Circulating tumour cells in non-metastatic breast cancer: a prospective study

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Summary

Background **The identification of circulating tumour cells correlate with poor prognosis in metastatic breast cancer, but there are few data describing the importance of circulating tumour cells in patients with non-metastatic disease. Our aim was to establish if circulating tumour cells predicted worse outcome in patients with non-metastatic breast cancer.**

Methods **We prospectively collected data on circulating tumour cells at the time of definitive surgery from chemonaive patients with stage 1–3 breast cancer from February, 2005, to December, 2010. We deemed eligible all patients with operable breast cancer presenting at The University of Texas MD Anderson Cancer Center (Houston, TX, USA). Patients were ineligible if they had bilateral breast cancer or any other malignancy within 5 years of the diagnosis of the present cancer. We measured circulating tumour cells with the CellSearch System (Veridex, Raritan, NJ). We correlated findings of circulating tumour cells with standard tumour characteristics, including tumour size and grade; oestrogen and progesterone receptor and human epidural growth factor receptor 2 (HER2) status; and axillary lymph node status with χ^2 or Fisher exact tests. We assessed outcomes at a median follow-up of 35 months. Log-rank test and Cox regression analysis was applied to establish the association of circulating tumour cells with progression-free and overall survival.**

Findings **No patients reported adverse events or complications from blood collections. We identified one or more circulating tumour cells in 73 (24%) of 302 patients. Detection of one or more circulating tumour cells predicted both decreased progression-free survival (log-rank p=0·005; hazard ratio [HR] 4·62, 95% CI 1·79–11·9) and overall survival (log-rank p=0·01; HR 4·04, 1·28–12·8).**

Interpretation **The presence of one or more circulating tumour cells predicted early recurrence and decreased overall survival in chemonaive patients with non-metastatic breast cancer. These results suggest that assessment of circulating tumour cells might provide important prognostic information in these patients.**

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Introduction

Patients with breast cancer are staged on the basis of primary tumour size, regional lymph-node status, and the presence or absence of obvious distant metastatic disease at the time of initial diagnosis. Axillary lymph node status remains the best predictor of outcome in operable breast cancer. However, about 25% of patients with breast cancer with localised disease and without axillary lymph-node metastasis will develop systemic relapse after successful primary treatment, whereas almost 30% of these patients with axillary lymph-node metastasis will not relapse within 5–10 years of primary treatment.[1–3] These disparate data suggest that micrometastatic haematogenous spread happens in a substantial number of patients with operable breast cancer and is independent of lymphatic involvement. In patients with breast cancer, early dissemination of tumour cells from heterogeneous breast tumours into the bloodstream is often undetectable by standard high-resolution imaging technologies, since these tumour cells are rare (as few as 1 in 1×10^6 cells). Only recently have advances in isolation, enrichment, and detection methods allowed clinical researchers to accurately and reproducibly detect occult tumour cells.

The number of circulating tumour cells in peripheral blood before the receipt of systemic treatment has been shown to be an independent predictor of progression-free survival and overall survival in metastatic breast cancer,[4] and the identification of circulating tumour cells at any time during systemic therapy correlated with increased mortality rates in patients with metastatic disease.[5] Circulating tumour cells have been reported in 10–60% of patients with stage 1–3 non-metastatic breast cancer by various types of detection assays, including density-gradient separation and cytokeratin-19 mRNA amplification,[6–8] density-gradient separation and subsequent cytokeratin immunostaining,[9] density gradient and immunomagnetic separation and subsequent cytokeratin immunostaining,[10,11] human epidural growth factor receptor 2 (HER2) immunostaining,[12] or the CellSearch (Veridex, Raritan, NJ) method.[13–17] With the CellSearch method, our group has previously identified circulating tumour cells in 31% of patients with T1 or T2 tumours, suggesting that occult dissemination can

happen early in disease progression.[18] So far, few data have been published on the prognostic importance of circulating tumour cells in early stage breast cancer.

