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Detection of disseminated tumor cells (DTCs) might improve prognosis of breast cancer patients

Nel I., Reinshagen A., Kreklau A., Aktas B.
University of Leipzig, Medical Center, Dept. of Gynaecology

Background

Despite successful treatment of the primary tumor recurrence occurs in about 30% of breast cancer patients. One reason might be hematogenous spread during early disease stages. Disseminated breast cancer cells preferentially migrate into the bone marrow (BM) where they become dormant (Fig.1). Due to low proliferation in this "steady state" DTCs are persistent against systemic chemotherapy and may cause metastatic relapse at a later stage. DTCs may serve as independent prognostic markers that are associated with impaired survival.

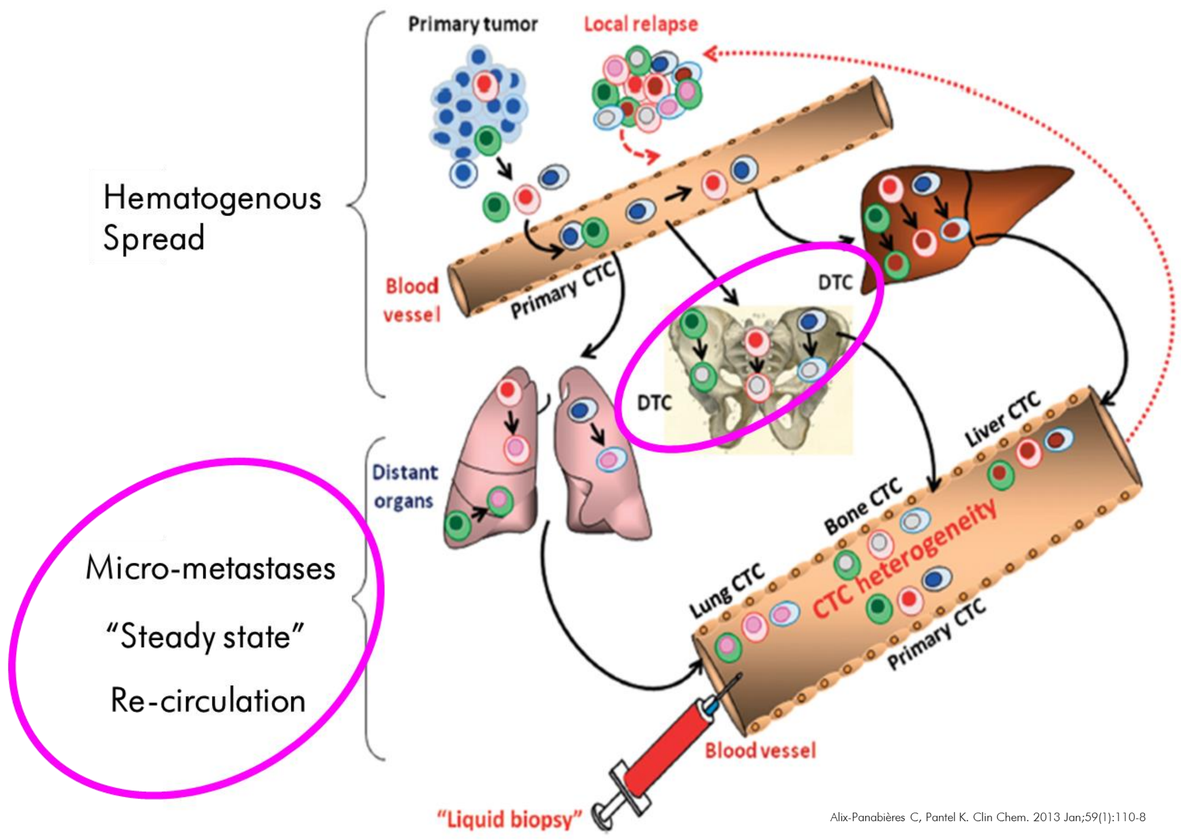


Fig. 1) Tumor cell dissemination.

