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Editorial

Circulating tumor cells: Isolation, quantification, and relevance of their translation into clinical practice

Células tumorales circulantes: aislamiento, cuantificación y relevancia de su traslación a la práctica asistencial

Occurrence of metastatic disease in solid tumors accounts for 90% of cancer-related deaths. Current understanding of hematogenous spread of tumors suggests that cancer cells clones crossing the endothelium circulate in bloodstream until they are removed by immune response mechanisms o they find a suitable microenvironment to remain in a latent status and eventually acquire the ability to proliferate at a later time. Thus, the process of generation of circulating tumor cells (CTCs) crossing the circulation and colonizing distant organs is inherent to the concept of metastasis. This ability of solid tumors to metastatize in a distant site has led researchers to propose that such tumors have "leukemic" phases similar to those known in lymphomas[1].

However, the mere finding of CTCs does not suffice to state that there are occult metastases. CTCs must pass through several phases before they are able to form metastatic colonies, namely: they must first extravasate from circulation to target organs and then, while proliferating, avoid tissue immune response and overcome metabolic difficulties. In fact, it is estimated that only one out of every 10,000 CTCs is able to form a metastasis.

Disseminated tumor cells (DTCs) reflect the true metastatic potential of tumors and are correlated to prognosis, but their detection requires invasive diagnostic procedures (biopsy, puncture or aspiration, etc.), which would impair the clinical value of CTCs because they are easily available in peripheral blood[2]. This objective reality is applicable to tumors located in urogenital organs.

CTC detection may not only show the aggressiveness of the primary cancer, as reflected by its ability to shed tumor cells into peripheral blood and its resultant potential to develop metastasis, but also its presence and its theoretical ability to start dissemination of a different CTC generation from the new metastatic sites.

CTCs dependent on primary tumor clones may be detected even before the tumor from which they originate, and they are even identified, and often persist, after the tumor has been removed. CTC measurement would thus be extremely helpful to assess essential aspects of oncology related to both regards diagnosis and staging and prognosis. It would also open new expectations for individualized, rapid, and accurate assessment of cancer response to different therapies (surgery, drug or radiation therapy) and for subsequent therapy modulation is required[3,4]. In this setting, CTC measurement obviously has a greater value as compared to the known tumor markers which, except in some cases, have a limited value for detection of both cancer tissue and its response to treatment because these proteins or antigens are also usually present in healthy patient and the cancer detection threshold is difficult to determine. Because of tumor heterogeneity, different markers would have to be used to improve their sensitivity and specificity.

CTC measurement at clinical laboratories has been an unmet objective for a very long time. Complexity of their detection is due to their low numbers in the bloodstream. Thus, as compared to the number of formed elements in peripheral blood, CTC numbers are extremely low, approximately one per every 10^6-10^7 white blood cells, and testing of samples 1,000 to 10,000 times greater than those usually drawn for a blood test would therefore be required. CTCs are thus rare events which have only been specifically detected to date at research laboratories using a combination of intracellular and surface markers. Fortunately, in the past decade different technological advances have allowed for optimized, reliable detection of CTCs, which is expected to have an increasing clinical relevance in the near future.

CTC detection methods use cytometry or nucleic acid analysis. For both methods, isolation and optimization of CTCs require that their expressiveness is increased using densitometric tests, immunomarkers and/or reverse transcriptase-polymerase chain reaction (RT-PCR). Current technological advances have allowed for development of different methods for CTC measurement at clinical laboratories, either morphological (ISET [Isolation by Size of

Epithelial Tumor cells], based on gradient density; Oncoquick) or immunomagnetic (MACS® [Magnetic Activated Cell Sorting system], AdnaTest®, RARETM® [RosetteSep-Applied imaging Rare Event], FAST® [Fiber-optic Array Scanning Technology], LSC® [Laser Scanning Cytometer], CellSearch®). Automated microscopes allowing for faster examination are also available (ACIS® [Automated Cellular Imaging System], CellSpotter, and ARIOL®)[5].

CTC detection methods based on free circulating DNA, RT-PCO, or preferably quantitative RT-PCR are more sensitive as compared to those based on cytometry, but uncertainty exists about the half-life of cells and nucleic acids in peripheral blood, which means that free circulating DNA reflects total nucleic acids, and not only nucleic acids from tumor cells. While this drawback could be obviated by measuring free RNA, which rapidly disappears from blood after cell death, specificity continues to be low and has prevented use of this method in clinical practice.

