor subtype [11]. Much of the information necessary to make a diagnosis is obtained from solid tumor biopsies and subsequent pathological analysis.

Solid tumor biopsies are invasive procedures that have associated risks for patients, including risk of bleeding, infection, allergic reaction to anaesthesia, cardiovascular complications of anaesthesia, nerve damage, and damage to other tissues enhancing morbidity. It is also an area of intense debate whether or not the physical act of obtaining tumor biopsies can cause cancer cells to "seed," meaning to dislodge and spread to regions they otherwise would not have spread as a result of the trauma of removing the biopsy needle [12]. Solid tumor biopsies also require a high level of technical skill to obtain and can require a repeat biopsy if performed poorly. Some tumors are located more centrally within patients, involving highly complex anatomy or dangerous vasculature that is difficult to see with conventional imaging, making these tumors not amenable or accessible to local biopsies without significant risk of complications. In order to determine whether solid tumor biopsy can be replaced purely by liquid biopsy and eliminate risks to the patient, it is prudent to initially use liquid biopsy as a complement to solid tumor biopsy and compare their results. In a recent study comparing molecular analyses from both solid and liquid biopsies across 351 patients with stage IV solid tumors, the authors found that in 86% of the patients, solid and liquid biopsies provided differing molecular information, leading them to suggest the two should be performed together routinely for complete tumor characterization [13].

Markers obtained via a liquid biopsy that have been studied include exosomes, microRNA, non-coding RNA, tumor-educated platelets, as well as circulating tumor DNA (ctDNA) and protein markers CA27-29 and CA15-3, for breast cancer, CEA for colorectal cancer, CA19-9 for pancreatic cancer, and PSA for prostate cancer [14, 15]. In a recent review, the authors evaluated literature comparing CTCs to circulating tumor DNA (ctDNA) in terms of their feasibility as a liquid biopsy in assessment of treatment response and resistance to chemotherapy. They speculated that while replacement of tumor biopsies with liquid biopsies seems unrealistic at present, ctDNA and CTCs will provide a useful tool when invasive biopsy is not feasible or when serial assessment of patients is required such as measuring a predictive biomarker for response to immunotherapy [16].

Enrichment, Isolation and Enumeration of Circulating Tumor Cells

As CTCs circulate in the bloodstream in very low levels, typically between 1 and 10 CTCs in 1 ml of whole blood of patients with metastatic disease, maximizing sensitivity and specificity as well as creating quick, easy sampling methods is important to sort out CTCs from the billions of other cell types also circulating in peripheral blood [17]. Detection is accomplished based on the process of enrichment, isolation, and enumeration which rely on physical properties of cells such as density and size, as well as immunogenic and surface properties.

Enrichment refers to the process of increasing number of tumor cells following capture in sample volume within a background of contaminating cells. Main forms of enrichment include immunocapture, size-based, and density-based, each with their own advantages and disadvantages. Positive enrichment of CTCs is accomplished with immunomagnetic devices or CTC surface markers (EpCAM, CD133, HER2, CSV, PSMA), and negative enrichment removes other cell types by targeting their cell surface markers with antibodies using filters with 7-8 pores that allow selective passage of CTCs [18].

In order to ensure precise and accurate diagnostic and prognostic data utilizing CTCs in various cancer subtypes, methods for detection need to be consistent among enrichment and detection steps. While there are numerous detection methods that vary in CTC isolation specificity, the most extensively studied and widely used method is CellSearch© by Veridex, as it has been FDA approved for breast, colorectal, and prostate cancer. This method enriches cells using a magnetic ferrofluid containing antibodies to the epithelial surface adhesion molecule [19]. The remaining cells are then stained for lack of expression of cytokeratin and CD45 [20-22]. This method, while advantageous for detecting CTCs, poses a challenge for identifying CTCs that lack the EpCAM surface marker [23]. Furthermore, unfortunately, cancer cells with a high potential for epithelial to mesenchymal transition and, consequently, metastatic behavior, tend to lose the EpCAM marker, which complicates detection of these cells using CellSearch© [24].

