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CIRCULATING TUMOR CELLS: A VALUABLE TOOL TO MONITOR THE CLINICAL COURSE OF PATIENTS WITH EPITHELIAL NEOPLASMS IN THE ROUTINE SETTING

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ABSTRACT

Background: Circulating tumor cells (CTC) in the peripheral blood of cancer patients (pts) is an indicator of a poor prognosis and have also been successfully used to monitor therapy (Tx) Currently, the CellSearch™ system (CS; Veridex, Raritan, NJ, USA) is the only FDA-approved technique for CTC detection. Despite its prognostic and predictive merits gauined in numerous trials, there are only a few data existing elucidating the value of CS in the routine setting. We thus report on our single institution experiences in the clinical use of CS in pts with various epithelial tumors.

Methods: A total of 394 samples have been analyzed (breast cancer, BC: 266; prostate cancer, PC: 70; colorectal cancer, CRC: 10; ovarian cancer, OC: 25; others, 23) CTC-negativity and –positivity were distinguished using a threshold of > 3 (CRC) or > 5 CTCs (all others) in 7.5 mL venous blood. 35 endocrine, cyctostatic, immunologic, or combined therapies (Tx) in BC (n=23), OC (n=7), and PC (n=3) were monitored by CS performed prior to and 6-8 weeks after Tx initiation. The first radiologic re-evaluation was performed 12 weeks after start of Tx and repeated every 3 months, if indicated. The response status was scored according to RECIST.

Results: In all but 4 cases (all BC), CS was considered as evaluable, resulting in an assay success rate of 99%. 19 BCs (7.1%) had 1-5 CTCs, and 21 (7.9%) had > 5 CTCs. The corresponding results were 4 (5.7%) and 10 (14.3%) for PC, and 4 (16%) and 0 (0%) for OC. In 35 pts monitored by CS, 19 progressed while 16 did not progress on Tx. All progression-free pts showed constantly normal or declining CTC values. In only one pt, the CTC count did not drop into the normal range. IN contrast, 13 of 19 pts showing disease progression had increasing CTC counts. Moreover, 3 pts with pathological CS did not normalize while being on Tx. Notably, a CTC increase within the normal range indicated progression in 2 cases, whereas a decrease within the normal range was associated with response to Tx in 4 pts.

Conclusions: CS is a valuable and robust tool to determine CTCs in the peripheral blood of pts with various epithelial malignancies in the routine setting. Contrasting its high specifity compared to other methods, the sensitivity of CS is relatively low which may result in a considerable number of false-negative measures. When regarding our own experiences, we thus conclude that the occurrence of any CTC detected by CS must be taken seriously.

INTRODUCTION

During the last decade, an increasing body of evidence has been emerged suggesting the occurrence of circulating tumor cells (CTCs) is a negative prognostic factor in patients (pts) with various epithelial neoplasms including breast cancer (BC), colorectal cancer (CRC), prostate cancer (PC), lung cancer, bladder cancer, and many others. In metastatic tumors, CTCs have also been successfully used to monitor various antineoplastic therapies (Tx). Currently, the CellSearch™ technology (CS, Veridex, Raritan, NJ, USA) is the only FDA-approved technique for CTC detection in pts with metastatic BC, CRC, and PC. Its high acceptance is mainly due to the fact that Tx monitoring by CS allows to discriminate potential responders and no-responders earlier than any other diagnostic means. Therefore, CS has already been incorporated in many phase II and phase III for both BC and PC. Cs is an immunomagnetic technique which uses the epithelial cell-adhesion molecule (EpCAM) as the primary target to capture CTCs which are then further characterized by expression of cytokeratines (CK), and various cell surface antigens such as HER-2/neu and others. Contrasting competing methods, CS is characterized by both a high specifity and a high positive predictive value offering an exceptionally low rate of false positive results. In pts with metastatic CRC, > 3 CTCs found in 7.5 µL peripheral venous blood is considered a positive result which is > 5 CTCs in all other indications. However, studies with CS in primary BC pts subjected to both neoadjuvant and adjuvant chemotherapy showed that the detection of only 1 CTC in 7.5 mL blood was associated with an impaired survival. Despite unequivocal prognostic and predictive merits, there is only limited information published so far, elucidating the value of CS in the routine oncologic setting. We hereby report on our single institution experience with the clinical use of CS in pts with various epithelial tumors.

METHODS

A total of 394 blood samples derived from patients with various epithelial tumors have been analyzed: BC, 266; PC, 70; CRC,10, ovarian cancer (OC), 25; miscellaneous, 23 (Figure 1). Both isolation and counting of CTCs were performed by using CS. This technology provides the immunomagnetic selection, fluorescence staining, concentration, and enrichment of CTCs. A total of 7.5 mL peripheral venous blood was collected in a CellSave™ Preservation Tube (Veridex), prefilled with an optimized EDTA-based preservative that stabilizes cells at room temperature for up to 96 hours. Another 7.5 mL sample which was taken simultaneously served as a backup. Immunomagnetic enrichment was performed automatically by using the anti-EpCAM Ferrofluid™ (Veridex). Isolated cells were then labeled fluorescently with the 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid dye and with monoclonal antibodies detecting CKs 8/18/19 and CD45. CTCs were identified as cells with the appropriate morphology as cytokeratin positive, DAPI positive, and CD45 negative. CTC-negativity and –positivity were distinguished using a threshold of > 3 (in CRC) or > 5 CTCs (all other tumor types) in 7.5 mL blood. A total of 35 antineoplastic treatments (endocrine, cytostatic, immunologic, or combined) in pts with overt metastastic tumors (BC, 23; OC, 7; PC, 2) were monitored by serial CS analyses performed prior to and 6-8 weeks after Tx initiation. Accordingly, radiological tumor imaging was performed prior to and 12 weeks after start of Tx. The response status was determined according to RECIST. In pts free from progression, additional radiological examinations were performed every three months, or at any other time upon the physician´s discretion (Figure 2).

