Commentary

Bone marrow micrometastases and circulating tumor cells: current aspects and future perspectives

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Abstract

Early tumor cell dissemination at the single-cell level can be revealed in patients with breast cancer by using sensitive immunocytochemical and molecular assays. Recent clinical studies involving more than 4000 breast cancer patients demonstrated that the presence of disseminated tumor cells in bone marrow at primary diagnosis is an independent prognostic factor. In addition, various assays for the detection of circulating tumor cells in the peripheral blood have recently been developed and some studies also suggest a potential clinical relevance of this measure. These findings provide the basis for the potential use of disseminated tumor cells in bone marrow or blood as markers for the early assessment of therapeutic response in prospective clinical trials.

Keywords: blood, bone marrow, breast cancer, disseminated tumor cells

Introduction

Although the presence of lymph-node metastasis is a negative prognostic factor for breast cancer and other cancers, it is still not possible to reliably identify, by means of only lymph node status at primary therapy, those patients who will relapse with metastatic disease. This indicates that other ways of metastatic tumor cell spread also have an important role [1]. Therefore, the availability of additional factors enabling individual risk assessment is desirable, to improve the identification of patients at risk for relapse. Immunocytochemical and molecular assays now enable the specific detection of metastatic tumor cells even at the single-cell stage and allow an approach to the important question of systemic tumor cell dissemination as one of the first crucial steps in the metastatic cascade. Several studies in breast cancer have shown that the presence of disseminated tumor cells (DTCs) in bone marrow seems to represent an additional clinical marker that might be useful for clinical decision making so as to establish risk-adapted adjuvant treatment strategies. Another important and – in comparison with other markers – unique application of the detection of DTCs could be the monitoring of therapeutic efficacy in the adjuvant setting with no measurable disease.

Detection of micrometastatic tumor cells in bone marrow

So far, most experience with bone marrow screening for occult metastatic breast cancer cells has been gained from immunocytochemical analyses with Ficoll density gradient centrifugation for tumor cell enrichment. The most recent studies [2–7] consistently reported that the presence of DTCs in bone marrow has a strong prognostic impact on patient survival. This was also confirmed in a pooled analysis by Braun and colleagues including 4199 patients with a 10-year follow-up [8]. However, even in these studies, a diversity of antibodies was used for the identification of epithelial cells in bone marrow, and the number of cells analyzed per patient varied or was not stated, thus introducing substantial variation into the methodology. To establish a standardized procedure for bone marrow examination, a prospective two-center study in primary breast cancer patients was initiated, using a validated immunoassay. Multivariate regression analysis verified that the presence of DTCs in bone marrow predicts poor prognosis independently of the lymph node status [2].

In this context, two more recent reports of the group of Naume and colleagues are also of considerable

CTCs = circulating tumor cells; DTCs = disseminated tumor cells.

importance. The first report [6] confirmed the independent prognostic value of DTC detection in bone marrow in a cohort of 817 patients. In addition, this study compared the information derived from the 'traditional' enrichment of mononuclear cells by Ficoll gradient with an immunomagnetic enrichment technique that used magnetic antibodies to deplete white blood cells and was able to increase the detection rate for DTCs. Interestingly, the higher rate of detection of positive patients with this technique did not improve the prognostic value of DTC detection.

In a second report [9], Naume's group showed that a morphologic evaluation of the cells detected by positive staining with an anti-cytokeratin antibody is important in determining their prognostic impact. In addition, the authors showed that an increasing number of DTCs in bone marrow per patient indicates an increasingly poor prognosis. This again highlights the need for standardized methodology in DTC detection.

Another important application of DTC detection might be the monitoring of therapeutic efficacy in the adjuvant setting. A report by Janni and colleagues suggested that the presence of DTCs after adjuvant treatment might be able to identify patients with an increased risk for recurrence [10]. In a larger study, Naume and colleagues confirmed this observation [11]; this examination might therefore be able to identify patients who need additional adjuvant therapy, for example a prolonged endocrine treatment that could offer additional benefit for some patients [12]. However, no study has so far established the best time point for the re-examination of bone marrow after primary treatment.

