Clinical Chemistry 65:10
000–000 (2019)

Editorials

Circulating Tumor Cells in Head and Neck Carcinomas

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Medical Need for Circulating Tumor Cell Analysis in Squamous Cell Carcinoma of the Head and Neck

Squamous cell carcinoma of the head and neck (HNSCC)[2] is 1 of the most common cancers worldwide *(1)*. Despite recent advances in systemic therapy [in particular, the use of anti-epidermal growth factor receptor (anti-EGFR) antibodies], the prognosis of HNSCC patients with recurrent or metastatic disease needs to be further improved. There is a lack of biomarkers that predict response to therapy and could be used for better stratification of therapies to individual patients.

More than 2 decades ago, pioneering work of our team and others indicated that HNSCC patients presented with blood-borne dissemination to the bone marrow, even in patients with apparently localized disease *(2)*. Because bone marrow aspiration is an invasive procedure, the focus has shifted from bone marrow to peripheral blood, which allows easy sample collection and monitoring of therapeutic responses *(3)*. Several techniques have been developed to detect and characterize circulating tumor cells (CTCs) in patients with solid tumors, including HNSCC, but their clinical utility is not yet fully established *(3)*. A recent meta-analysis in HNSCC demonstrated that the presence of CTCs was significantly associated with shorter disease-free survival, and concluded that CTC detection is extremely specific but not very sensitive in HNSCC *(4)*. This conclusion was also supported by the report of Groebe and coworkers *(5)*; using the EpCAM-dependent CellSearch® system as the most standardized system for CTC detection in various tumor entities *(6)*, CTCs were detected in only 12.5% of patients with early squamous cell carcinoma of the oral cavity surgically resected without neoadjuvant therapy. Despite this relatively low detection rate, univariate and multivariate analyses revealed that CTCs were strong independent predictors of recurrence-free survival. There remains, however, an urgent need for enhancing the sensitivity of CTC detection in HNSCC.

CTC Analysis in the CIRCUTEC Study

In this issue of *Clinical Chemistry*, Garrel and coworkers *(7)* performed an exploratory prospective study to detect and monitor CTCs in the peripheral blood of patients with recurrent or metastatic HNSCC treated by chemotherapy plus the anti-EGFR antibody cetuximab. The CIRCUTEC study focused on patients with advanced HNSCC, and 3 complementary assays (CellSearch, EPISPOT, and flow cytometry) were applied for CTC detection. In 65 patients suitable for analyses, peripheral blood was taken at day 0 (D_0), D_7, and D_{21} of treatment. At D_0, CTCs were detected with EPISPOT, CellSearch, and flow cytometry in 69% (45 of 65), 21% (12 of 58), and 11% (7 of 61) of patients, respectively. In the patients tested with all 3 methods, EPISPOT identified 92% (36 of 39), 92% (35 of 38), and 90% (25 of 28) of all positive samples at D_0, D_7, and D_{21}, respectively. The EPISPOT assay uses depletion of leukocytes by anti-CD45 antibodies to enrich for CTCs in blood samples, whereas CellSearch depends on EpCAM-based positive enrichment of CTCs. EpCAM is not expressed on all HNSCCs, which might explain the higher sensitivity of the EPISPOT assay; moreover, EpCAM expression has been recently identified as a positive prognosticator of clinical outcome in locally advanced HNSCC *(8)*.

CTC detection was also related to clinical outcome in the study of Garrel et al. Median progression-free survival time was significantly lower in patients with increasing or stable CTC counts, in particular those detected with the EPISPOT assay based on EGFR released by viable tumor cells. Interestingly, the changes in CTC counts between baseline (D_0) and D_7 were more relevant for outcome than the changes at D_{21}. One would assume that changes over a period of 3 weeks should affect CTC counts more than the short-term changes occurring within 1 week. Another surprising finding was that the presence and number of CTCs were not related to the metastatic progression but rather to locoregional progression. Although this could be explained by the assumption that CTC counts might be a mere indicator for total tumor burden, it could support the hypothesis that tumor cells can recirculate from distant sites back to the original site of tumor growth *(9)*.