We present a prospective study designed to establish if circulating tumour cells predict survival in patients who are chemonaive with non-metastatic breast cancer. We postulated that identification of circulating tumour cells within the blood would independently predict shorter survival, irrespective of axillary lymph node status or standard tumour markers. If the presence of circulating tumour cells were to contribute independently to the currently available prognostic factors, this information might be useful in disease staging and in identifying patients who might benefit from additional adjuvant therapies.

Methods

Participants

Between February, 2005, and December, 2010, we assessed chemonaive patients with stage 1–3 breast cancer undergoing surgery for their primary tumour. We deemed eligible all patients with operable breast cancer presenting at The University of Texas MD Anderson Cancer Center (Houston, TX, USA). We ruled out non-response bias by comparing differences in presenting clinical stages between participants and those who elected not to participate. Patients with bilateral breast cancer or any other malignancy within 5 years of diagnosis of the present cancer were ineligible. Our original protocol approved by the institutional review board was amended, and is ongoing, to increase accrual to allow for subgroup analysis and to permit sequential circulating tumour-cell measurements during routine follow-up visits. We obtained informed consent from all patients before collecting their blood. The institutional review board at The University at Texas MD Anderson Cancer Center approved this prospective study.

Randomisation and masking

Results for individual patients were masked from investigators by use of a random number system as the unique patient identifier.

Procedures

We designated the primary TNM staging (primary tumour [T], regional nodes [N], distant metastases [M]) in accordance with the criteria set by the American Joint Commission on Cancer[19] and tumour grade with Black's nuclear grading system.[20] We defined clinical stage as the TNM stage established at the time of the first diagnostic procedure confirming the invasive component of the tumour. We established axillary lymph node status by the presence or absence of lymph node metastasis as reported at the time of operation for the primary tumour.

We immunostained tumour sections for oestrogen and progesterone receptors, and HER2 in accordance with previously published procedures.[18] We judged

	Number of patients overall (%); n=302	One or more circulating tumour cells	
		Number of positive patients (%); n=73	Number of negative patients (%); n=229
Age (years)	54 (12)	53 (12)	54 (12)
Median follow-up (months)	35 (3–96)	35 (3–96)	35 (4–84)
25% follow-up (months)	22	21	22
75% follow-up (months)	51	63	50
Tumour size			
<2 cm (T1)	161 (53%)	34 (47%)	127 (55%)
2–5 cm (T2)	110 (36%)	29 (40%)	81 (35%)
>5 cm (T3)	18 (6%)	5 (7%)	13 (6%)
Skin or chest-wall infiltration (T4)	13 (4%)	5 (7%)	8 (3%)
Pathological nodal status			
Node negative	184 (61%)	44 (60%)	140 (61%)
1–3 lymph nodes	93 (31%)	21 (29%)	72 (31%)
>3 lymph nodes	20 (7%)	7 (10%)	13 (6%)
Missing	5 (2%)	1 (1%)	4 (2%)
Histological tumour grade			
Low grade (grade 1)	49 (16%)	12 (16%)	37 (16%)
Intermediate grade (grade 2)	150 (50%)	28 (38%)	122 (53%)
High grade (grade 3)	102 (34%)	32 (44%)	70 (31%)
Missing	1 (0%)	1 (1%)	0
Receptors			
Oestrogen-receptor positive plus progesterone-receptor positive	198 (66%)	43 (59%)	155 (68%)
Oestrogen-receptor positive plus progesterone-receptor negative	33 (11%)	11 (15%)	22 (10%)
Oestrogen-receptor negative plus progesterone-receptor positive	5 (2%)	2 (3%)	3 (1%)
Oestrogen-receptor negative plus progesterone-receptor negative	66 (22%)	17 (23%)	49 (21%)
HER2-receptor positive	35 (12%)	11 (15%)	24 (10%)
Ki-67 >35%	39 (13%)	14 (19%)	25 (11%)
Missing*	158 (52%)	36 (49%)	122 (53%)
Tumour phenotype			
Luminal A	219 (73%)	50 (68%)	169 (74%)
Luminal B	17 (6%)	6 (8%)	11 (5%)
Only HER2 positive	18 (6%)	5 (7%)	13 (6%)
Triple negative	14 (16%)	12 (16%)	36 (16%)
Type of surgery			
Segmental mastectomy	183 (60%)	39 (53%)	144 (63%)
Total mastectomy	87 (29%)	22 (30%)	65 (28%)
Modified radical mastectomy	24 (8%)	9 (12%)	15 (7%)
Other	7 (2%)	2 (3%)	5 (2%)
Missing	1 (0%)	1 (1%)	0
Adjuvant chemotherapy	200 (66%)	51 (70%)	149 (65%)
Missing	3 (1%)	1 (1%)	2 (1%)
Post-menopausal women	201 (67%)	45 (62%)	156 (68%)
Missing	4 (1%)	2 (3%)	2 (1%)