Circulating tumor cells in the peripheral blood

Peripheral blood would be an ideal source for the detection of DTCs because of ease of sampling. However, the prognostic significance of circulating tumor cells (CTCs) is much less clear than for DTCs in bone marrow. For tumor cells, blood is only a temporary compartment, and it is not known whether a significant proportion of CTCs survive and are subsequently capable of forming detectable metastases. One report by Mehes and colleagues observed that a large proportion of CTCs in breast cancer patients are apoptotic [13]. However, it was shown that the presence of CTCs in breast cancer patients detected by immunocytochemical or molecular methods was correlated with stage and course of the disease [14,15] and there are also more recent reports showing a prognostic impact of CTCs. Stathopoulou and colleagues described a negative prognostic impact of CTCs detected by reverse transcriptase polymerase chain reaction (PCR) for cytokeratin 19 in the blood of non-metastatic breast cancer patients before adjuvant therapy [16], and one report by Weigelt and colleagues, who used a multimarker PCR assay in metastatatic patients, also found a negative prognostic impact [17]. These findings indicate that CTCs have malignant potential.

Using conventional Ficoll gradient centrifugation and immunocytochemistry, Piegra and colleagues have recently shown a correlation between the detection of CTCs in the blood and DTCs in bone marrow of patients with primary breast cancer, but only the presence of DTCs in bone marrow provided significant prognostic information [7]. However, the optimal time point for the blood examination is not clear and, in addition, Ficoll gradient centrifugation of blood might not provide sufficient enrichment of malignant cells. An important advance therefore seems to be the development of new enrichment systems for CTCs [18,19]. Hayes and colleagues showed in a prospective study that CTC detection with the use of an automated enrichment system provided significant prognostic information in patients with metastatic breast cancer [19]. However, the clinical application for such a detection system has to be demonstrated in a study that is able to a show a benefit for patients, for example by changing therapy regimens in patients with increasing CTC numbers and thereby improving their outcome. It would also be important to explore this test system in patients with non-metastatic breast cancer.

Genetic characterization of disseminated cancer cells

Recent technical developments permit the examination of the genome of single disseminated cancer cells. Using a combination of immunocytochemistry and fluorescence *in situ* hybridization, several groups have reported numerical chromosomal aberrations in cytokeratin-positive cancer cells in bone marrow and blood, indicating the malignant origin of these disseminated cells [20–22]. The availability of new protocols for the amplification of the whole genome has enabled a more detailed analysis of DTCs. Using single-cell comparative genomic hybridization, Klein and colleagues were able to demonstrate that cytokeratin-positive cells in bone marrow of breast cancer patients without clinical signs of overt metastases (stage M0) are genetically heterogeneous [23,24]. Surprisingly, these cells bore little resemblance to their respective primary tumor [24]. Besides potential technical problems, a possible interpretation of this finding is that the disseminated cancer cells might have separated from their primary tumor at an early stage and evolved independently, influenced by the specific selective pressures of the bone marrow environment. Although this hypothesis adds a new perspective to the currently debated models of metastasis, the study by the group of Klein and colleagues [24] did not address the viability and proliferative potential of the disseminated cells that were demonstrated in another study [22]. It is therefore unclear whether the cells analyzed are capable of developing into metastases and which of the genomic alterations observed in DTCs are clinically relevant.

Conclusions and perspectives

Various immunocytochemical and molecular methods have been applied to detect occult hematogenous tumor cell spread in breast cancer patients. International consensus is now urgently needed on quality-control issues and criteria for acceptable technical assay performance, to permit comparisons between different assay platforms. In addition, marker implementation into current risk classification systems, such as the tumor–node–metastasis (TNM) classification system, is needed. The most recent TNM classification for breast cancer [25] does not qualify the presence of CTCs in peripheral blood or DTCs in bone marrow as metastasis, but it optionally reports the presence of such cells together with their detection method, e.g. M0(i+) for immunocytochemical detection or M0(mol+) for detection by molecular methods.

In addition to the detection of DTCs in bone marrow and CTCs in blood discussed here, the detection of micrometastases in the lymph nodes as another independent prognostic indicator is significant and could have a role in tumor staging.

Beyond adding another prognostic factor in breast cancer, the potential of occult hematogenous tumor cell spread as a tool for predicting or monitoring the efficacy of systemic therapy is of great importance. In contrast to lymph nodes, which are generally removed at primary surgery, bone marrow and blood can be obtained repeatedly during post-operative treatment. The therapeutic efficacy of adjuvant systemic therapy can currently be assessed only retrospectively in large-scale clinical trials after an observation period of at least 5 years. Consequently, progress with this form of therapy is extremely slow and it is also not possible to tailor therapy to an individual patient. The potential of a surrogate marker assay that permits the immediate assessment of therapy-induced cytotoxic effects on occult metastatic cells is therefore evident. Prospective clinical studies are now required, to evaluate whether the eradication of DTCs in bone marrow and blood after systemic therapy translates into a longer disease-free period and overall survival. An additional important future goal is the possibility of identifying specific targets for metastatic tumors to improve chemotherapy regimens.

Competing interests

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