Abstract

Material and methods A prospective study was conducted to determine the value of changes in circulating tumour cell (CTC) levels prior to and after the first cycle of neoadjuvant treatment in early prediction of pathologic response in locally advanced breast cancer (LABC). Two blood samples were obtained from 72 eligible LABC patients to isolate and enumerate CTCs before neoadjuvant chemotherapy started on day 1, and on day 21, immediately before second cycle administration.

Results Sixty patients (83.3%) had <1 CTC in the first sample and response rates in this cohort were pathologic complete response (PCR) in 2 patients (5%), partial response (PR) in 35 (87.5%), stable disease (SD) in 2 (5%) and progressive disease (PD) in 1 (2.5%). Twelve patients (16.7%) had >2 CTCs in the first sample; these patients were more likely to have triple negative tumours. All 12 had fewer CTCs in the second sample. Response rates in this second cohort of 12 patients were PCR in 4 (34%), PR in 6 (50%), SD in 1 (8%) and PD in 1 (8%). PCR rate was markedly better in this second cohort (p<0.0042; OR 14.5, 95% CI 2.3–92).

Discussion This study suggests that the presence of CTCs prior to neoadjuvant therapy might be a predictor of response to this therapy.

Keywords Locally advanced breast cancer (LABC) · Circulating tumour cells (CTCs)

Introduction

Breast cancer is a major public health problem because of its high incidence, prevalence, and morbidity and mortality rate. Prognostic factors that estimate risk and select optimal therapy for breast cancer patients are well defined. These include tumour stage, histological grade, hormonal receptors and HER2/neu gene amplification [1]. However, greater understanding of cancer biology has led to research into more effective, individual anti-cancer therapies to improve patient outcome. Knowledge of diagnostic procedures to evaluate early response to treatment in breast cancer patients provides an opportunity to stratify patients in research studies and offer new treatment if patients fail to benefit from therapy. Consequently, more individualised treatments may be available.

The existence of circulating tumour cells (CTCs) is a novel prognostic factor in breast cancer [2, 3]. The system is based on enumerating epithelial cells that are separated from the blood by antibody-coated magnetic beads and identified with the use of fluorescent-labelled antibodies to cytokeratins, fluorescent nuclear stain and fluorescent cytokeratin antibodies [4]. Currently available data strongly support this and it should be tested in clinical trials [5, 6].

Locally advanced breast cancer (LABC) remains a clinical challenge as most patients diagnosed with LABC...
develop distant metastases despite appropriate therapy. Surgery and radiotherapy following anthracycline- and taxane-based neoadjuvant chemotherapy are the standard therapy for patients with LABC. Neoadjuvant chemotherapy aims for pathologic complete response (PCR) in breast and axillary lymph nodes, which is the best predictor of improved outcome and prolonged survival. In addition to clinical and radiologic procedures to monitor response to neoadjuvant treatment, new strategies are needed. Circulating tumour cells may constitute a good approach to early assessment of the efficacy of neoadjuvant therapy in LABC and might prove useful in clinical practice.

We conducted an experimental study to establish whether changes in pre- and post-first cycle neoadjuvant chemotherapy CTC levels might predict pathologic response in LABC.

**Methods**

**Study design**

This is a prospective experimental study to ascertain CTC level changes before and after adriamycin or docetaxel neoadjuvant regimens for LABC.

The primary endpoint was to assess the relation between changes in pre- and post-neoadjuvant chemotherapy CTC levels and pathologic response following neoadjuvant therapy. The secondary endpoint included assessing CTC incidence in various subtypes of LABC.

**Patients**

The study population included women (>18 years of age) with histologically proven LABC who received anthracycline-taxane-based neoadjuvant chemotherapy. Enrolment criteria included Eastern Cooperative Oncology Group performance status 0, adequate organ function and baseline echocardiogram with left ventricular ejection fraction above the lower limit for normal (>50%). Metastasic breast cancer patients and those who failed to meet inclusion criteria were excluded. Informed consent was required from all patients. The study was performed in accordance with Local Ethics Committee procedures.

**Method of isolating circulating tumour cells**

Two 7.5-ml samples of peripheral blood were collected from patients. The first sample was collected immediately before the first neoadjuvant cycle; the second immediately before the second of four programmed neoadjuvant cycles. Blood was drawn into a 10-ml test tube with EDTA and cellular preservatives (Cellsave preservative tubes by Veridex™) and maintained at room temperature for a maximum of 72 h before processing. Blood (7.5 ml) was mixed with a ferrofluid coated with antibodies specific for epithelial cell detection (EpCAM). Isolated cells were fluorescent-stained with nucleic acid dye 4.6-diaminodino-2-phenilindole (DAPI) and labelled with monoclonal antibodies specific to leukocytes (CD45) and epithelial cells (cytokeratin 8, 18, 19) (CK-PE). They were analysed with the Cellspotter Analyzer (Veridex™ LLC). Analysis showed cell images that were verified as CTCs as they met the following criteria: round to oval morphology with a diameter of over 4 μcytoplasm positively stained for CK-PE and negative for CD45 and more than 50% of the nucleus stained with DAPI inside the cytoplasm. Results are expressed as number of CTCs/7.5 ml of blood. Results were independently interpreted by five specifically trained specialists, blinded to patients’ clinical characteristics.

In our research and in one previously reported study, the number of CTCs in healthy volunteers is usually 0, rarely 1 and exceptionally >1. In the present study, we consider finding at least ≥2 CTCs in every sample to be clinically relevant [7]. Similarly, we consider finding only 1 CTC not significant. Therefore, we chose a cut-off point of ≥2 CTCs to define the test as positive for CTC presence.

**Neoadjuvant chemotherapy**

Patients received 75 mg/m² adriamycin or 100 mg/m² docetaxel every 21 days in 4 courses. Heart function was monitored as deemed necessary by the researcher. Treatment could be reduced or delayed in response to toxicity events. Following neoadjuvant chemotherapy, patients underwent surgery. Subsequently, patients were crossed over to receive the other drug (adriamycin or docetaxel) in the adjuvant setting. Both supportive granulocyte colony-stimulating factors and prophylactic levofloxacin were administrated with docetaxel cycles. Patients completed adjuvant radiotherapy, antioestrogen and/or trastuzumab adjuvant therapy depending on original specimen immunochemistry.

**Statistical methods**

Sample size was calculated according to probability of CTC occurrence in breast cancer patients, as reported by Cristofanilli [2]. To determine the size, two groups were defined taking as reference the detection of 5 or more cells per 7.5 ml of whole blood from fewer than 5 circulating tumour cells per 7.5 ml (percentage of exposed patients in 1st group (P1)=0.5; percentage of exposed patients in 2nd group (P2)=0.75; Zα=1.96 for error α=0.05 for a bilateral study. Zβ=1.2816 for an error β=0.1). Sample size (N)=[(P1(1−P1)+P2(1−P2))/(Zα+Zβ)2][(P1−P2)2]. According to these data the sample size was 73 patients.

Symmans et al.’s classification was used for the pathological specimen evaluation following neoadjuvant chemo-