Cellular Immunodiagnostics: Circulating Tumour Cells - CTCs Enrichment and Quantification

One of the most promising developments in cancer medicine is the detection of circulating tumour cells (CTCs) as a minimally invasive multifunctional biomarker. CTCs in peripheral blood originate from solid tumours and are responsible for metastasis. Quantification of CTCs can help to estimate and predict the course of disease in patients. It can thus be seen as a **'liquid biopsy'** and real-time marker for tumour progression and survival prognosis.

It must be possible to detect CTCs in concentrations millions of times lower than those of leukocytes. Detection of 30-100 cells in 7.5ml EDTA blood is already regarded as prognostically unfavourable according to existing studies. However, this number varies depending on the type of tumour. This shows the great necessity of a very sensitive test procedure as well as a preceding enrichment for the detection of CTCs.

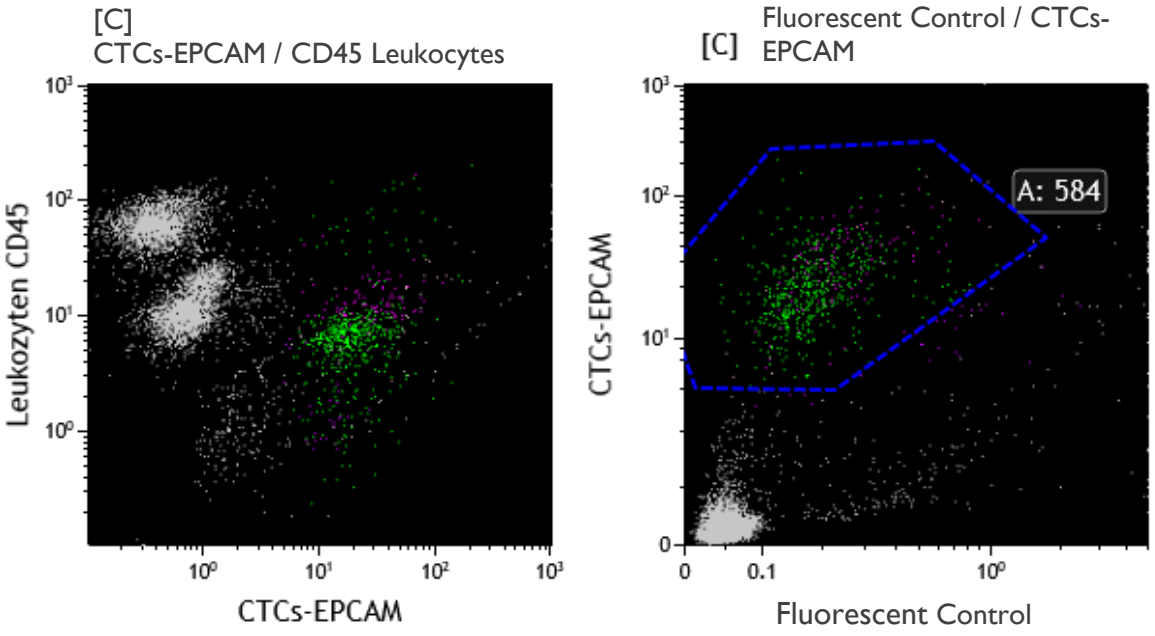
Enrichment of CTCs by immunomagnetic cell separation

Nearly all tumour cells express **EPCAM** (**ep**ithelial cell-**a**dhesion **m**olecule = **HEA h**uman **e**pithelial **a**ntigen) on their surface. Using this antigen, the cells are thus ferromagnetically labelled and adhered to an iron carrier applying a magnetic field, then washed and finally eluted after removing the magnetic field. Enrichment factors of 1000 to 2000-fold can be achieved in this way.

Analytical quantification of the CTCs

By subsequent labelling of the antigens with fluorescence dye-conjugated antibodies, the CTCs can be analytically detected and quantified. Various fluorescence microscopic, semi-automated methods are available for this purpose.

