EpCAM negative circulating tumor cells in metastatic lung cancer enriched by filtration

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Background

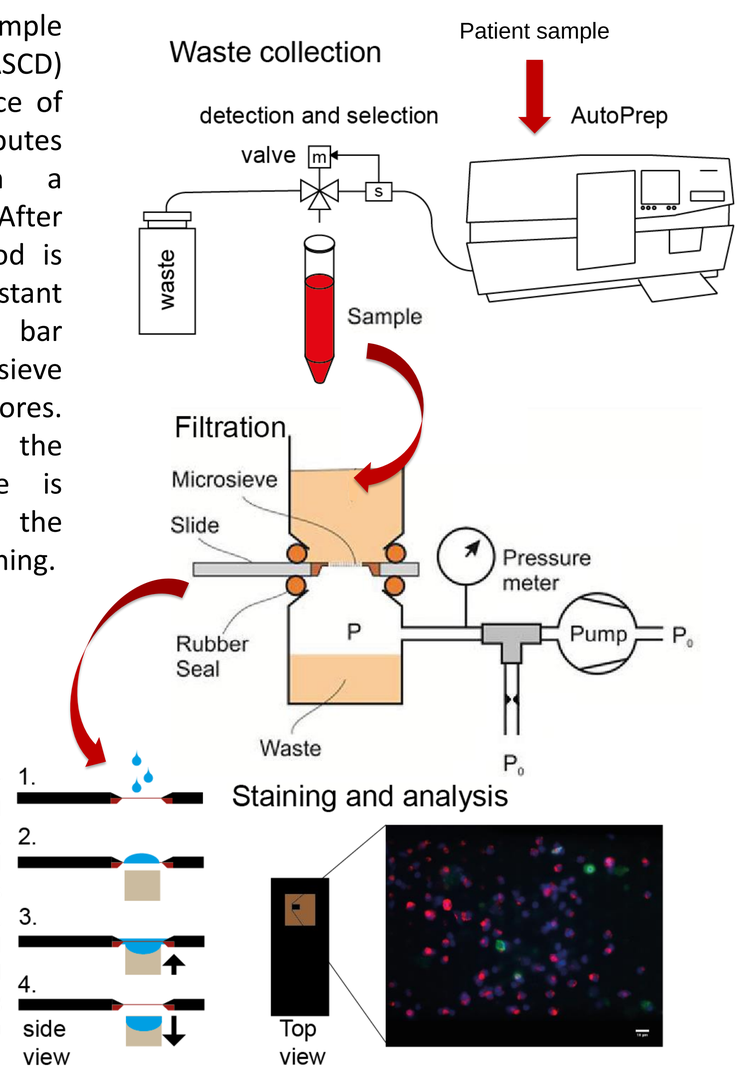
Presence of circulating tumor cells (CTC) in patients with lung cancer is associated with poor survival. The frequency of CTC in lung cancer patients enriched by the CellSearch system is very low, raising the question whether EpCAM-negative CTC can be found that are missed by the CellSearch system. Blood discarded by CellSearch after the EpCAM enrichment was collected and filtered for CTC enrichment and enumeration after immunofluorescent labelling.

Study design

To investigate EpCAM-negative CTC in lung cancer patients, a device was designed that collects the sample material of the individual samples that is discarded by CellSearch. EpCAM positive CTC were isolated using the CellSearch system and EpCAM-negative CTC were isolated from blood discarded by the CellSearch Autoprep using filtration. Extra cytokeratin (CK) markers were added to the CellSearch system to broaden the coverage of all CK-positive CTC .

Methods

The Autoprep Sample Collection Device (ASCD) detects the presence of blood and distributes each sample in a colonical tube. After collection, the blood is passed with constant pressure of 100 bar through a microsieve containing 5 µm pores. After filtration of the sample, the slide is removed from the consumable for staining.



Staining cocktail: nuclear dye DRAQ5, two PE-labeled antibodies targeting CK 4-6, 8, 10, 13, 18 & 19 (C11 and A.53B/A2), three antibodies targeting CK 1-8, 10, 14-16, 19 & 20 (AE1/AE3, LP5K and KS20.10) labeled to FITC and one antibody targeting CD45 (HI30) labeled with Brilliant Violet.

Figure 1 *Overview of methods for analysis of patient samples with Waste filtration and staining on the microsieve.*

The staining of cells is performed on the sieve. Permeabilization was initiated with PBS/saponin 0.15%, followed by incubation at 37°C for the staining cocktail. After fixation, the sieve was covered with a mounting medium and subsequently sealed with a cover slip for fluorescent microscope analysis or storage at -20°C.

Cell lines

The performance of the ASCD and microsieve filtration was tested using pre-stained EpCAM-positive (SW480 and SK-BR-3) and EpCAM-negative cell lines (COLO 320, T24 and NSCLC cell line NCI-H1650). Spiking experiments showed that the majority of EpCAM-positive cells could be detected with the CellSearch system, whereas very few were detected with EpCAM-low or negative cells. The recovery of these cells on the microsieves depended strongly on the size of the

	Relatively large		Relatively small			Negative control
	T24 $EpCAM^{low}$	SK-BR-3 $EpCAM^{high}$	COLO 320 $EpCAM^{low}$	SW480 $EpCAM^{high}$	NCI-H1650 $EpCAM^{neg}$	
EpCAM molecules	4.9×10^3	1.5×10^6	2.0×10^3	2.3×10^6	1.35×10^2	
Size	16 µm	16 µm	11 µm	11 µm	12 µm	
Prestained cells (n=5)						
CS recovery	2% (±1)	87% (±12)	2% (±2)	91% (±13)	0.2% (±0.3)	
MS recovery	59% (±9)	2% (±1)	18% (±6)	6% (±7)	60% (±7)	
Antibody Staining (n=4)						
CS recovery	15% (±5)	nd	nd	nd	0.1% (±0.1)	0.6 CTC (±1.2)
MS recovery	23% (±7)	nd	nd	nd	27% (±1)	0 CTC (±0)