More importantly, it should be recognized that CTC measurement by cytometry is not only intended to achieve identification and quantification of these cells in peripheral blood, but also CTC isolation and genetic and molecular characterization to determine their true biological significance and origin (primary tumor or metastasis), which represents an added benefit as compared to CTC detection methods based on DNA-RNA (RT-PCR).

Epithelial tumors account for approximately 80% of all cancers. CTCs come from epithelium and are not found in the peripheral blood of patients with no neoplastic disease. Most technologies use EpCAM (a pan-epithelial differentiation antigen which is expressed in epithelial cells and in virtually all carcinomas) or other similar antigens to identify CTCs.

Currently, the possibility of measuring and quantifying CTCs using only 7.5 ml of blood from cancer patients may be a reality at clinical laboratories now that the clinical relevance and technical feasibility of the procedure has been shown.

The semi-automated system CellSearch Epithelial Cell Kit (Veridex®) for CTC measurement is the only one currently approved by the FDA (US Food and Drug Administration) as an independent predictor of overall and progression-free survival in patients with metastases of breast, colon, and prostate cancer when used in the specific clinical setting. This system uses expression of the epithelial cell adhesion molecule, EpCAM, and enriches the sample with ferric fluid and antibodies directed against EpCAM bound to immunomagnetic bands. Circulating epithelial cells are isolated using magnetic fields and subsequently stained with DAPI (fluorescent nucleic acid dye, 4,2-diamidino-2-phenylindole dihydrochloride).

Fluorescein-marked monoclonal antibodies specific to white blood cells (CD45-allophycocyanin) and epithelial cells (cytokeratins 8, 18, 19-phycoerithrin [CK-PE]) will be used to distinguish epithelial cells from white blood cells. CTCs are subsequently identified and enumerated using the CellSpotter analyzer, a semi-automated fluorescent microscope that allows for CTC recognition using specific software[4,5].

Results obtained in the different currently available clinical trials on CTCs suggest that the ability to shed cells into the bloodstream is a property inherent to the tumor, and that their presence may start at early stages in tumor development and progression. Tumor cells may be detected in blood and bone marrow from cancer patients with no clinical or histopathological signs or even no evidence of metastasis, and therefore provide unique information which, when adequately used, may allow for individualized treatment of the patient[2].

Current methods are far from perfect, and those based on EpCAM expression in cancer cells have as disadvantage that EpCAM is not homogeneously present in all tumors, and implicit dependence on a sufficient EpCAM expression by the different tumors that allows for its immunomagnetic uptake could therefore affect its measurement in some cancers. However, although no gold standard method currently exists for measuring CTCs, other alternative methods for CTC measurement have been developed and are under validation. Such new methods include microchips, microfluids, and microfilters[6,7].

Researchers from the Massachusetts General Hospital (MGH) have recently developed, in collaboration with the Research Center of Bio-micro-electromechanical Systems (Bio-MEMS), a silicon chip (CTC-chip) which is able to isolate, count, and analyze CTCs. The microchip surface, of the size of a credit card, has approximately 80,000 tubular microposts coated with antibodies which are able to detect the proteins expressed by different solid tumors and are geometrically arranged so that when blood from the sample circulates between them at a flow and velocity preset using a pneumatic pump, they grab tumor cells by their molecular footprint. The few clinical trials conducted to date show that this method is highly reliable[6].

These technological advances in CTC measurement are also an alternative to invasive tumor detection procedures such as biopsies, as well as for monitoring of treatments administered, which was previously only possible by imaging tests at predefined intervals. Thus, testing of a single blood sample would allow for real time monitoring of the different therapies and the response to them based on an increased or decreased CTC count. This would allow for early discontinuation of ineffective treatments to try other therapeutic modalities.

Rapid technological development of methods for measuring CTCs opens a new area, specific therapy. Such methods not only allow for CTC identification, but also for their isolation, which enables study of their genetic information. Very recently, different researchers have reported the results of genomic analysis by FISH (fluorescence in situ hybridization) of viable isolated CTCs[8].

Non-invasive tumor biopsies could thus become a reality, and treatments could be followed up as frequently as needed. Tumor genotype could also be monitored during treatment. CTCs isolated using these technologies may be assimilated to a "liquid biopsy" allowing for an individualized molecular study (mutations, known drug resistance genes, new tumor markers, etc.) specific for each patient.

There is currently increasing evidence showing the clinical significance of CTC detection in peripheral blood from cancer patients. CTCs have been shown to be a relevant prognostic and predictive biomarker after treatment[1-4].

Part of such evidence comes from studies conducted on urogenital organs[4,9], which supports the need for promoting multicenter translational research in urology and warrants the contents of this editorial comment.

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