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Circulating tumour cells of solid tumours – a key to the novel clinical staging of genito-urinary neoplasms

Krążące komórki nowotworowe guzów litych – klucz do nowej klasyfikacji stopnia zaawansowania raków układu moczowo-płciowego

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Summary

This is a current review on the circulating tumour cells (CTC) in patients with renal cell carcinoma as a potential diagnostic tool that will allow for more accurate assessment of advanced disease and the monitoring of treatment effects. Using current diagnostic methods, we are still unable to identify patients especially prone to metastases. Assaying for levels of CTC may become a useful diagnostic tool, which will help to assess disease stage with more accuracy as well as monitor treatment outcomes. The introduction of "liquid biopsy", for screening and treatment monitoring, forms a very attractive outlook, where cases could be easily found by a blood test.

Key words: circulating tumor cells (CTC), solid tumors, genito-urinary system

Streszczenie

Opracowanie zawiera współczesne poglądy na temat oznaczania liczby krążących we krwi komórek nowotworowych (CTC) u chorych na raka nerkowokomórkowego, jako potencjalnego narzędzia diagnostycznego, które pozwoli na dokładniejszą ocenę stopnia zaawansowania choroby i monitorowania efektów leczenia. Stosując obecne metody diagnostyczne nie potrafimy wyodrębnić grupy pacjentów szczególnie narażonych na wystąpienie przerzutów. Oznaczanie liczby krążących we krwi pacjentów CTC może stać przydatnym narzędziem diagnostycznym, które pozwoli na dokładniejszą ocenę stopnia zaawansowania choroby i monitorowanie efektów leczenia. Bardzo kusząca jest wizja wprowadzenia do diagnostyki przesiewowej i oceny skuteczności leczenia Liquid biopsy, która pozwala na szybkie wykrycie nowotworu na podstawie badania krwi.

Słowa kluczowe: komórki nowotworowe, guzy lite, układ moczowo-płciowy

INTRODUCTION

Circulating Tumour Cells (CTC) are a population of cells that have detached from the tumour mass and are able to disseminate via the vascular system and create metastatic foci in the body (1). Initially, the immune system eliminates the circulating cancer cells from the bloodstream, but over time, the cells remain there for longer periods and become identifiable. The first published report of circulating tumour cells dates back to 1869 (2).

Prospective studies have demonstrated the effectiveness of identifying and quantifying CTCs in the prediction of malignant tumour treatment outcomes, including cancers of the breast, colon and prostate (3). Currently, intensive research is conducted on the importance of CTC in the biology and therapy of renal cell carcinoma (RCC) and transitional cell carcinoma (TCC). CTCs can be isolated from patients' blood in the course of most cancers due to the ease of identification of the epithelial phenotype of the cancer.

The presence of such cells in the blood coexists with micrometastases, which are clinically silent, and/or precedes the clinical emergence of metastases (4). CTCs are suspected of being instrumental for dissemination of the cancer during surgery, which involves mechanical manipulation and traumatising of the primary tumour (5).

The circulating cells acquire properties of mesenchymal cells, i.e. they are able to function independently. This is known as Epithelial to Mesenchymal Transition (EMT) (6). As a result of changes in their adhesion molecules, the cells separate from the primary cell population and gain the ability to migrate independently within the extracellular matrix and, eventually, outside it (7). The reverse process, known as Mesenchymal to Epithelial Transition (MET), is one of the most important stages of the development of cancer metastases. Armstrong et al. demonstrated that 80% of circulating cells of prostate or breast cancer express mesenchymal and epithelial markers (8). The authors suggested that the ratio of these markers could be a valuable prognostic parameter.

Circulating cancer cells are gradually gaining importance in clinical medicine. Many studies have especially been published on the subject of prostate cancer, where authors underscore the potential role of CTC in the prediction of survival and treatment outcomes. The presence of CTC in the patient's bloodstream is consistent with disease that is refractory to a given treatment, which therefore requires modification (9). It has been demonstrated in an animal model that approximately 10 CTC cells are sufficient to develop a new tumour in the case of renal or prostate cancer (10). Identifying CTC may in future help to discern patients who, according to standard diagnostic methods, are not qualified to undergo further radical treatment due to unfavourable prognosis regarding cure (11).

The isolation and identification of CTC is a challenge for the biotechnological industry. The main issue remains the small number of cells available for study. Normally, blood is drawn in samples of 5 to 10 ml. In a majority of cancers, the available methods are able to identify no less than 10 CTC in 1 ml of blood (12). These data regard cut-off thresholds, hence they cast doubt on experimentally applied norms. Moreover, current diagnostic methods do not allow for identification of CTCs in frozen or preserved blood. This excludes potential retrospective studies, which would certainly accelerate the wide introduction of CTC-based assays into clinical practice.

CellSearch is currently the only system with FDA (Food and Drug Administration, USA) approval for use in determining the number of circulating cancer cells (13). Nonetheless, many new devices for isolating CTC have been presented. Clinical trials are under way to assess the effectiveness of electric biosensors used to rapidly identify CTC in a small volume of blood (14,15). Progress in the isolation and identification of CTC, as well as the understanding of the value of CTC in the

prevention and treatment of solid tumours, may in future allow for a "liquid biopsy", which would identify CTC in the patient's blood sample and help to plan individualised treatment (16).