We apply **flow cytometry**: The cell eluate is labelled with EPCAM antibodies, the leukocyte antigen marker CD45 and a vital dye. The CTCs we are scanning for are positive for EPCAM, negative for CD45 (Fig., green) and stain with the vital dye when they are no longer alive (Fig., red).



Are all detected CTCs actual tumour cells?

The current methodology does not allow definitive confirmation that these are actual tumour cells, as circulating tumour cells do not carry a tumour-specific antigen on their surface. They can only be quantified by their epithelial cell (EPCAM+) property and their negativity for the CD45 leukocyte antigen. In healthy volunteers, virtually no epithelial cells would usually circulate in the blood. However, exceptions are possible, such as in the event of injuries, postoperatively, or in certain diseases (e.g. diverticulosis, polyps, Crohn's disease, ulcerative colitis or endometriosis lesions in the colon). We, therefore, do not refer to tumour cells in our results, but to **tumour suspect cells**.

Requirement

EPCAM, profile 7893
Blood sampling preferably Monday to Thursday
Specification of clinical results, type of tumour, treatment, etc.
Material: 2x EDTA blood

Results and interpretation

Number of EPCAM-positive tumour suspect cells detectable in 7.5 ml EDTA blood.
Progressions are displayed graphically and support the evaluation of disease and therapy progression.

We recommend the following interpretation for initial measurements:

- 0- approx. 30 cells / 7.5 ml EDTA blood: no repeat measurement indicated
- approx. 30-100 cells / 7.5 ml EDTA blood: repeat measurement indicated after approx. 3 months
- From approx. 100 cells / 7.5 ml EDTA blood: further tumour diagnosis indicated

Price

350 TP (not covered by health insurance)

Complementary diagnostics

NK cell test, basic profile 2830
Blood sampling preferably Monday to Thursday
Material: Heparin blood, fresh

The NK cell test can be recommended to additionally map the ability of killer cells as primary, non-MHC-dependent immune defence against tumour cells. It measures the number, degranulation ability as an indicator of cytotoxicity, and stimulability by interleukin 2 in the basic profile. Additional tests for modulators can be requested.

Detailed information can be found in the information for physicians (Arztinfo) available in the menu item Service Download Centre ('Service-Downloadcenter') on our homepage.

References:

1. Lopresti et al.: Sensitive and easy screening for circulating tumor cells by flow cytometry; JCI Insight. 2019;4(14):e128180. https://doi.org/10.1172/jci.insight.128180.
2. Warawdekar et al.: A versatile method for enumeration and characterization of circulating tumor cells from patients with breast cancer; J Cancer Metastasis Treat 2017;3:23-33.
3. Kerklaan et al.: EpCAM-based flow cytometry in cerebrospinal fluid greatly improves diagnostic accuracy of leptomeningeal metastases from epithelial tumors; Neuro-Oncology 18(6), 855–862, 2016; doi:10.1093/neuonc/nov273.
4. Watanabe et al.: Multicolor Detection of Rare Tumor Cells in Blood Using a Novel Flow Cytometry-Based System; Cytometry PartA 85A, 2014, 206-213.
5. Simsek et al.: Determination of Circulating Tumor Cells in Peripheral Blood By Flow Cytometry; Niche, 2014; 3: 0-0 • DOI: 10.5152/niche.2015.246
6. Parkinson et al.: Considerations in the development of circulating tumor cell technology for clinical use; Journal of Translational Medicine 2012, 10:138.
7. Man et al.: Currently Used Markers for CTC Isolation - Advantages, Limitations and Impact on Cancer Prognosis; J Clinic Experiment Pathol 2011, 1:1.
8. Allard et al.: Tumor Cells Circulate in the Peripheral Blood of All Major Carcinomas but not in Healthy Subjects or Patients With Nonmalignant Diseases; Clinical Cancer Research Vol. 10, 2004, 6897–6904.