Following enrichment, CTCs must be enumerated, or singled-out from other cell types potentially still present in the sample. Various cytometric or nucleic acid-based strategies can be employed based on a certain marker, then enumerated for more specific detection [25]. Another method called Size of Tumor cells, ISET© detects CTCs based on size [26]. Screencells© increases sensitivity, however, it shows decreased specificity for targeting CTCs [27]. Politaki *et al.* described a method their team developed involving a real-time quantitative polymerase chain reaction (RT-qPCR) assay for detection and Ficoll isolation for CK19 mRNA for metastatic breast cancer CTC detection, which identifies a set of patients with worse prognosis [28-30]. Also, this group also used immunofluorescent assay using immunofluorescent microscopy using A45-B/B3 and CD45 antibodies. Another method recently developed by Kamal *et al.* utilizes a size-based exclusion principle for CTC enrichment which allows more sensitive capture of CTCs with minimal biological bias [31].

Circulating Tumor Cells in Cancer Detection, Diagnosis and Staging

Breast cancer is the most commonly diagnosed malignancy among women globally, and treatment guidelines place a strong emphasis on prevention and recurrence [32]. Numerous studies have been conducted to determine the utility of CTCs in the diagnosis of breast cancer. A study in 108 metastatic breast cancer patients was done to investigate the use of CTCs in diagnosis and prognosis using the label-free microfluidic platform Clear CellFX. The study authors found a detection rate of 75.9% prior to neoadjuvant therapy, with significantly worse progression free survival (PFS) in patients with CTC counts ≥ 5 CTCs/7.5 ml blood than those with <5 CTCs/7.5 ml blood (median PFS 4.3 vs 7 months; p = 0.037). The prognostic relevance was more significant in HER2 positive patients (median PFS 4.1 vs 8.3 months; p = 0.032) and patients who had received neoadjuvant therapy (median PFS 4.2 vs 7 months; p = 0.02). Baseline CTC levels (HR 1.84, p = 0.02) and pre-treatment status (HR 1.87, p = 0.05) were independent prognostic factors in this study [33].

While staging of breast cancer is primarily performed through physical exam, imaging, and clinical and surgical pathology, there is a growing body of evidence for assessing patient breast cancer stage using blood biomarkers, including the role CTC count could eventually play. However, more research needs to be done before this can become the gold standard, as it is problematic that CTC count varies with diagnosis and response to treatment. As a consequence of this irregularity, since 2007, ASCO has recommended CTC count not be used for diagnostic interpretation or treatment modifications [34].

Lung cancer is the 2nd most common type of cancer worldwide and is the leading cause of cancer deaths, causing more deaths in 2017 than breast, prostate, colorectal, and brain cancers combined [35, 36]. There are various methods of detecting lung CTCs, which makes knowing which system to use in diagnosis, treatment, and monitoring treatment response difficult. There is also a large number of biomarkers used in liquid biopsy for lung cancer detection, making it difficult to know how CTCs stand out in comparison to other biomarkers, and how the cost and slow turnaround time in detection of CTCs will inform treatment decisions [37]. Multiple clinical trials have been conducted in order to determine the best method of detection in non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) [38-40]. Interestingly, patients with SCLC have 10 times as many CTCs in their blood than any other cancer type, which should make CTC isolation easier than in other cancer types [41].

Pancreatic cancer, the majority of which is pancreatic ductal adenocarcinoma (PDAC), has a 5-year survival rate of only 7%, with very few new diagnostic methods or therapies to improve survival in the last 30 years [42] Multiple studies have assessed CTC use in the diagnosis of pancreatic cancer. In a study involving 964 patients, CTCs were successfully detected in pancreatic cancer patients, though in lower numbers than patients with other cancer subtypes such as lung cancer [43]. Pancreatic cancer primary lesions are notoriously difficult to access due to their complex anatomical surroundings, which makes the gold standard US-guided FNA biopsy difficult to accomplish. Thus, CTCs may prove crucial in alleviating the challenge of pancreatic cancer diagnosis [44]. To our knowledge, there have been no publications thus far demonstrating the ability to use CTCs in PDAC diagnosis, staging, or screening, although, encouragingly, two large clinical trials are currently ongoing in Rouen, France (NCT02072616) (Link) and Columbia, Missouri (NCT03551951) (Link).