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Received July 17, 2019; accepted July 25, 2019.
Previously published online at DOI: 10.1373/clinchem.2019.309609

[2] Nonstandard abbreviations: HNSCC, squamous cell carcinoma of the head and neck; EFGR, epidermal growth factor receptor; CTC, circulating tumor cell; D, day; CK, cytokeratin.

The authors have used 2 interesting markers [cytokeratin (CK) 19 and EGFR] for the detection of CTCs in their EPISPOT assay. CK19 has been explored as a biomarker in oral squamous cell carcinoma *(10)* and used successfully for the detection of CTCs in blood of breast and colon cancer patients *(3)*. Despite being a cytoskeleton protein, CK19 can be actively released by viable tumor cells and, therefore, used as a marker in the EPISPOT assay *(11)*. The function of keratins in tumor cell biology is diverse and still underinvestigated. In HNSCC, a recent report presented the first evidence for the existence of 2 parallel pathogenic pathways in oral squamous cell carcinomas, characterized by the expression of low and high molecular weight CKs; a significant association was reported between the expression of low molecular weight CK8/18 and CK19 and a high-tumor grade, β- and γ-catenin expression, and deregulated cell cycle proteins *(12)*.

In the present report, CK19 release by CTCs alone, however, was not a prognostic marker; the addition of released EGFR as a second marker was important to obtain significant information *(7)*. Expression of EGFR on subsets of CTCs has been reported *(13)*, whereas the specificity of EFGR as a CTC marker has been less explored. Examination of mixed populations of peripheral blood mononuclear cells did not detect EGFR expression. However, functional EGFR was detected in subpopulations of human peripheral blood monocytes in the context of noncancer diseases *(14)*. Garrel et al. did not observe any positive signals in their 15 healthy controls. Besides the transmembrane EGFR form, there is evidence of the existence of truncated/soluble EGFR isoforms containing the EGF binding site but lacking the membrane-spanning region and the kinase domain. This allows the use of EGFR in the EPISPOT assay and adds another level of complexity in the definition of how the fine regulated EGFR network works. Two mechanisms are discussed for the generation of soluble EGFR: (*a*) the alternative splicing of the full-length receptor mRNA that gives rise to a protein isoform with a shorter COOH-terminal sequence and lacking the intracellular domain; and (*b*) the site-specific proteolysis (shedding) that involves the cleavage of the full-length receptor to release the extracellular *(15)*.

Future Steps of Liquid Biopsy Research in HNSCC

In conclusion, the demonstration of prognostic value of CTC detection in HNSCC patients is an important step toward the clinical validation of liquid biopsy in this important tumor entity. The results of the exploratory study by Garrel and coworkers are promising and deserve further clinical validation in larger independent clinical cohorts. The high sensitivity of the EPISPOT assay might also allow the detection and monitoring of CTCs in patients at earlier stages of HNSCC. Besides enumeration of CTCs, further molecular characterization for therapeutic targets and resistance mechanisms may provide relevant information for the personalized treatment of individual HNSCC patients.

There are several potential future applications of CTC analysis: (*a*) a tool to provide early evidence of occult cancer spread; (*b*) a quantifiable risk factor for the development of distant metastatic disease and the associated prognosis; (*c*) the potential molecular analysis of CTCs for targeted treatment strategies or radiotherapy/chemotherapy sensitivity; and (*d*) the establishment of a biomarker for cancer surveillance to detect minimal residual disease *(3)* and monitoring of tumor responses to therapy *(16)*. All of these applications may lead to interventional clinical studies for which clinical decision-making will be based on the result of the CTC assay.

Beyond CTCs, other promising biomarkers of the liquid biopsy family, such as circulating cell-free nucleic acids (in particular DNA and microRNAs), extracellular vesicles, or tumor-educated platelets, might be included into a composite biomarker that reveals the maximum amount of information from 1 blood tube. Standardization and quality assurance will be important, and international consortia such as the CANCER-ID network and the European Liquid Biopsy Society are now able to fulfill this important task.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

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