Data are mean (SD), median (IQR), or n (%). HER2=human epidermal growth factor receptor 2. *Ki-67 not routinely assessed in all patients with breast cancer.

Table 1: **Patient characteristics**

	Number of patients	Number of relapses or deaths (%)	Progression-free or overall survival rate at 2 years (95% CI)	p value by log-rank test
Progression-free survival				
One or more circulating tumour cells				0·005
Positive status	73	11 (15%)	87% (0·77–0·93)	
Negative status	229	7 (3%)	99% (0·96–1·00)	
Two or more circulating tumour cells				0·001
Positive status	29	7 (24%)	79% (0·59–0·90)	
Negative status	273	11 (4%)	98% (0·95–0·99)	
Three or more circulating tumour cells				<0·0001
Positive status	16	5 (31%)	69% (0·40–0·86)	
Negative status	286	13 (5%)	97% (0·95–0·99)	
Overall survival				
One or more circulating tumour cells				0·010
Positive status	73	7 (10%)	94% (0·85–0·98)	
Negative status	229	5 (2%)	99% (0·96–1·00)	
Two or more circulating tumour cells				<0·0001
Positive status	29	6 (21%)	89% (0·70–0·96)	
Negative status	273	6 (2%)	99% (0·96–1·00)	
Three or more circulating tumour cells				<0·0001
Positive status	16	5 (31%)	81% (0·52–0·94)	
Negative status	286	7 (2%)	99% (0·96–1·00)	

Table 2: **Progression-free and overall survival in patients with circulating tumour cells**

primary breast tumours that expressed nuclear staining in 1% or greater of the cells as having positive expression for oestrogen, progesterone, or both receptors. We scored immunostaining results for HER2 as 1+ when less than 10% of the tumour cells had complete membranous staining; as 2+ when weak-to-moderate, membranous staining was present in greater than 10% of tumour cells; and as 3+ when strong, complete membranous staining was present in greater than 30% of tumour cells. We assessed all 2+ and 3+ cases by fluorescence in-situ hybridisation for *HER2* (also known as *ERBB2*) gene amplification with the PathVysion HER2 DNA probe kit (Abbott Laboratories, Abbott Park, IL, USA). We judged a ratio of HER2 to chromosome enumeration probe 17 greater than 2·2 as positive for *HER2* gene amplification. We defined triple-negative breast cancer as the absence of primary tumour oestrogen and progesterone receptor expression and *HER2* gene amplification. Tumours were immunostained and judged Ki-67 positive when greater than 35% of tumour cells exhibited Ki-67 staining.