According to 2018 data from GLOBOCAN, colorectal cancer (CRC) is the 3rd most common cause of cancer death worldwide, and the 4th most commonly diagnosed cancer type, with approximately 2 million new cases and 1 million deaths occurring in 2018 [36, 45]. While advances are being made for screening and targeted therapy, mortality and metastatic disease risk remains high. Methods for diagnosis and treatment of CRC are currently limited to invasive procedures including colonoscopy, flexible sigmoidoscopy, and double-contrast barium enemas, with conventional MRI and CT utilization contributing to increased costs. Fecal occult blood testing, a non-invasive method to screen for disease demonstrated a high false-positive rate [46].

Baek *et al.* conducted a prospective study using CTCs to aid in diagnosis and prognosis in 88 newly diagnosed CRC patients scheduled to undergo surgery from 2014-2018 at a single institution in South Korea. While there have been previous studies in which CTCs have been used for CRC diagnosis and prognosis, this study included a system, fluid-assisted separation technique (FAST) enrichment and CTC detection with fluorescence microscopy in 74/88 patients (84.1%), which is a 10.5-36.2% improvement in positivity rates using the standard CellSearch© system. Using the cutoff value of 5 CTCs/7.5ml blood, sensitivity and specificity compared to healthy volunteers was 75% and 100%, respectively. In patients with CTCs ≥5, vascular invasion was frequently identified (p = 0.0035), and all patients with stage IV disease were positive for CTCs, with patients with ≥5 showing poor OS and PFS, though the differences were not statistically significant, over a follow up period of 19.5 months. The authors also determined that the FAST method (75% sensitivity rate) is comparable to guideline-recommended screening tests: 62-79% for guaiac-based fecal occult blood tests, 73-88% for fecal immunochemistry alone, 92% for stool DNA plus fecal immunochemistry, and 75-93% for colonoscopy for CRC [47, 48]. Given the increasing prevalence of CRC, its reputation for poor outcomes, and the highly invasive, inconvenient nature of screening, diagnosis, and treatment, these studies encourage the eventual use of CTCs in the early management of CRC going forward.

Circulating Tumor Cells in Determination of Prognosis, Treatment Response and Surveillance

Many studies have been done to assess the role of CTCs in assessing patient outcomes and prognosis. A study conducted by Stathopoulou *et al*. enrolled 148 patients with operable breast cancer. CTCs were detected in in the blood of 30% of patients with early stage disease and 52% of patients with metastatic disease, compared to 3.7% of healthy blood donors. This study found a correlation to prognosis (early stage pre-treatment: reduced PFS (p = 0.007) and overall survival (p = 0.01)), with multivariate analysis determining CTCs were an independent prognostic factor for disease relapse and death [49]. Another, more recent study with 118 patients from October 2004 - July 2006, detecting CTCs in 23% pre-adjuvant and 17% post-adjuvant patients, concluded that CTC presence after 18- month follow up following chemotherapy

was an independent prognostic factor for a shorter relapse-free survival ($p = 0.017$), although CTCs were not correlated to primary tumor response to treatment. In a review conducted by Bidard *et al.* the authors suggest that CTC count provides a prognostic factor as a biomarker in metastatic cancer, though in early breast cancer, CTC count is not correlated with currently known prognostic factors [50].

This same group conducted a meta-analysis in over 1500 nonmetastatic breast cancer patients treated with neoadjuvant therapy to assess CTC validity as a prognostic marker, and they discovered that CTCs were present in 25.2% before neoadjuvant chemo. Additionally, the number of CTCs correlated to a detrimental impact on overall survival ($p<0.001$), distant disease-free survival ($p<0.001$), and locoregional relapse-free interval ($p<0.001$), but not on pathological complete response. In 861 patients, adding CTC detection before neoadjuvant chemotherapy increased the multivariable prognostic models for overall survival ($p<0.001$), distant disease-free survival ($p<0.001$), and locoregional relapse-free interval ($p = 0.008$). Cristofanelli *et al.* was the first to report a cutoff of 5 CTC/7.5 ml of blood in a study with 517 patients, showing that patients with a CTC level at or above this cutoff displayed shorter PFS (2.7 vs. 7 months $p<0.001$) and shorter overall survival (OS) (10.1 vs. >18 months, $p<0.001$) compared to those with fewer than 5 CTC/7.5 ml [20].

