

5. Methodology tip – circulating tumor cells as a biomarker

Tumor cells circulating in the bloodstream can be used as a measure of solid tumors present in specific organs, and as a prognostic tool for survival to that solid tumor. It has been reported that all types of solid tumors give rise to circulating tumor cells, and that in all types of solid tumors, some of these find residence in the bone marrow (61). Once residing in the bone marrow, these cells may persist over many years and eventually disseminate into other organs. Hence, studies using tumor cells as a biomarker use PBMCs as well as bone marrow as the source of cells. Circulating tumor cells can be measured directly, using an immunoassay that employs antibodies and a microscope, or indirectly, using PBMCs with detection of tumor cells by a PCR-based method (62). PBMCs, used as a source of unpurified lymphocytes by immunologists, also contain circulating tumor cells.

A number of studies of colorectal cancer, for example, have used the polymerase chain reaction (PCR) for measuring the number of tumor cells present in the bulk of unpurified peripheral blood mononuclear cells (PBMCs). Iinuma et al. (63) used PCR for quantifying tumor cells, where the target genes were carcinoembryonic antigen (CEA) and cytokeratin 20 (CK20). In a careful methodological study, these authors found a difference in circulating tumor cell counts, when comparing normal control subjects with cancer patients. Iinuma et al. (64) report a detection limit of one tumor cell in 3 million PBMCs.

6. Cytokeratin as a soluble protein biomarker for colon cancer – the Koelink study

The following study concerned the tumor antigen cytokeratin. The type of cytokeratin that was measured was CK18-Asp396, a degradation product of cytokeratin-18.

Koelink et al. (65) demonstrated that soluble cytokeratin is elevated in the blood plasma of patients with colon cancer and that elevated cytokeratin is correlated with reduced survival. Outcome was according to the endpoint of disease-free survival (DFS). In this study, patients were divided into two groups, namely, those with cytokeratin greater than the median plasma concentration (for the group of patients), and those with cytokeratin lower than the median concentration (for the group of patients).

⁶¹ Riethdorf S, Wikman H, Pantel K. Review: biological relevance of disseminated tumor cells in cancer patients. *Int J Cancer*. 2008;123:1991–2006.

⁶² Vogelaar FJ, Mesker WE, Rijken AM, et al. Clinical impact of different detection methods for disseminated tumor cells in bone marrow of patients undergoing surgical resection of colorectal liver metastases: a prospective follow-up study. *BMC Cancer*. 2010;10:153 [7 pages].

⁶³ Iinuma H, Okinaga K, Egami H, et al. Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. *Int J Oncol*. 2006;28:297–306.

⁶⁴ Iinuma H, Okinaga K, Egami H, et al. Usefulness and clinical significance of quantitative real-time RT-PCR to detect isolated tumor cells in the peripheral blood and tumor drainage blood of patients with colorectal cancer. *Int J Oncol*. 2006;28:297–306.

⁶⁵ Koelink PJ, Lamers CB, Hommes DW, Verspaget HW. Circulating cell death products predict clinical outcome of colorectal cancer patients. *BMC Cancer*. 2009;9:88.