We collected peripheral blood (7·5 mL) at the time of primary tumour surgery (but before any surgical manipulation of the primary tumour). Circulating tumour-cell status was established with the CellSearch System within 72 h of blood collection. This semi-automated technology enriches blood samples for cells expressing the epithelial cell adhesion molecule with antibody-coated magnetic beads, labels the nuclei of these enriched cells with the fluorescent dye 4,2-diamidino-2-phenylindole dihydrochloride, and stains the enriched cells with a combination of cytokeratin 8, 18, and 19, and CD45 fluorescent antibodies. We used a semiautomated fluorescence-based microscope system to identify circulating tumour cells; nucleated cells positive for cytokeratin and negative for CD45, as described elsewhere.[4] All results were reviewed by a qualified laboratory technician from whom all patient data was masked. Only restricted data were available on circulating tumour-cell detection levels in patients with early-stage breast cancer with the CellSearch method when we started our study in 2005. Therefore, we did circulating tumour-cell analysis on three 7·5 mL tubes of blood in a previously published report.[18] In that study, we noted negligible tube-to-tube variability in circulating tumour-cell detection for each patient sample; the circulating tumour-cell detection rate κ inter-rater agreement was 0·88 between the three 7·5 mL tubes of blood, which is in agreement with published data.[4,21] Therefore, here we report circulating tumour-cell detection levels as the number of circulating tumour cells per single 7·5 mL tube of blood. We report circulating tumour-cell levels only for the patient group; based on our previous institutional experience, we did not compare them with levels in a control group of healthy volunteers. The first published report of the CellSearch method for circulating tumour cell measurement in patients with metastatic breast cancer included a multi-institutional analysis of circulating tumour cells.[4] The University of Texas MD Anderson Cancer Center was the primary institution for that study, and the technician who undertook the circulating tumour cell analyses in that study was the same person who did all the circulating tumour-cell analyses in our present study. In the original study, circulating tumour cells were measured in 145 so-called normal, healthy individuals as well as in 200 women with benign breast conditions. Circulating tumour cells were rare in healthy women (mean 0·1 [SD 0·2] per 7·5 mL blood) and in patients with benign breast disease (mean 0·1 [0·9] per 7·5 mL blood). None of the normal control participants had two or more such cells per 7·5 mL blood. These so-called normal and benign samples were masked during assessment. Those results were similar to those of another study,[21] in which it was reported that circulating tumour cells were rare in healthy volunteers (n=145 samples, mean circulating tumour cell 0·1 [0·2] per 7·5 mL blood) and in patients with benign disease (n=199 samples, mean circulating tumour cell 0·1 [0·3] per 7·5 mL blood).

Statistical analyses

We used χ^2 or Fisher exact tests to assess associations between presence of circulating tumour cells and primary tumour characteristics. We used Fisher's exact test when any one of the observed frequencies in the two by two contingency table was less than five.

We defined progression-free and overall survival as time elapsed between date of diagnosis and either the date of clinical disease progression, death, or the last follow-up. We used log-rank tests to detect significant differences between groups. Kaplan-Meier curves were derived with STATA/IC 11.2 (StataCorp, College Station, TX, USA) for comparison of groups defined by different counts of circulating tumour cells. We used the Cox proportional hazards regression model to establish univariate HRs for progression-free and overall survival.[22] p values were two-tailed, and we judged values of less than 0·05 to be significant statistically. We used S-Plus v8.04 software (TIBCO Software Inc, Palo, Alto, CA, USA), and the Grambsch and Therneau test to rule out any violations for the proportionality of hazards (PH) assumption.[23]

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. AL, CSH, SK, AKL, AB, AEA, LX, IB, and HMK had access to the raw data. The corresponding author had full access to all of the data in the study at the time of analysis and had final responsibility for the decision to submit for publication.

Results

We enrolled 302 patients into our study (table 1). Mean age was 54 years, and median follow-up time was 35 months (table 1). No adverse events or complications were reported from blood collections. 73 patients (24%) had one or more circulating tumour cells, 29 patients (10%) had two or more circulating tumour cells, and 16 patients (5%) had three or more circulating tumour cells per 7·5 mL of blood (table 2). No primary tumour characteristic predicted the presence of one or more circulating tumour cells (table 1). 16 patients (5%) developed metastases to liver, lung, distant lymph-nodes, brain, and bone and there were 12 deaths during the follow-up period.

Patients with at least one circulating tumour cell showed decreased progression-free survival at 2 years (figure 1, table 3). A greater proportion of patients who had at least one or more circulating tumour cells relapsed compared with patients who had no circulating tumour cells (table 2). The progression-free survival rate at 2 years was much lower in the group that had at least one or more circulating tumour cells than in patients with none (figure 1, table 2).

As the number of circulating tumour cells increased, so did the HRs for disease progression. Patients with two or more circulating cells showed significantly decreased progression-free survival (figure 1, table 3); the progression-free survival rate at 2 years was lower in this group than in patients who had no circulating tumour cells (figure 1, table 2).