RESULTS

In all but 4 cases (all BC), CS was considered as evaluable, resulting in an assay success rate of 99%. The main reason of non-evaluability was an excessive formation of cell aggregates occurring in the test tubes which impaired the immunomagnetic cell capture. Figure 4 illustrates the distribution of pathological and pre-pathological CS results in BC, PC, and OC. In BC, 19 samples (7.1%) showed 1-5 CTCs, and 21 samples (7.9%) had > 5 CTCs. The corresponding results were 4 (5.7%) and 10 (14.3%) for PC and 4 (16%) and 0 (0%) for OC. The relatively low frequency of samples with at least 1 detectable CTC reflected the high proportion of non-metastatic pts in the population investigated.

In 35 pts treated for over metastatic disease which were monitored by repeated CS measurements, 19 progressed, while 16 did not-progress while being under observation. As shown in Figure 5, all progression-free pts showed constantly normal or declining CTC counts during Tx. In only one pt out of this group, the CS result did not completely normalize although throughoutly improving. In contrast to these findings, 13 of 19 pts with progressive disease had increasing CTC counts. Additionally, 3 pts out of this group did not normalize although showing declining CTC counts during Tx. Notably, a CTC increase within the normal range indicated progression in 2 cases whereas a decrease within the normal range was associated with response to Tx in 4 individuals.

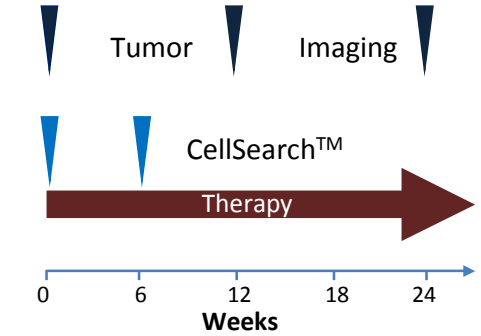

Figure 2: Evaluation scheme applied in 35 patients systemically treated for overt metastatic disease

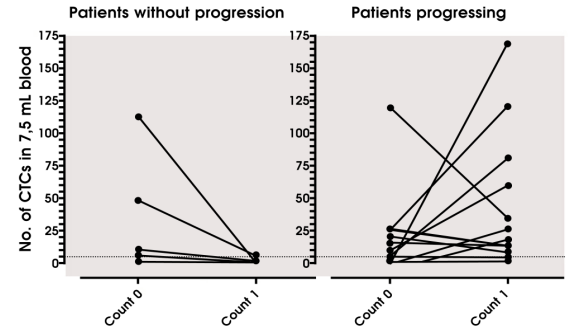

Figure 4: Results of sequential CTC counts performed by CellSearch™ in tumor patients exposed to antineoplastic therapy for overt metastatic disease

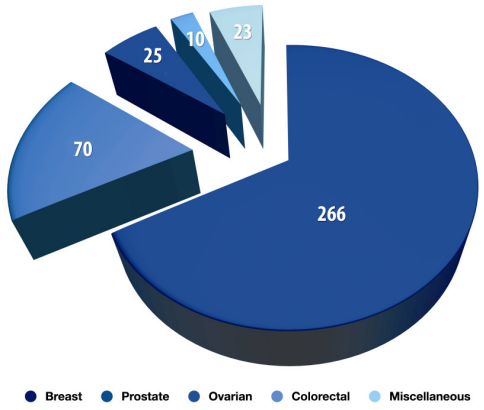

Figure 1: Distribution of tumor types tested for circulating tumor cells by using the CellSearch™ technology

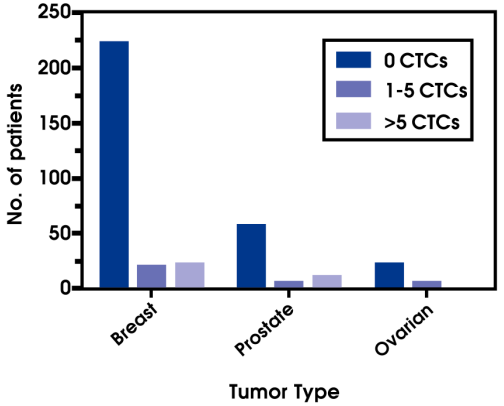

Figure 3: Distribution of CellSearch™ results in breast, prostate, and ovarian carcinomas

CONCLUSIONS

- The FDA-approved CellSearch™ technology is a valuable and robust tool to determine circulating tumor cells in the peripheral blood of cancer patients in the routine setting
- The assay evaluability rate is approximately 99%, particularly focusing on its methodological safety.
- CellSearch™ offers a high specifity and, accordingly, a high positive-predictive value, suggesting that a cell under suspicion detected by this technology is most likely a real tumor cell.
- The overall CTC detection rate in our population was relatively low reflecting the high proportion of non-metastatic cases.
- In patients treated for metastatic breast, prostate, or ovarian cancer, constantly normal or declining CTC counts were associated with tumor response whereas increasing CTC counts indicated tumor progression in the majority of cases.
- Changes of CTC counts within the normal were of particular interest. In our population of patients, an increase of CTCs within the normal range was associated with disease progression in 2 patients whereas a decrease within the normal range indicated response to therapy whatsoever in 4 individuals.
- In regard to the high specifity of the CellSearch™ system and our own experiences reported hereby, we conclude that the appearance of any CTC detected should be taken seriously because it may indicate subclinical metastatic tumor burden.

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