Methods

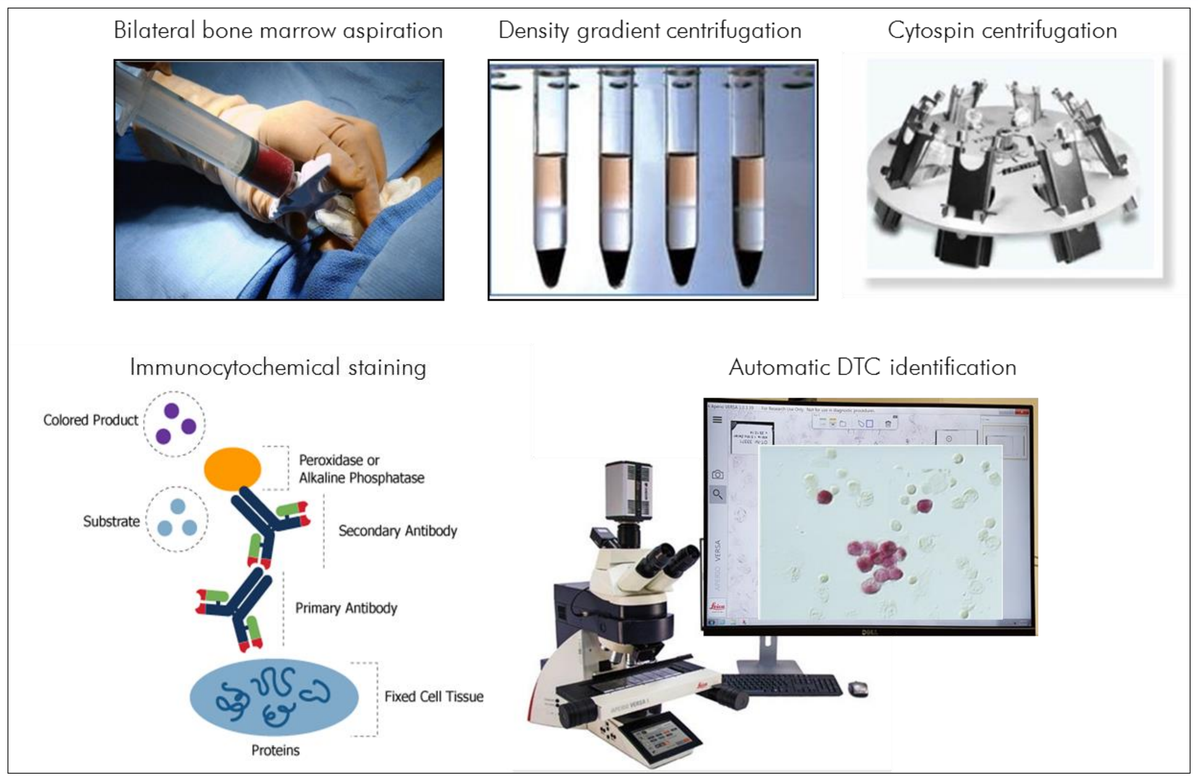
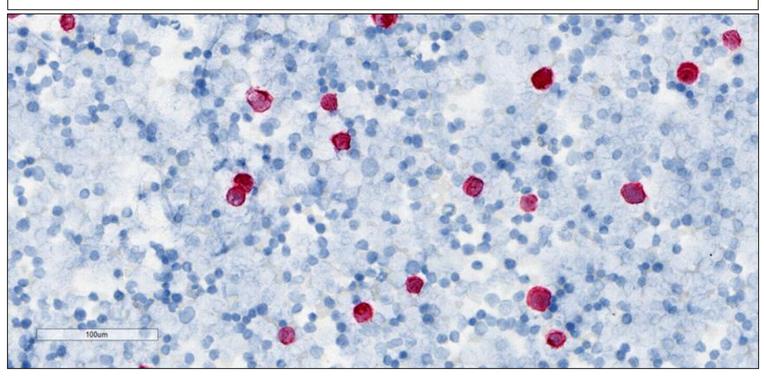


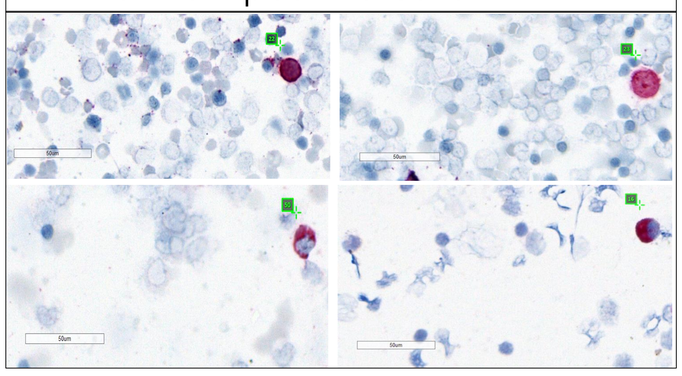
Fig.2) BM aspirates were collected from the anterior iliac crest during surgery. After density gradient centrifugation cell suspensions were transferred onto glass slides and subjected to immunocytochemical staining against pan-cytokeratin. DTCs were visualized in pink using alkaline phosphatase and short counterstaining with hematoxylin which colored the nuclei light blue. DTCs were semi-automatically detected and enumerated using the Aperio Versa microscope-based scanning system with rare events software that selected DTC candidates according to color, shape, intensity and size. Reference slides with a mix of bone marrow cells and a defined number of HCT116 cells were used as a positive control. Between February and November 2019 BM aspirates from 79 breast cancer patients were collected. Per patient about 4 million BM cells were analyzed.

Results

A) Positive Control:
BM cells mixed with HCT116 cells.