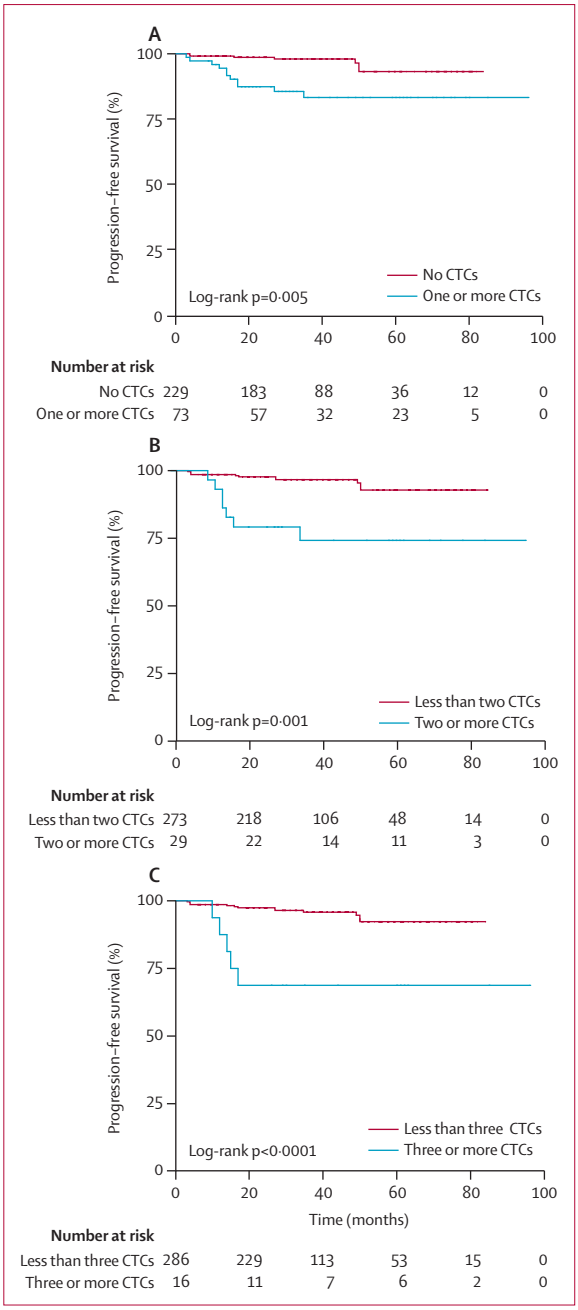

Figure 1: **Kaplan-Meier survival estimates of progression-free survival according to circulating tumour cells in operable breast cancer**
Probability of progression-free survival in patients with one or more (A), two or more (B), and three or more circulating tumour cells (C). CTC=circulating tumour cell.

16 patients with three or more circulating tumour cells had an HR of 6·74 for disease progression at 2 years (figure 1, table 3). The progression-free survival rate was much lower in patients with three or more circulating tumour cells than patients with less than three circulating tumour cells (figure 1, table 2). Progression-free survival rates at 2 years were only 64% and 55% (data not shown)

