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Focus On

The role of disseminated and circulating tumour cells in breast cancer

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Abstract Occult dissemination of tumour cells in patients with operable breast cancer is a crucial step in formation of metastasis, yet conventional tumour staging does not reveal it. To identify individual tumour cells that have successfully escaped from the primary tumour and invaded secondary organs, several research groups established sensitive immunocytochemical and molecular assays. Aside of the well documented prognostic impact of lymph node metastasis and micrometastases, respectively, bone marrow plays a prominent role as a determinant for haematogenous micrometastatic organ involvement. In the past decade, several groups have documented the independent prognostic impact of the presence of bone marrow micrometastases, which, in a recent pooled analysis of individual patient data from over 4000 breast cancer patients with Stage I–III disease, could be confirmed for the entire study population, and, in addition, provided data for challenging hypotheses to be tested in future adjuvant therapy trials of clinically relevant subgroups of breast cancer patients. Although the availability of a simple blood test would be highly desirable, no prognostically relevant data so far exists for early breast cancer patients. Options for the applicability of such approaches to detect disseminated (to bone marrow) and circulating tumour cells (in blood) are ample, both in clinical trial settings and for basic as well as translational research. In this overview we provide a brief summary of the prognostic role and the potential clinical utility of bone marrow micrometastases in breast cancer patients.

Keywords: Bone marrow; Breast cancer; Circulating tumour cells; Disseminated tumour cells; Immunocytochemistry; Micrometastases; Polymerase chain reaction

Background

When Steven Paget published his theory of 'seed and soil' in 1889 [1], the idea of haematogenous tumour-cell dissemination was born. More than a century later, with the use of molecular tools, new clinical findings have resulted in explanations of hither-to unexplainable phenomena, such as that donor-derived cancer in recipient organ allografts [2] and viable single tumour cells in secondary organs were both the descendants of a known primary tumour [3] and the potential precursors of subsequent metastasis [4].

Currently, the genesis of overt metastasis in breast cancer is based on the idea that tumour cells dissociate from the primary cancer and gain access to circulation either directly into blood vessels or after transit in lymphatic channels. Thus, detection of such cells in patients with newly diagnosed solid tumours has been an appealing strategy to provide evidence of future metastasis [5].

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Evidence of malignant nature

Overall, the existence of circulating tumour cells (CTC) and the settlement of these cells in secondary

organs, such as liver, bone, and lungs, as disseminated metastatic tumour cells (DTC) is generally accepted. These cells are believed to be rare members among the cellular population of primary tumour cells [6]. This model, viewing CTC and DTC as rare and late events during primary tumour progression, has been challenged by recent expression profiling studies, in which a more ubiquitous 'metastatic phenotype,' that can be assessed by gene expression analysis [7–9].

On the other hand, most readers will not be familiar with this word analyses of numerical chromosomal changes and altered gene expression of single disseminated tumour cells demonstrated that the majority of DTCs have genetic aberrations compatible with malignancy, and therefore, are most likely direct descendants of the primary tumour, although the genetic changes generally were incongruent with the dominant genotype of the corresponding primary tumour [10–12].

Prognostic and potential clinical role

The actual presence of tumour cells outside the primary tumour and in organs relevant for subsequent metastasis formation, such as bone and bone marrow, serve three purposes that could be clinically useful (1) as unambiguous evidence for an early occult spread of tumour cells; (2) as a relevant risk factor for subsequent metastases and, thus, a poor prognosis; and (3) as a marker for monitoring treatment efficacy. Finally, and perhaps as importantly in the long run, genotyping and phenotyping of CTC and DTC should provide detailed insight into the metastatic process and permit direct exploration of targeted treatment strategies [5].

Is detection of CTC or DTC prognostic in early-stage breast cancer? The currently available literature regarding the prognostic relevance of the presence of DTC in bone marrow is controversial, and without clear conclusions if viewed globally. However, a substantial number of studies do not meet essential criteria for quality assurance, adequate controls, and/or clinical trial design, and therefore should be excluded from the debate. To date, sufficient data are available from several large studies that unambiguously demonstrate the independent poor prognostic influence of DTC present in bone marrow on outcome in patients with Stage I, II, or III breast cancer [13–19] add Cote *et al*. [20]; Wong *et al*. [21]. In a recent pooled analysis of individual patient data from over 4700 breast cancer patients with Stage I–III disease, we were able to confirm the independent prognostic impact for the entire study population, and, in addition, provided data for challenging hypotheses to be tested in future adjuvant therapy trials of clinically relevant subgroups of breast cancer patients [22].

Although a blood test specifically designed for patients with Stage I–III breast cancer would be highly desirable, preliminary data suggest that that findings on CTCs and DTCs in peripheral blood and bone marrow, respectively, do not provide congruent results. In contrast to DTC detection in bone marrow of patients with early-stage disease, CTC analysis appears to be less sensitive and less prognostic [23]. On the other hand, a recently reported, highly rigorous study clearly showed that, in breast cancer patients, with metastatic disease the number of CTCs permit prediction of progression-free and overall survival as well as response to treatment [24]. As a major finding of their study, the opportunity to predict response as early as 3–4 weeks after initiation of treatment reflects an important step towards individualized treatment decisions in patients with metastatic disease.

Methodological aspects and potential pit-falls

In contrast to the simplicity of the technology, the influence of confounding variables of the immunocytochemical assay on detection of bone marrow should not be underestimated [25–27]. Thorough and critical evaluation of each process step of sample preparation, immunostaining, and analysis is required to avoid misinterpretation. Before unrestricted routine use of the technology, results of an ongoing process of methodological improvement have to be awaited. Thus, the immunocytochemical technology, which ultimately has turned out to be technically demanding, has induced implementation of seemingly easier molecular solutions, such as the reverse-transcriptase polymerase chain reaction technique [28–31]. However, the same quality control issues that were raised for immunocytochemistry are pertinent for this technology as well, and there are concerns that many of the reported studies may overestimate the importance of the findings [32–35]. As a hallmark and further essential requirement for upcoming studies using molecular techniques for DTC detection, comparison with the benchmark technique of DTC detection (i.e., immunocytochemistry with anticytokeratin antibodies) would be essential.

Conclusion

The prognostic value of DTC in bone marrow of breast cancer patients can be viewed as a statistically valid and clinically useful prognostic marker. Beyond mere prognostic estimation, and perhaps even more important, it may be assumed that presence of DTC can serve as a predictive marker. In order to individualize decision-making on adjuvant therapy and to find out the prognostic relevance of CTCs in comparison

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to DTCs, we need well designed, highly powered, prospective clinical trials using DTCs and CTCs as candidate surrogate markers for the various clinical settings currently under investigation question, such as secondary adjuvant endocrine treatment and dose-dense or otherwise intensified cytotoxic therapy.

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