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A Comparative Evaluation of the Results of Immunofluorescence and Hemofiltrocytological Testing in Cancer Patients

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Abstract:

Immunofluorescence is a unique immunochemical technique that combines the detailed morphological testing and the use of the specificity of an immunological response. The advantage of immunofluorescence is its high sensitivity and resolution. We have studied cell smears of the venous blood filtrate of cancer patients by immunofluorescence, and the isolation of the circulating tumor cells (CTCs) by the device for blood microscreening confirmed the results of immunofluorescence.

Key Words: immunofluorescence, circulating tumor cells, the device for blood microscreening.

Introduction

Modern science and technology achievements have significantly expanded the capabilities of recognizing malignant tumors (MT), however, due to the constant search for new more advanced diagnostic methods, the interest of researchers in circulating cancer cells (CTCs) does not wane.

Unfortunately, it is not always possible to detect cancerous cells in the circulating blood. Such traditional biological method for detecting carcinemia as inoculation experimental animals with tumor by blood indicate that carcinemia is a natural phenomenon. In some cases, tumor blood transplantability reached 100% [1]. However, given that the number of CTCs is very small, the reliable systems with high reproducibility will allow accurately identifying and monitoring them in dynamics. To date, there are few such systems suitable for clinical use. One of them is the CellSearch system — a semi-automatic device that uses immunomagnetic sorption to detect CTCs. The advantages of this system are the reproducibility of data in different laboratories and the ability to identify CTCs in various types of cancer [2]. Newer technologies take advantage of specific epithelial-coated antigens like epithelial cell adhesion molecule (EpCAM) to selectively capture CTCs. CellSearch is the only clinically validated, FDA-cleared system for identification, isolation, and enumeration of CTCs for prediction and treatment monitoring in metastatic breast, prostate and colorectal cancer. The method is based on immunomagnetic separation [3, 4, 5], when EpCAM conjugated magnetic beads are used to capture EpCAM-positive CTCs from the blood under the influence of a magnetic field. And although many clinical studies have confirmed its predictive value, the analysis is expensive and labour-intensive, which, unfortunately, limits its wider use.

Isolation of CTCs from venous blood based on cancer cell size through microfiltration has proven to be a potentially effective, inexpensive, and rapid method for isolating cancer cells [6, 7, 8, 9].

Materials and Methods

We carried out the hemofiltrocytological test in patients with abdominal and lung tumors to detect CTCs in peripheral venous blood (patent for invention No. 2425385 of the Federal Service for Intellectual Property, Patents and Trademarks). The technique we used consisted in microfiltration of peripheral venous blood followed by a cytological examination of the smears obtained from venous blood filtrate in order to determine the CTCs in it. Microfiltration was carried out using a device for blood microscreening, which has a filter with a pore diameter of 6000 nanometers (patent for invention No. 2414710 of the Federal Service for Intellectual Property, Patents and Trademarks). To carry out a hemofiltrocytological test we drew 12 ml of venous EDTA-stabilized blood of patients with cancer of various locations, and passed it through a nanofilter with a pore diameter of 6000 nm. Cells retained on the filter were placed on a glass slide. 3 smears were obtained from each filtered blood sample. The result of the test was considered positive (+) if circulating tumor cells were detected in the smears and, accordingly, negative (-) if circulating tumor cells were not found in them.

Using immunofluorescence technique, we assessed the presence of EpCam (CD326)-positive cells in 24 smears obtained from a device for blood microscreening. For identification of EpCam (CD326)-positive cells we used the Axiostarplus fluorescence microscope (CarlZeiss, Germany). For this purpose, fixed smears were treated with proteinase K (Dako, Denmark) for 15 min at 37°C, washed with PBS, then incubated with fluorescent nuclear dye PI (Invitrogen, USA) and monoclonal antibodies against human EpCam (CD326) (clone B29.1 (VU-ID9)), labeled with FITC (Abcam, UK) at a concentration of 5 μg/ml. The result was considered positive, when there were cells with nuclear red staining (PI) together with a green label (EpCam-FITC).

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Patients	Age	No. of Smears	The results of testing on	
			CTCs	EpCAM-positive cells
1	64	1-a	+	+
		1-b	-	-
		1-c	+	+
2	40	2-a	-	-
		2-b	+	-
		2-c	-	-
3	75	3-a	+	+
		3-b	+	+
		3-c	+	+
4	40	4-a	+	+
		4-b	+	+
		4-c	+	+
5	53	5-a	+	+
		5-b	+	+
		5-c	+	+
6	46	6-a	+	+
		6-b	-	-
		6-c	+	+
7	43	7-a	+	+
		7-b	-	-
		7-c	+	+
8	76	8-a	+	+
		8-b	+	-
		8-c	-	-

Results and Discussion

As the Table 1 shows, the hemofiltrocytological testing detected circulating tumor cells in the peripheral venous blood of eight cancer patients. As mentioned above, 3 smears were obtained from the filtered blood sample of each patient.