While there is no current use for CTCs in monitoring lung cancer prognosis, in a single-institution prospective study with 81 patients with stage IV NSCLC, CTCs were collected across multiple timepoints across treatment regimens after 139 lines of therapy from 392 peripheral blood samples using a non-enrichment based high-definition single cell assay. The authors found that while CTCs were identifiable in most patients with stage IV NSCLC, there was weak correlation between the absolute number of CTCs at a single timepoint of therapy and patient outcomes (OS $p = 0.0754$). However, in the 81 patients, CTCs were detected in 51 (63%) of patients on initiation of therapy, and the changes in CTC counts were predictive of survival in patients with metastatic NSCLC receiving chemotherapy [51]. In a case report reported by Horton *et al.*, a patient with stage III NSCLC had a primary tumor that responded to chemoradiation, yet unexpectedly showed an increased CTC count post-treatment. PET/CT revealed liver metastasis, so in this case the CTC accurately predicted the patient's prognosis, highlighting the need for exploration of CTC count as a supplemental tool to conventional imaging [52].

With regard to prognosis, in a 9-cohort meta-analysis involving 623 patients, 268 (43%) were classified as CTC positive and displayed poor PFS (HR = 1.89, $p<0.001$) and OS (HR = 1.23, $p<0.001$) compared CTC negative group, with no difference between Asian and Caucasian populations ($p<0.05$). Kulemann *et al.* conducted a study involving 58 patients with PDAC from 2012 – 2014. The authors found that 29 patients (67%) showed CTC clusters or single CTCs, with 2 patients (3.4%) having cytology suspicious for CTCs, and 17 patients (29.3%) negative for CTCs. The presence of CTCs had no influence on overall survival ($p = 0.23$, 12 vs 8 months), though higher numbers in the bloodstream were associated with poorer overall survival, with patients having 3 or more CTC/ml blood ($n = 16$) experiencing median overall survival of 11.5 months and patients with 0.3-3 CTCs/ml blood ($n = 23$) surviving 20 months ($p = 0.12$). Interestingly, they also determined there was significant discordance in KRAS mutations between the primary tumor and the CTCs analyzed in the patients, highlighting a potential challenge in targeting CTCs [53]. Another study conducted by Effenberger *et al.* including 69 patients showed a median overall survival of 11 months, with 58 patients receiving gemcitabine. CTCs were present in 23 patients (33.3%), with a range from 1-19 cells per patient. PFS and OS were significantly reduced in CTC positive patients in univariate ($p = 0.009$, PFS, $p = 0.030$, OS) and multivariate analysis (HR = 4.543, CI 1.549-13.329; $p = 0.006$, PFS, HR = 2.093, CI 1.081-4.050, $p = 0.028$, OS). Interestingly, in the patients that had received chemotherapy, PFS was significantly reduced in CTC positive patients in univariate ($p = 0.013$) and multivariate (HR = 4.203, CI 1.416-12.471, $p = 0.010$) analysis.

There is strong evidence that CTCs are related to outcomes in both metastatic and non-metastatic CRC [54, 55]. Bork *et al.* studied 287 patients with curable CRC, including 239 patients stages I-III at a single institution from May 2009-August 2012. CTC levels were measured in blood preoperatively and days 3 and 7 postoperatively, with metastatic patients analyzed separately. After 28 months of follow up, patients with ≥ 1 CTC/7.5 ml blood were associated with worse overall survival (49.8 vs 38.4 months, $p<0.001$) in the non-metastatic group and in the complete cohort (48.4 vs 33.6 months, $p<0.001$). On multivariate analysis, CTC count was the strongest prognostic factors in non-metastatic patients (HR = 5.5; 95% CI 2.3-13.6) and in the entire study group (HR = 5.6; 95% CI 2.6-12.0). In a prospective, multicenter study including 430 patients with metastatic CRC, CTCs were measured at various timepoints of treatment. Patients were divided into an unfavorable prognostic group (>3 CTCs/7.5 mL) or favorable prognostic group (≤3 CTCs/7.5 mL). The unfavorable group had shorter median PFS (4.5 vs 7.9 months, $p = 0.0002$) and OS (9.4 vs 18.5 months, $p<0.0001$). At up to 20 weeks of therapy, the favorable group, in addition to patients converting from the unfavorable to the favorable group, demonstrated longer PFS and OS at all the various timepoints measured. These authors also found that CTC count positively correlated to progression of disease status as measured using conventional imaging [55].