B) Patients samples:
Pan-CK positive cells



C) Patient samples:
Pan-CK positive cell clusters

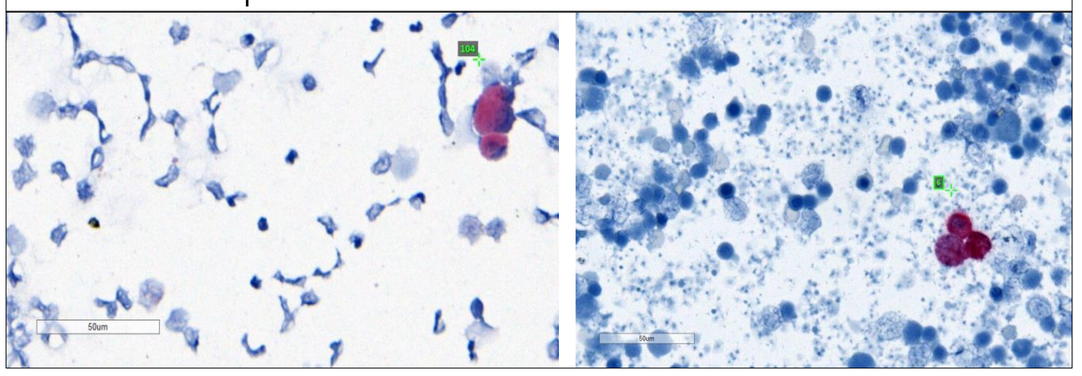


Fig. 3 A-C) Samples were stained with Pan-CK antibody AE1/AE3, visualized with alkaline phosphatase and detected using Aperio Versa System and Rare Event algorithm.

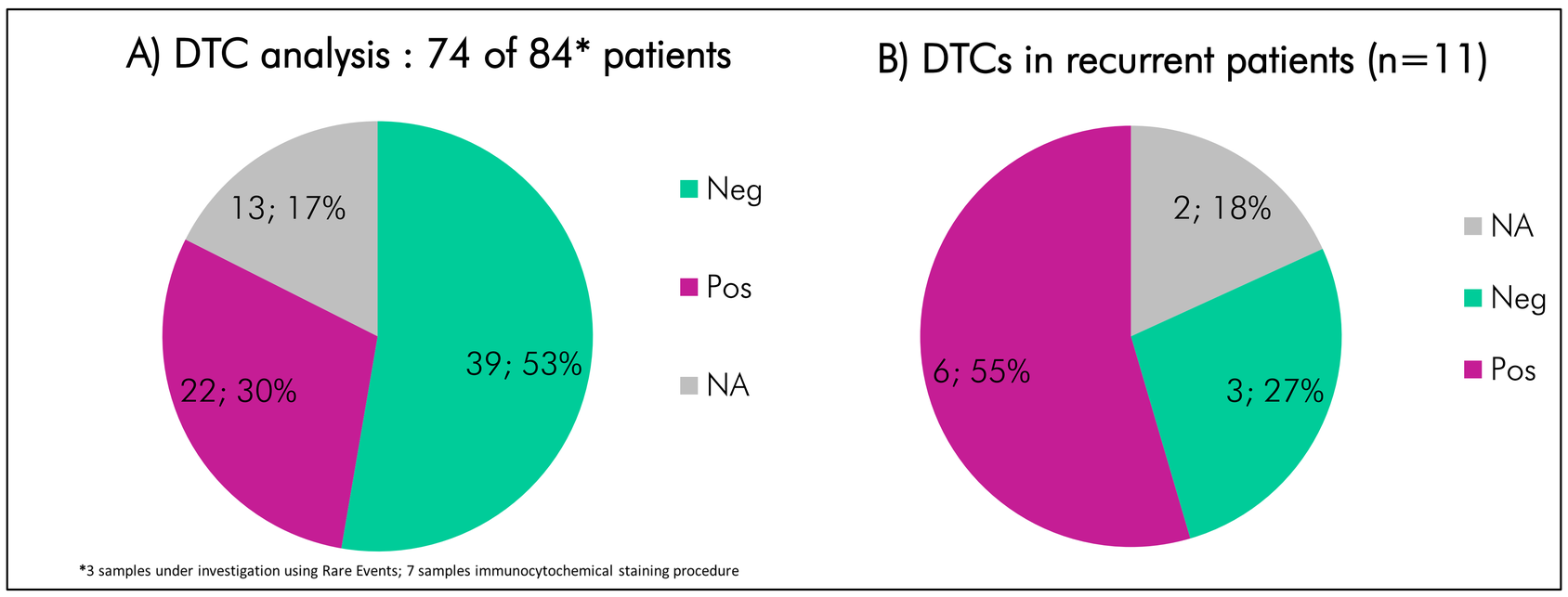


Fig. 4) DTC status: A). Pan-CK positive cells were detected in 30% of the analyzed cases (n=74). 53% were negative and 17 % were not applicable or still under review. B) 11 patients were recurrent when BM was aspirated and processed. Of the relapsed patients 55% had a positive and 27% a negative CTC status. 2 of the samples are still being analyzed.

Outlook

A very promising approach to eradicate DTCs is the use of bisphosphonates (BP). Breast cancer patients that were tested positive for DTCs could benefit from BP intake and hence better prognosis even years after first diagnosis. Based on DTC status, patients with high risk for relapse can be identified and treated accordingly.

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