PROSTATE CANCER

The number of identified CTC in prostate cancer patients may in the near future become a valuable parameter used to assess the disease stage and improve treatment outcomes. In a clinical study, Stott et al. demonstrated that on average approximately 100 CTCs were found in patients with prostate cancer. The number of CTC fell in all patients on the first day following radical prostatectomy (17).

In prostate cancer patients, the number of CTC correlates with the PSA level (18). Twenty per cent of patients with an elevated PSA in the range of 2.5 to 10 ng/ml were found to have CTC in peripheral blood (19). The same study revealed that CTC were present in 21% of patients who had undergone radical prostatectomy. Moreno et al. demonstrated that the presence of at least 5 CTC in 7.5 ml of peripheral blood in patients with metastatic prostate cancer correlates with poor survival (20). Twenty three patients of 37 had more than 5 CTC. Similar results were obtained by Okegawa et al., who showed that the finding of at least 5 CTC in patients with a hormone-refractory prostate cancer is associated with reduced survival (21).

In a large group of patients with prostate cancer, Olmos et al. showed that the reduction of CTC below 5, following chemotherapy, correlates with longer survival compared to a reduction of CTC to a value above 5 cells (22). Besides survival prediction, CTC can help qualify patients to undergo appropriate treatments. Goldman et al. found that the number of CTC reflected the tumour's hormone sensitivity and also helped to assess the risk of the emergence of hormone resistance (23). Okegawa et al. determined a higher susceptibility of the prostate cancer to induced hypogonadism in patients who were found to have over 5 circulating prostate cancer cells (24).

RENAL CANCER

In 2009, 3650 cases of renal cancer were diagnosed in Poland. According to predictions, the incidence of this tumour will grow by an annual rate of about 2.5% (1, 25).

The universal availability of imaging studies serves to significantly increase the fortuitous identification of malignant renal tumours in various stages. It is characteristic for renal cancer to frequently generate local relapses and distant metastases, which are found even years following primary surgical treatment. Patients with this tumour require prolonged follow up, which should exceed 5 years after surgery (26).

CTC can be found in patients with renal cell carcinoma. The identification of CTC in the patients' bloodstream precedes the emergence of clinically identifiable lesions. The circulating renal carcinoma cells are

characterised by the presence of cytokeratins 8, 18, 19 and surface glycoprotein CD 44 (27). Not many published clinical studies have addressed correlations between the number of CTC in the patients' blood and their prognosis or treatment outcomes. **Bluemke et al. are the only authors to have shown a correlation between the presence of CTC in renal cancer and the disease stage or the risk of metastases (28).**

TRANSITIONAL CELL CARCINOMA OF THE BLADDER

In Poland, transitional cell carcinoma of the bladder has the fourth highest incidence among cancers in men and the eighth in women. The majority of cases are non-invasive tumours, of which 75% develop multiple relapses following sparing surgical treatment (29). According to estimates, over 50% of patients with non-invasive cancers will sustain a disease progression to N1 within 2 years. This is a consequence of the unavailability of an easily accessible method of identifying malignant infiltration that exceeds the bladder epithelium and threatens with dissemination of cancer cells in the vascular system (30). The detection of CTC in a patient's bloodstream may effectively supplement current management and disease progression monitoring. CTC are present in patients with urothelial cancer. **Naoe et al. were among the first to publish results of assaying for CTC levels in patients with this tumour. In their study, they found a significant difference in CTC levels between patients with non-invasive vs. invasive and metastasising cancer (31).** This is a valuable observation, which may broaden the portfolio of available diagnostic parameters used to stage the disease

and select methods and duration of treatment. Rink et al. assessed the prognostic value of CTC identification in a larger group of patients with invasive, non-metastatic bladder cancer (T3, N0) and demonstrated that the number of CTC correlates with survival following surgery. Out of the 50 subjects, 15 were found to have approx. 33 CTC in 7.5 ml of blood. The majority of CTC-positive subjects died within a year from implementation of radical surgical treatment (32). The number of CTC precisely reflects the number of metastases found in PET and MRI studies (33). The study by Okegawa et al. found CTC in all patients with TCC in stages N1 and N2. On the other hand, no CTC were found in the bloodstream of any of the subjects with a stage N0 tumour (34). While these results are promising, data are still lacking to determine a cut-off level below which the number of CTC could be deemed "safe" (35). Studies are also needed to assess the levels of CTC in patients with non-advanced bladder cancer

SUMMARY

Using current diagnostic methods, we are still unable to identify patients especially prone to metastases. Assaying for levels of CTC may become a useful diagnostic tool, which will help to assess disease stage with more accuracy as well as monitor treatment outcomes. The introduction of "liquid biopsy", for screening and treatment monitoring, forms a very attractive outlook, where cases could be easily found by a blood test. The CTC are among the major markers applied in clinical trials of "liquid biopsy" systems based on biochips (36).

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