Circulating Tumor Cells: What Goes Around, Comes Around

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When invasive tumors are merely a few millimeters, they have the capacity to infiltrate normal tissue, create a reactive stroma, and generate a neovascular response. The tumor neovascularization creates an angiogenic support for cellular proliferation and vascular entry that leads to tumor dissemination. Despite excision of the primary neoplastic mass, residual tumor cells remain occult to physical or radiologic examination or serologic testing until recurrent malignancy is later identified in a metastatic site (1). These isolated tumor cells may have invaded the lympho-vascular system as circulating tumor cells (CTCs) or achieved an isolated tumor focus designated as disseminated tumor cells (DTCs), possibly detected in a bone marrow biopsy (2–4). Occult malignancy or minimal residual disease is thought to be the source of cancer relapse in patients whose postsurgical status represents no evidence of disease. Given that hematogenous metastases occur through a complex biologic pathway of vascular invasion, circulation, identification of a favorable metastatic site, and clonal expansion, it appears intuitive that identification of the leukemic phase of CTCs in peripheral blood or the deposition phase of DTCs in vascular-rich environments may be prognostic for tumor recurrence and metastasis.

DTCs at the time of diagnosis predict a poor prognosis, and persistence of DTCs is associated with an increased risk for relapse (2,5,6). Although DTCs serve as a valuable prognostic tool, the invasive nature and pain associated with a bone marrow biopsy has encouraged researchers to seek other options to act as a surrogate for minimal residual disease. One of the more promising alternatives is the identification of CTCs in the peripheral blood (4,7,8). The basis for adjuvant chemotherapy is to reduce these residual tumor cells and prevent or prolong the time for these malignant cells to proliferate, disseminate, and generate clonal expansion in metastatic sites. The risk or hazard of tumor recurrence and formation of metastases is dependent on the biology of the tumor and may be predicted based on a set of surrogate markers. Prognostic factors have traditionally included tumor burden and extent (stage) and pathologic features and molecular expression indicative of relative aggressive behavior. The identification of CTCs represents an additional biomarker that provides insight into clinical behavior. Studies have shown that identification of CTCs reflects minimal residual disease and may offer prognostic information for disease-free survival (DFS) and overall survival (9–12).

CTCs are present in the buffy coat of a centrifuged blood sample with enhanced detection using a detecting antibody (surface epithelial cell adhesion protein) attached to magnetic beads within a magnetic field. The tumor cells are defined as nucleated cells with epithelial cytokeratin markers (CK 8, 18, 19) and lacking hematopoietic antigens (CD45) (13,14). Although CTCs may represent a spectrum of enumerated tumor cells within each sample, many studies merely convert the findings to a binary result of positive or negative. Although the presence relative to the absence of CTCs tends to predict a poor outcome, even studies with existing metastatic disease only demonstrate a minority of patients with positive CTCs.

Several studies using the Cell Search System, a US Food and Drug Administration–approved technique for detecting CTCs in breast cancer patients, have demonstrated prognostic relevance for distant DFS and overall survival, but the number of patients in these studies was small. In this issue of the Journal, Dr. Bridgette Rack and colleagues, on behalf of the SUCCESS Study Group, report the results of CTC analysis within a phase III study comparing fluorouracil-epirubicin-cyclophosphamide followed by docetaxel vs gemcitabine (15). The authors evaluated peripheral blood samples from 2026 early breast cancer patients and identified CTCs in 21.5% after primary tumor resection and before chemotherapy. As expected, node-positive case patients were more likely CTC positive (22.4% N1–3; 19.6% N0); however, CTCs were not associated with histologic immunophenotype. After adjuvant chemotherapy, CTC analysis on 1492 patients identified 22.1% that were CTC positive. The authors used the CellSearch System (Veridex, Raritan, NJ), as described above. The patients were followed for a median of 35 months.

One hundred fourteen patients suffered a disease relapse (6%), and 54 died of breast cancer during follow-up. At 36 months, the presence of CTCs was associated with disease-free probability (88% CTC negative; 94% CTC positive). In multivariable proportional hazards model, CTCs were predictive of DFS and overall survival. As expected, other traditional prognostic markers, such as nodal status, receptor status, tumor size, and grade were also prognostic for survival outcomes. Persistence of measurable CTCs before and after chemotherapy was a poor prognostic indicator. Although CTC positivity was initially defined as any identifiable tumor cell, the authors were able to demonstrate that the hazard ratio for survival endpoints increased with CTC count. A Kaplan–Meier comparative analysis for DFS plotting absent CTCs and CTCs equal to or greater than five showed a respective survival probability of 93% vs 72%, whereas, for any number of CTCs, the DFS was approximately 88%.

The article by Rack and colleagues (15) is notable for the large cohort involved in the study and the focus on patients in the early course of their breast cancer and CTC analysis both before and after chemotherapy. An acknowledged limitation is the relatively short
follow-up for breast cancer, especially given that the hazard plot for breast cancer recurrences tends to peak at year 2 to 3, depending on pathobiologic factors. Although it is clear that the CTC count is prognostic for DFS and overall survival, the qualitative assessment of the CTCs—namely, their targeted therapy phenotype or their acquisition of multidrug resistance programming—may be additionally meaningful for therapeutic manipulation (16,17). Given the CTC result, what are the algorithms for decision analysis by the treating oncologist (9,18–20)? Will an early-stage breast cancer with absent measurable CTCs obviate the need for chemotherapy? Should the CTC assay be performed after initial cycles of chemotherapy and/or during a periodic schedule given the dynamic nature of the metastatic potential? The test characteristics need to be reproducible and reliable in a variety of institutions and clinical settings.

The test characteristics for the analysis of CTCs needs continued attention. Given the fragility and short lifetime of CTCs, improvements in test sensitivity and specificity (false positive rate = 4.9%) are needed. Many patients with metastatic disease do not demonstrate expected CTCs, and several case patients with CTC positivity did not have recurrence within the 3-year follow-up. Is there a meaningful reproducible cutoff level for CTC positivity? The assessment of CTCs is based on their cellular epithelial characteristics and absent hematopoietic antigens. Might some types of higher-grade breast cancers be missed by the choice of epithelial markers? Will additional antibodies demonstrating an epithelial-to-mesenchymal transition or expression of stem cell features provide additional meaningful diagnostic and therapeutic information? Is epitope expression uniform among CTCs for meaningful assessment?

The authors have demonstrated that CTCs may provide clinical prognostic information in a large cohort of early breast cancer. Additional studies and clinical experience will be needed to show that CTC assessment as a biomarker will have a satisfactory cost–benefit ratio and provide useful information for the management of breast cancer patients.

References

Notes
The authors declare no conflicts of interest.

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