With regards to treatment response, studies have shown that CTC detection and quantity can predict whether a patient responds to treatment and whether the patient is at risk for relapse. Pierga *et al.* found that in breast cancer patients ≥1 CTC/7.5 ml blood correlated to PFS ($p<0.0001$) and ≥5 CTCs/7.5 ml blood correlated to PFS and OS ($p = 0.03$) on multivariate analysis, independent of other serum markers including CA 15-3, CEA, and LDH. Additionally, CTC detection rates were 65% ≥1 CTC/7.5 ml and 44% ≥5 CTCs/7.5 ml and this detection was independent of breast cancer subtypes (luminal, triple negative, HER2+). They also found that elevated CTC levels at second cycle of chemotherapy, were indicative of poor PFS and OS. This suggests that there is potential to use CTC count to monitor treatment response [56]. As a follow up to this study, with 177 more patients with metastatic breast cancer, Hayes *et al* determined that in patients with ≤ 5 CTCs/7.5 ml of blood, median PFS were longer than PFS in patients with ≥ 5 CTCs/7.5 ml blood at various time points (3-5 months, 5, 6 to 8, 9 to 14, and 15-20 weeks follow up), leading them to conclude that detection of elevated CTCs at any point during therapy is an accurate indication of subsequent rapid disease progression and mortality [57].

A meta-analysis conducted by Zhang *et al.* from January 1990 - January 2012, included 49 eligible studies and 6,825 patients. The authors found prognostic significance of CTC value in both early (DFS: HR = 2.86; 95% CI, 2.19-3.75; OS: HR = 2.78; 95% CI, 2.22-3.48) and metastatic breast cancer (PFS: HR = 1.78; 95% CI, 1.52-2.09; OS: HR = 2.33; 95% CI, 2.09-2.60), with further subgroup analysis demonstrating stable results even irrespective of detection method or time point of blood withdrawal [58]. Paoletti *et al.* determined in a clinical trial investigating estrogen receptor mutational status in breast cancer alongside CTC detection with CellSearch© suggested multiple mechanisms of resistance to endocrine therapy, as well as better outcome predictions by characterizing CTCs in addition to circulating tumor DNA [59, 60]. Stefanovic *et al.* recently concluded that both intact and apoptotic CTC counts (reported in 52-79% of CTC-positive metastatic breast cancer patients) should be enumerated as each has its own prognostic significance over the course of therapy [61, 62]. The authors found downregulation of apoptotic CTCs in luminal ($p = 0.038$) and triple negative ($p = 0.035$) patients and correlated these changes to mRNA-assessed intrinsic subtype change between primary tumor and metastatic sites. Other studies have investigated utilizing CTC expression profiles in assessing prognosis and therapeutic intervention responses across different breast cancer phenotypes [63, 64].

While CTCs have not been used thus far to monitor treatment response in pancreatic cancer patients, van der Sijde *et al.* investigated circulating conventional biomarkers CA 19-9, CEA, and single nuclear polymorphisms, circulating tumor DNA, long non-coding RNAs, and markers of inflammatory responses [65]. Surveillance of pancreatic cancer recurrence for high risk individuals is typically done with conventional imaging such as ultrasound or MRI and various biomarkers such as CEA and CA19-9. A meta-analysis of 16 studies reporting 1551 familial high-risk patients determined that many patients received unnecessary surgery (68.1%, CI 59.5-76/7, $p<0.001$), which suggests other methods of surveillance should be pursued to reduce this number of unnecessary surgeries for pancreatic cancer going forward [66].

While the role of CTCs in prostate cancer is not here discussed in detail, a notable study investigated CTCs in prostate cancer. Specifically, the response to treatment was analyzed using the FDA-approved CTC predictive biomarker ARV7, which is associated with prostate cancer. Nuclear ARV7 expression in the CTCs of prostate cancer patients predicts a better outcome on patients treated with taxane and worse outcomes on patients treated with anti-hormonals [67, 68].