The results were as follows: CTCs were detected in all 3 smears of three patients (No. 3a-3c; 4a-4c and 5a-5c), in 2 smears of four patients (No. 1a, 1c; 6a, 6c; 7a, 7c; 8a and 8b), and in 1 smear of one patient (No. 2b). Such difference in results is explained by the stages of cancer in the examined patients. There is a direct connection between the stage of the disease and the severity of carcinemia: the

higher the stage, the greater the number of CTCs per unit volume of the peripheral vascular bed [10].

The stage of cancer, as well as the presence or absence of metastases in regional lymph nodes in the studied patients, were confirmed by the results of pathohistological examination of the surgical specimens.

We used immunofluorescence technique to study 24 cell smears obtained from a device for microscreening of venous blood, which has a filter with a pore diameter of 6000 nm. Using mouse monoclonal antibodies against human EpCAM (CD326) (clone D29.1 (VU-ID9)) labeled with FITC, and nuclear fluorescent dye PI, we detected circulating tumor cells in smears on an Axiostarplus microscope (CarlZeiss, Germany). Since there were 24 smears, a total of 24 samples were tested using the immunofluorescence. The study found that 16 out of 24 samples contained EpCAM-positive cells.

6 samples of smears (No. 1-b, 2-a, 2-c, 6-b, 7-b and 8-c) with (-) results for CTCs when examined by immunofluorescence also showed negative results. The immunofluorescence of two samples of smears (No. 2b and 8b with (+) results for CTCs) didn't found EpCAM positive cells. Consequently, the percent agreement of all results for the two compared diagnostic techniques, both positive and negative for CTCs, was 91.6%.

Thus, the results of immunofluorescence confirm the high efficiency of isolating circulating tumor cells from venous blood using a microscreening device with a nanofilter having a pore diameter of 6000 nm.

Conclusions

1. Hemofiltrocytological testing, compared to immunofluorescence, is an inexpensive diagnostic technique.

2. Hemofiltrocytilogical technique is simple to perform and gives 2.5 times faster results compared to the immunofluorescence.

3. Hemofiltrocytological testing is an effective diagnostic technique, not much inferior to the immunofluorescence in terms of accuracy in detecting circulating tumor cells.

References

1. Napalkov N. P. *Obshchaya onkologiya: Rukovodstvo dlya vrachey* [General Oncology: A Guide for Doctors]. Ed. by N. P. Napalkova. Leningrad : Meditsina Publ., 1989. P. 161 (In Russ.).
2. Allard W. J., Matera J. et al. Tumor Cells Circulate in the Peripheral Blood of All Major Carcinomas but Not in Healthy Subjects or Patients with Nonmalignant Diseases. *Clin. Cancer Res.* 2004; 10(20): 6897 – 6904.
3. Benez A., Geiselhart A.et al. Detection of Circulating Melanoma Cells by Immunomagnetic Cell Sorting. *J. Clin. Lab. Anal.* 1999; 13(5): 229–233.
4. Racila E., Euhus D. et al. Detection and Characterization of Carcinoma Cells in the Blood. *Proc. Natl. Acad. Sci. U.S.A.* 1998; 95(8): 4589–4594.
5. Riethdorf S., Fritsche H.et al. Detection of Circulating Tumor Cells In Peripheral Blood of Patients with Metastatic Breast Cancer: a Validation Study of the Cell Search System. *Clin. Cancer Res.* 2007; 13 (3): 920–928.
6. Kahn H. J., Presta A. et al. Enumeration of Circulating Tumor Cells in the Blood of Breast Cancer Patients after Filtration Enrichment: Correlation with Disease Stage / H.J. Kahn. *Breast Cancer Res. Treat.* 2004; 86(3): 237–247.
7. Pinzani P., Salvadori B.et al. Isolation by Size of Epithelial Tumor Cells in Peripheral Blood of Patients with Breast Cancer: Correlation with Real-Time Reverse Transcriptase-Polymerase Chain Reaction Results and Feasibility of Molecular Analysis by Laser Microdissection. *Hum. Pathol.* 2006; 37(6): 711–718.
8. Vona G., Sabile A.et al. Isolation by Size of Epithelial Tumor Cells — A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells. *Am. J. Pathol.* 2000; 156 (1): 57–63.
9. Vona, G., Beroud C. et al. Enrichment, Immunomorphological, and Genetic Characterization of Fetal Cells Circulating in Maternal Blood. *Am. J. Pathol.* 2002; 160(1): 51–58.
10. Helo P., Cronin A. M., Danila D. C. et *al*. Circulating Prostate Tumor Cells Detected by Reverse Transcription-PCR in Men with Localized or Castration-Refractory Prostate Cancer: Concordance with CellSearch Assay and Association with Bone Metastases and with Survival. *Clin. Chem.* 2009; 55: 765–773.