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LASER SCANNING CYTOMETRY AND MAGNETIC BEAD ENRICHMENT FOR RAPID QUANTITATIVE ENUMERATION OF MINIMAL NUMBERS OF TUMOR CELLS IN PERIPHERAL BLOOD AND BONE MARROW IN BREAST CANCER PATIENTS

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Laser canning cytometry, a new method to quantify, enumerate and relocate cells carrying a defined phenotype or genotype, was used to detect individual disseminated breast cancer cells in patients in different stages of disease by immunofluorimetry. After staining whole blood or bone marrow with antibodies specific for epithelial antigens only epthelial cells were stained. Automatic counting of stained and unstained cells allowed quantification of the aberrant cells. Relocation in addition enabled discrimination of tumor cells from unspecifically stained events and from normal skin epidermal cells staining specifically. In cases of very low numbers of aberrant cells magnetic bead enrichment allowed to detect even minimal numbers of tumor cells and enrichment was up tp 10 000 fold. In contrast to molecular genetic methods requiring tumour specific markers and flow cytometry, which is neither sensitive nor unequivocal Laser Scanning Cytometry provides an automated procedure for screening up to 5x104 cells in suitable time and, most importantly, for rescreening positive events for morphological verification. We have applied this method together with the enrichment procedure to an artificial system, mimicking seeding of tumour cells into the periphery by diluting breast cancer cell line cells into peripheral blood. One positive cell was unequivocally detectable in 104 cells without previous enrichment and it was possible to reliably recover 50 out of 60 tumour cells added to a 20ml blood volume, equal to 1-2 cells in 107, after magnetic bead enrichment. Data from blood and bone marrow samples of ten normal controls and 50 breast cancer patients in different stages of disease are presented. This method allows quantitation of tumour cells in peripheral blood and bone marrow in reasonable time and will, for the first time, enable extensive quantitative investigation of the seeding behaviour of breast tumours.

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