While most cancer subtypes have few, if any, studies evaluating surveillance or outcomes by utilizing CTCs, clinicians believe that switching treatments based on CTC status instead of relying of traditional clinical signs of progression may provide an opportunity for patients to receive beneficial treatment. However, more work needs to be done to determine the role of CTCs in assessing response to treatment and surveillance for most cancer types.

Role of Circulating Tumor Cells in Immune System Evasion

A wealth of literature supports the possibility for CTCs to escape destruction by hosts' immune system. Santos *et al.* used a flow cytometric assay to measure natural killer (NK) cell cytotoxicity toward target cells in 74 patients with metastatic breast, colorectal (CRC), or prostate cancer. The authors found decreased NK cell cytotoxic activity in patients with relatively high numbers of CTCs in peripheral blood (>5 CTCs for breast and prostate, and >3 CTCs for CRC) compared to patients with lower CTC levels. This conclusion was further supported by the decreased observed expression levels of toll-like receptors [69]. Though overall NK cell number increases in metastasis, CTCs evade their activity via inhibitory cytokines, increasing platelet number and activity, and neoangiogenesis [70, 71]. Also, programmed death ligand 1 (PD-L1), which binds PD-1 on T-cells to induce immune tolerance, was found on CTCs from HER2 positive breast cancer patients [72]. Furthermore, it appears certain breast CTCs with surface Fas Cell Surface Death Receptor Ligand (FASL) can bind to FAS on T-cells and induce apoptosis in the T-cells [73]. These data together help suggest why CTCs are elevated in cancer patients across a broad spectrum of cell lineages, and point to the potential broad utility of CTC measurement as a prognostic biomarker.

Cost Effectiveness

Given the large number of potential options for solid and liquid biopsy, a comparison of the cost effectiveness of CTCs to other options is needed. While very few cost-effectiveness analyses have been done to assess using circulating tumor cells as an adjuvant to gold standard monitoring and treatment of cancer, there have been a few recent studied published to investigate cost-effectiveness in the utilization of CTCs as a biomarker.

Goodman *et al.* recently assessed whether CTCs could be used to predict benefit of radiotherapy (RT) following breast conserving surgery (BCS) among women with low-risk, early-stage breast cancer and subsequently conducted a cost utility analysis for this biomarker-directed treatment recommendation. They found the total direct and indirect cost was lowest for CTC-negative women not requiring RT ($1,074) and highest for CTC-positive women requiring RT ($7,202). The 5-year quality-adjusted life years (QALY) was highest in CTC-negative women for whom RT was omitted (4.80) and lowest for CTC-positive women who received RT (4.49), with women receiving standard of care having a 5-year QALY of 4.58. With cost-effectiveness analysis, the CTC-directed RT was $4,441 less expensive than the total cost of RT with or without a boost and gained 0.19 incremental QALY in 5 years. Thus, this study indicated a preference for biomarker-directly therapy over standard of care for cost-effectiveness [74].

Another study investigated the cost effectiveness of using MRI vs endoscopic ultrasound in surveillance of high-risk pancreatic cancer patients. The authors demonstrated that MRI was most cost-effective, however, only in a population with 5-fold higher pancreatic cancer relative risk compared to the United States general population. Although conventional imaging and various biomarkers are used currently in surveillance of pancreatic cancer recurrence, given the simplicity and low-risk of a blood draw, more studies to understand the role CTCs could play in the future to aid in surveillance, though no studies have been done at this time [75].

While research on the economic impacts of CTC detection is currently lacking, using CTCs as an adjuvant to current therapy has proved cost-effective in the situations studied. With this evidence, as well as the increased popularity of personalized medicine and the probability that further technological progress will further reduce the cost of CTC analysis, careful analysis should be done and continually updated to determine if and when CTC detection should become customary.