	Hazard ratio (95% CI)	p value
Progression-free survival		
Primary tumour >2 cm	2·73 (0·97–7·67)	0·06
Pathological node-negative versus		
1–3 lymph nodes	3·90 (1·17–12·9)	0·03
>3 lymph nodes	13·1 (3·51–48·8)	<0·0001
Histological grade 3	4·72 (1·68–13·3)	0·003
Oestrogen-receptor positive	0·45 (0·18–1·15)	0·09
Progesterone-receptor positive	0·45 (0·18–1·15)	0·09
Segmental mastectomy versus		0·04
Total mastectomy	2·51 (0·84–7·47)	
Modified radical mastectomy	5·43 (0·65–17·9)	
HER2 positive	0·41 (0·05–3·11)	0·39
Adjuvant chemotherapy	1·08 (0·38–3·04)	0·89
One or more circulating tumour cells	4·62 (1·79–11·9)	0·002
Two or more circulating tumour cells	5·50 (2·11–14·2)	<0·0001
Three or more circulating tumour cells	6·74 (2·40–19·0)	<0·0001
Overall survival		
Primary tumour >2 cm	3·03 (0·82–11·2)	0·09
Pathological node-negative versus		
1–3 lymph nodes	1·92 (0·39–9·51)	0·43
>3 lymph nodes	17·4 (4·14–72·8)	<0·0001
Histological grade 3	5·30 (1·42–19·5)	0·01
Oestrogen-receptor positive	0·27 (0·08–0·85)	0·03
Progesterone-receptor positive	0·20 (0·05–0·73)	0·02
Segmental mastectomy versus		0·15
Total mastectomy	1·35 (0·32–5·65)	..
Modified radical mastectomy	4·74 (1·27–17·8)	..
HER2 positive	0·64 (0·08–4·94)	0·66
Chemotherapy	1·14 (0·31–4·22)	0·85
One or more circulating tumour cells	4·04 (1·28–12·8)	0·02
Two or more circulating tumour cells	8·18 (2·63–25·5)	<0·0001
Three or more circulating tumour cells	11·5 (3·64–36·3)	<0·0001

HER2=human epidermal growth factor receptor 2.

***Table 3*: Cox regression analyses of survival associated with presence of circulating tumour cells**

in patients with at least four or five circulating tumour cells respectively.

We identified a significant difference in overall survival between the patients who had at least one circulating tumour cell compared with patients who had none (figure 2, table 3). The overall survival rate at 2 years was lower in this group than in patients who had no circulating tumour cells (figure 2, table 2). HRs increased with rising numbers of circulating tumour cells. Two or more circulating tumour cells predicted significantly worse overall survival (figure 2, table 3). Patients with two or more circulating tumour cells had a higher death rate than patients with less than two circulating tumour cells (table 2). Similarly, patients with three or more circulating tumour cells had lower overall survival rates than patients with less than three circulating tumour cells (figure 2, table 3).

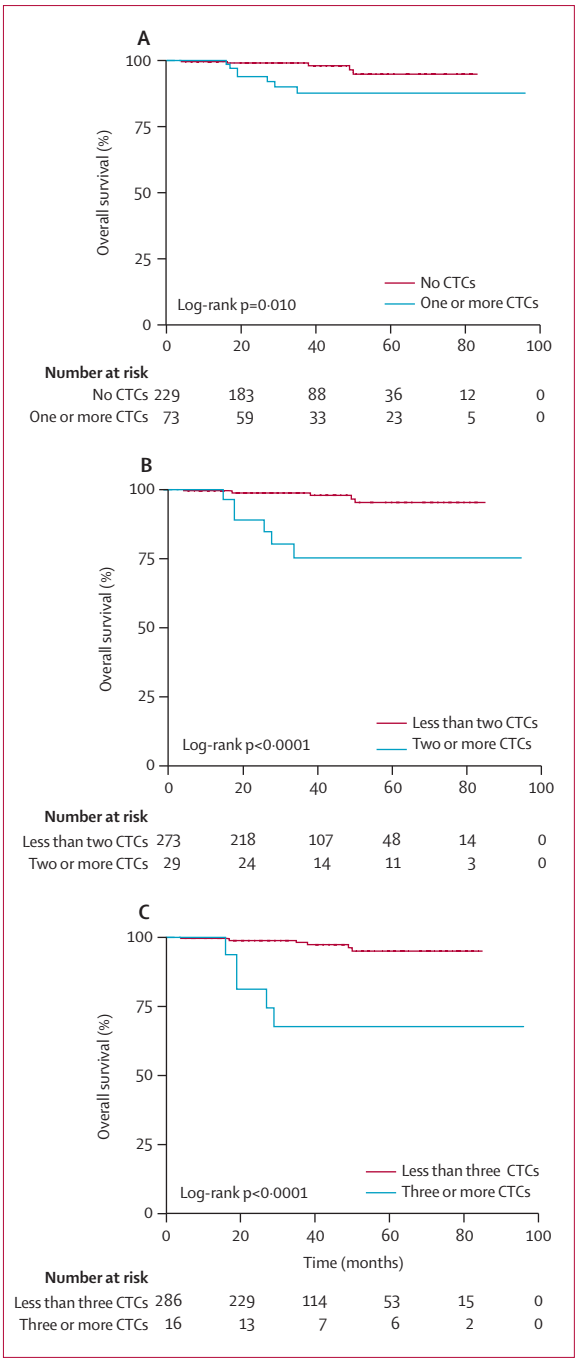

***Figure 2*: Kaplan-Meier survival estimates of overall survival according to circulating tumour cells in operable breast cancer**
Probability of overall survival in patients with one or more (A), two or more (B), and three or more circulating tumour cells (C). CTC=circulating tumour cell.