Table 1 *Recovery of cell lines spiked in blood of 5 donors and processed by CellSearch (CS). The blood discarded by CS was collected and filtered through a micosieve (MS). The cells in the CS cartridges were counted on the CellTracks Analyzer and the cells on the MS by standard fluorescent microscope.*

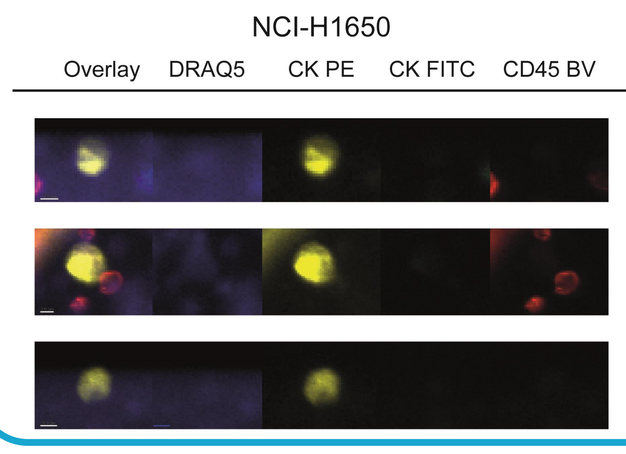


Figure 2 *NSCLC cell line NCI-H1650 filtered with a microsieve from the CellSearch Waste and stained with the staining cocktail (CK PE), with extra cytokeratin antibodies (CK FITC). CD45 shows some white blood cells.*

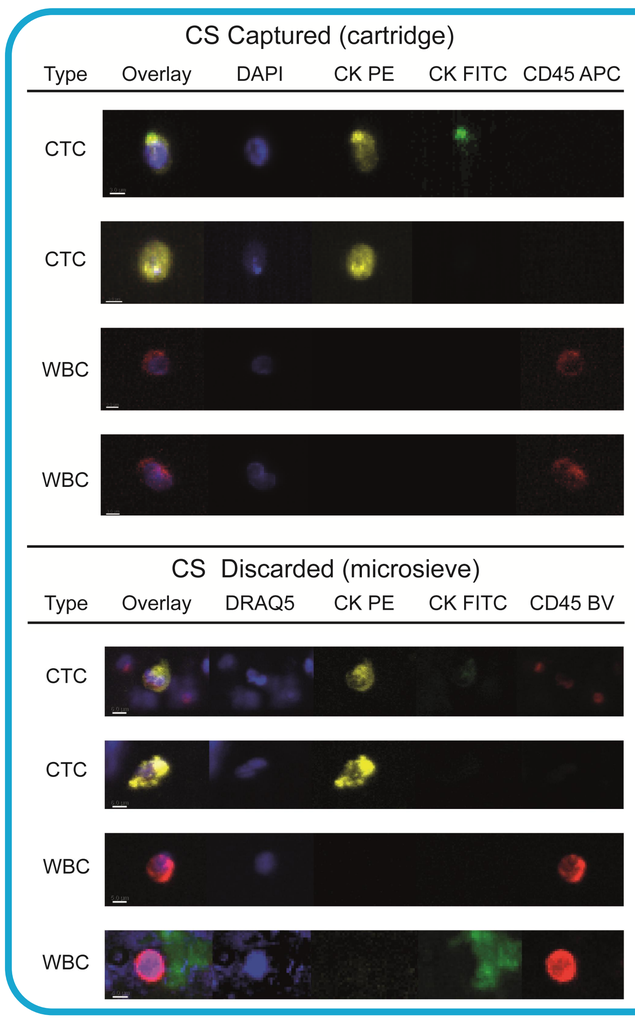


Figure 3 *NSCLC patient CTC found with CellSearch (upper panel) and EpCAM-negative CTC detected on the sieve from CellSearch Waste (lower panel).*

Patient data

In patients with CTC, we found more EpCAM-negative CTC in CellSearch Waste than EpCAM-positive CTC in CellSearch. The additional CK markers show that the expression of CK is heterogeneous in the CTC population. When examining the discarded blood with the use of additional cytokeratin antibodies, CTC counts increase. However, there is no correlation between the number of both types of CTC in each sample with a Spearman's Rho of 0.022.

CTC in patient samples (N=29)	≥1	≥3	≥5	≥10
CTC in CellSearch	41%	17%	14%	10%
Patients extra due to sieved CTC	35%	31%	28%	11%
CellSearch CTC & sieved CTC	76%	48%	41%	21%
CTC in patient samples (N=28)	≥1	≥3	≥5	≥10
CellSearch CTC	43%	18%	14%	11%
Patients extra due to added CK	11%	7%	4%	0%
CellSearch CTC & extra marker	54%	25%	18%	11%
All CTC CS CTC (CK PE or CK FITC) and CS Waste CTC	**83%**	**59%**	**41%**	**24%**

Table 2 *Overview of CTC found in 29 lung cancer patients by CellSearch (CS), on the microsieves after filtration of the CS Waste and in CS using additional cytokeratins (CK).*

Conclusion

The number lung cancer patients in which CTC could be detected, and the number of CTC detected in these patients, is doubled by expanding the CellSearch assay by filtration of the blood discarded by the CellSearch system and the cytokeratin coverage. The relation between the presence of these CTC populations and clinical outcome will need to be established to determine the clinical relevance of this observation.

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