Circulating Tumor Cells and Surgery Risk

When evaluating CTCs as a biomarker, it is important to also consider the effects of surgery on primary tumor and metastatic sites in relation to patient outcomes. Importantly, it has been speculated that surgical removal of primary tumors can lead to increased CTC circulation in the blood, thus increasing the potential for metastatic disease. In a pre-clinical breast cancer mouse model, CTC counts were measured before and six weeks following interventions including tumor compression such as would be conducted during mammography, punch biopsy, or surgery. The authors found no significant CTC increase in the mice undergoing palpation but found significant increase in CTCs immediately following punch biopsy ($p = 0.02$) with consistent elevation after six weeks, and decreased CTC counts immediately following surgery ($p = 0.03$), with CTC recurrence after six weeks [76]. In a multicenter, prospective clinical study including 29 patients with T1b-2N0M0 NSCLS, 16 patients were positive for CTCs prior to surgery and remained positive for CTCs. Additionally, 4 patients who were previously CTC free prior to surgery became positive after surgery [77].

Furthermore, in 139 patients with hepatocellular carcinoma (HCC), CTC detection incidences increased from before surgery (43.9%) to 3 days postoperatively (54%). Interestingly, the mean CTC counts did not show a clear trend from before (1.54, range 0-42) to after surgery (1.13, range 0-26), with data not statistically significant ($p = 0.1158$). In this patient cohort, compared with the preoperative counts, postoperative CTC counts increased in 58 (41.7%) patients, decreased in 35 (25.2%) and remained unchanged in 46 (33.1%) patients, and the postoperative increase was associated with the presence of macroscopic venous thrombus ($p = 0.012$) [78]. A single-institution prospective study investigating postoperative CTCs and outcomes in adenocarcinoma of the colon or rectum with hepatic metastases was conducted in 20 patients who underwent hepatic resection or ablation. The study authors concluded that after 2 patients possessed preoperative CTCs (mean count 3.9, range 0-56), one additional patient had CTCs postoperatively (count 1, range 0-9). They found that detection of CTCs preoperatively (OS $p = 0.446$) or intraoperatively (DFS $p = 0.248$, OS $p = 0.798$) was not predictive of DFS or OS, conflicting with prior work done by Koch *et al.* However, postoperative CTC presence was predictive of DFS ($p = 0.036$) and OS ($p = 0.036$) [79, 80].

In lung cancer, a group recently proposed that CTC counts in patients undergoing video-assisted thoracic surgery (VATS) or open surgery for primary lung cancer are influenced by hemodynamic changes caused by surgery and manipulation. The authors found CTCs in 58/138 samples across 31 patients, of which CTCs were more often found in the pulmonary vein samples (70%) compared to samples from the radial artery (22%, $p<0.01$) and observed higher counts ($p<0.01$). This result was consistent following surgery, suggesting central CTC clearance and no changes with surgical approach [81]. Ultimately, although numerous studies have been done to investigate how CTC detection and counts change over time, including before and after primary tumor biopsy or surgical intervention, more work should be done in order to determine optimal timing to measure CTC in patients undergoing invasive procedures for realistic prognostic predictions.

Weaknesses, Limitation and Pitfalls

Given that CTCs comprise only a small fraction of the total circulating cell types in the bloodstream, high sensitivity and specificity, as well as consistency, are paramount when isolating CTCs. These characteristics are crucial for reproducibility and accurate determination of patient outcomes [82]. Unfortunately, the heterogeneity between different cancer types, as well as the scarcity of CTCs in patients' blood make a "one size fits all" approach to using CTCs across numerous cancer subtypes exceptionally difficult. While mainly focused on ctDNA and pediatric solid tumors, a recent review makes an important point about the limitations of liquid biopsies clinically [83]. In this setting, it is plausible that levels of ctDNA vary with age, comorbidities preventing clearance of the ctDNA, and presence of multiple cancer types pose issues with reliability. These limitations could very well be applicable to CTCs as well.

Summary

CTCs have shown tremendous potential in the recent literature for their utilization in diagnosis, staging, determining patient prognosis, predicting response to treatment, and monitoring or surveillance following treatment in cancers of the breast, lung, pancreas, colon, and others. This review aims to provide a concise summary to demonstrate the recent success in developmental methods of detection of CTCs, how CTC detection compares to other biomarkers for a potential "liquid biopsy" in terms of feasibility, sensitivity, specificity, and cost-effectiveness, as well as their potential to guide clinicians in patient care decisions for optimizing outcomes. A comprehensive understanding of CTCs will provide more opportunities for insight into their clinical utility.

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Conflicts of Interest

None.

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