We did not identify any significant correlation between circulating tumour cell count and pathological axillary lymph-node status. This lack of significant association persisted after stratifying lymph-node positive patients into one to three, or more than three positive lymph nodes.

Discussion

Our findings show that the presence of one or more circulating tumour cells per 7·5 mL blood is an independent predictor of relapse and death in chemonaive patients with non-metastatic breast cancer. At present, clinical practice does not use this information to make treatment decisions or to stage disease (panel). In our sample cohort, we identified that 25% of patients had at least one circulating tumour cell. Neither tumour size nor any other primary tumour characteristic reliably predicted circulating tumour cells. Remarkably, even with a relatively short follow-up there were significantly worse outcomes in patients with one or more circulating tumour cells. Furthermore, the identification of one or more circulating tumour cells showed greater HRs for both relapse and death than almost all of the variables currently used to assess prognosis in patients with operable breast cancer; higher numbers of circulating tumour cells carried HRs as prognostically powerful as lymph-node metastasis. Additional prognostic information from circulating tumour cells might be useful in identifying early-stage patients at risk, especially since many of these patients will undergo only restricted lymph-node removal with the acceptance of the American College of Surgeons Oncology Group (ACOSOG) Z0011 data showing no reduced local control or survival for patients who had sentinel-node biopsy alone with limited axillary-nodal disease.[24] Furthermore, complete lymph-node removal results in complications for a substantial number of patients whereas drawing blood has few sequelae.[25] Most patients in our study had early-stage disease, suggesting that advanced disease is not necessary for cancer cells to spread haematogenously and compromise survival.

The 25% circulating tumour-cell positivity rate in our study is in agreement with those reported in the SUCCESS trials,[16] which assessed circulating tumour cells before and after adjuvant therapy. In the first SUCCESS study, more than one circulating tumour cell was identified in 19% of patients before the start of systemic adjuvant therapy. The recently released SUCCESS-A trial reported a 21·5% circulating tumour cell positivity rate.[16] The circulating tumour-cell positivity rate we report also agrees with the German GeparQuattro study, in which 22% of patients had circulating tumour cells before the start of neoadjuvant chemotherapy;[14] and the French REMAGUS 02 trial, which reported a 23% circulating tumour-cell positivity rate.[13,15]

So far, the data identifying the prognostic significance of circulating tumour cells are limited. Preliminary studies from the REMAGUS 02 trial reported that the presence of one or more circulating tumour cells before the start of systemic chemotherapy was an independent predictor of both metastasis-free and overall survival rates in 118 patients with stage 2 and 3 breast cancer.[13,15] In the REMAGUS studies, 55% of patients were classified as T1 or T2, and only 38% were axillary lymph-node negative. In our study, we classified 89% of patients as T1 or T2 and 63% axillary lymph-node negative. SUCCESS-A trial data suggests that the detection of one or more circulating tumour cells after surgical resection, but before the start of adjuvant therapy, was an independent predictor of both progression-free and overall survival rates in patients with non-metastatic breast cancer.[16] In the SUCCESS-A trial, 41% of the patients were classified as T1 and only 34% were axillary lymph-node negative. Although 63% of our patient cohort was axillary lymph-node negative, one or more circulating tumour cells predicted shortened progression-free and overall survival. We are prospectively comparing our circulating tumour cell results to predictive gene signature analyses of primary tumour such as OncotypeDX, to ascertain whether these tests provide information independent of each other in early-stage, node negative, oestrogen-receptor positive patients. Thus far we have not identified any significant association, probably because of the small numbers of events in oestrogen-receptor positive, lymph-node negative patients—longer follow-up is needed. Identifying the prognostic significance of circulating tumour cells would be particularly important in this subgroup since it might assist in clinical decisions regarding the use of systemic chemotherapy. Since our

