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Original Research

Prognostic relevance of disseminated tumour cells from the bone marrow of early stage breast cancer patients – Results from a large single-centre analysis

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Abstract ***Background:*** This is the largest single-centre study to determine the prognostic relevance of disseminated tumour cells (DTCs) from the bone marrow (BM) of stage I-III breast cancer patients. Additionally, we aimed to analyse the impact of DTC detection on adjuvant bisphosphonate (BP) treatment efficacy.
Methods: BM aspirates were collected during primary surgery for early breast cancer (EBC; T1–4, N0–2, M0) at Tuebingen University, Germany, between January 2001 and January 2013. DTCs were identified by immunocytochemistry (pancytokeratin antibody A45/B-B3) and cytomorphology. We retrospectively estimated the influence of DTC detection and BP treatment on disease-free survival (DFS) and overall survival (OS) using univariate (log-rank test) and multivariate (cox regression) analysis.
Findings: BM aspirates were available from 3141 patients. In 803 (26%) of these, DTCs were detectable. As compared to DTC-negative patients, DTC-positive patients more frequently had larger tumors ($p < 0.001$), lymph node involvement ($p < 0.001$), hormonal receptor positive tumours ($p < 0.001$) and HER2-positive tumours ($p = 0.048$). DTC-positive patients were at an increased risk of relapse (hazard ratio (HR) 1.74, 95% confidence interval (CI) 1.34–2.25, $p < 0.001$) and death (HR 1.44 95% CI 1.13–1.86, $p = 0.004$). In the multivariate analysis

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DTCs were an independent predictor of DSF and OS. Additionally, BP treatment had no significant influence on DFS or OS in DTC-negative patients, while it was significantly associated with increased DFS ($p < 0.001$) and OS ($p = 0.006$) in DTC-positive patients.
Interpretation: These data confirm the clinical validity of DTCs from the BM for prognostication of early breast cancer patients. Further studies are warranted to determine whether DTCs are predictive for adjuvant treatment efficacy using bisphosphonates.

1. Introduction

Early breast cancer (EBC) can relapse even years after successful treatment of the primary tumour. It has therefore been hypothesised that individual tumour cells spread to secondary sites in the body where they can persist for long periods of time before initiating metastatic growth [1]. This phenomenon is termed minimal residual disease (MRD), and the aim of all adjuvant therapies is to eradicate MRD before it becomes clinically evident.

Evidence has been increasing that cytokeratin (CK)-positive disseminated tumour cells (DTCs) in the bone marrow (BM) of breast cancer patients may reflect the presence of MRD [2]. DTCs are detected in the BM of 30–40% of EBC patients and associated with a worsened prognosis [3–7]. The strong independent prognostic significance of DTCs at primary diagnosis was confirmed in a large pooled analysis of more than 4700 patients published by Braun and colleagues in 2005 [7].

Additionally, the persistence of DTCs during adjuvant treatment of stages I–III breast cancer predicts an increased risk of disease relapse and death [8,9]. Thus, the detection of DTCs might serve as an indicator of systemic treatment efficacy, helping to identify patients in need of additional adjuvant treatment. However, chemotherapy regimes often fail to eliminate DTCs, which can persist in the BM in a dormant, non-proliferative state for years [10]. Another approach to eradicating DTCs from the BM might be the use of bisphosphonates (BPs), which primarily target the skeletal system [11]. However, the role of BPs in EBC treatment remains unclear. A large meta-analysis investigating the addition of BPs to adjuvant therapy in patients with EBC found that menopausal status was a predictor of BP treatment efficacy [12]. Additionally, recent findings indicate that successful eradication of DTCs with BP therapy improves prognosis in patients with EBC [11,13].

Although the results highlighting the potential usefulness of DTC detection in the BM of patients with EBC have been encouraging, DTC determination has not thus far been recommended for routine clinical use by guidelines or expert panels due to a lack of consensus on certain methodological and institutional problems. The pooled analysis by Braun et al. [7] covered seven different techniques for DTC detection. Moreover, while patient enrolment in the largest published studies ended in 2002, the past decade has seen a number of significant changes in the treatment of patients with EBC, including primary systemic therapy and HER2-targeted therapy.

Against this background, we conducted the present large single-centre study to investigate the impact of DTCs on prognosis in patients with early stage I–III breast cancer. DTCs were determined according to a standardised method recommended by expert consensus [14]. We moreover determined the impact of DTC detection on adjuvant BP efficacy by analysing survival outcomes in DTC-negative and DTC-positive patients treated with or without BPs.

2. Methods

2.1. Study population, setting, design, and ethics

Women undergoing primary surgery for EBC (T1–4, N0–2, M0) at the Department of Obstetrics and Gynaecology at Tuebingen University Hospital, Tuebingen, Germany, between January, 2001, and January, 2013, were eligible for this retrospective study. Patients with recurrent or metastatic disease, bilateral breast cancer, R1 resection or a previous history of secondary malignancy were excluded. All patients provided written informed consent for BM aspiration. The analysis was approved by the ethics committee of the University of Tuebingen (reference number 560/2012R).

2.2. Systemic treatment

Systemic treatment was based on national treatment guidelines (www.ago-online.de). Most patients were treatment-naïve at the time of DTC determination. However, as bone marrow aspiration was performed exclusively during primary surgery, a small proportion of patients received preoperative treatment (ie, neoadjuvant chemotherapy) before being evaluated for DTC status. In patients who were treatment-naïve at the time of surgery, tumour stage (ie, tumour size and nodal status) was determined by pathology. If patients received neoadjuvant chemotherapy, tumour size and nodal status were determined by clinical examination and imaging modalities before the first treatment cycle. The preferred imaging modality was ultrasound, but magnetic resonance imaging and mammography served as alternatives if ultrasound findings appeared not to be valid.