Panel: Research in context

Systematic review

In 2004, a study showed that the identification of five or more circulating tumour cells per 7·5 mL of blood independently predicted progression-free and overall survival in patients with metastatic breast cancer.[4] We postulated that circulating tumour cells would be identified in a significant number of patients with non-metastatic breast cancer and would be prognostically significant. Before we started our study in 2005, we searched PubMed for reports published from 1990 to 2005 with the search terms "operable breast cancer" and "circulating tumor cells"; seven articles met these search criteria. We did not limit our search by language. Five of the seven articles, with various methods, focused on circulating tumour-cell detection, and two preliminary reports reported on circulating tumour-cell detection as well as outcomes data. Since 2005, with the exception of a few published studies and abstracts, there remains a lack of work describing the prognostic significance of circulating tumour cells in patients with non-metastatic breast cancer. Thus we designed our study to assess identification and prognostic significance of circulating tumour cells in patients with non-metastatic breast cancer.

Interpretation

Our findings, in congruence with previous reports, suggest that circulating tumour cell assessment provides important prognostic information for a significant number of patients with non-metastatic breast cancer. These studies identified that both progression-free and overall survival were worse in patients with one or more circulating tumour cells. At present, the American Society of Clinical Oncology guidelines for biomarker analysis do not recommend routine measurement of circulating tumour cells to assist in decision making for patients with breast cancer. However, the growing body of published work, including our study, suggests that assessment of circulating tumour cells might provide important prognostic information in these patients. Clinicians should await the results of multi-institutional studies that might identify subgroups wherein information on circulating tumour cells assists in the clinical decision-making process.

original study design was not powered for subgroup analysis, we are continuing to enrol patients on this protocol to enable us to do these analyses. Longer follow-up is also warranted because oestrogen-receptor positive patients often develop recurrences later than oestrogen-receptor negative patients.[26]

Our survival-curve distributions are consistent with expected outcomes in patients with non-metastatic breast cancer, with long, flat lines indicative of the low number of recurrences at periods longer than 20 months. The Eastern Cooperative Oncology Group (ECOG) study[27] reported on annual hazard rates for breast cancer recurrence after primary therapy in 3585 operable patients; the peak hazard for disease recurrence was in the interval between 1 and 2 years after surgery and tapered off slowly thereafter. Similar findings were reported in another large study published the same year.[28] This study reported on time distribution of the recurrence risk in 1173 operable patients who were treated with mastectomy alone; peak recurrence was at 18 months after surgery, then slowly diminished in a "plateau-like tail extending up to 15 years post-surgery".[28] Peak recurrence rates in both studies was within 1–2 years after surgery, irrespective of whether or not patients received systemic therapy. These data suggest it would be useful to obtain follow-up circulating tumour-cell measurements at sequential timepoints both post-operatively and after completion of any adjuvant therapies. We have amended our approved institutional review-board protocol and are at present collecting follow-up samples. However, since the protocol was amended a year ago, longer follow-up times will be needed to establish if follow-up circulating tumour-cell assessments provide additional important prognostic information.

None of the patients included in our report received chemotherapy before circulating tumour-cell assessment. Therefore, we could not establish the effects of chemotherapy on circulating tumour-cell identification. However, results obtained from a larger concurrent study of patients with stage 1–3 breast cancer from our group (which included patients who received neoadjuvant chemotherapies, adjuvant therapies, as well as chemonaive patients) suggested that circulating tumour cells are identified at similar levels in neoadjuvant treated versus chemonaive patients, or in patients treated with adjuvant therapies selected on the basis of primary tumour characteristics. Published reports have established that circulating tumour cells are often dormant, as shown by Ki-67 immunostaining.[29] Preliminary reports have also suggested that circulating tumour cells exhibit a putative cancer stem-cell phenotype;[30,31] this phenotype is associated with the upregulation of multidrug-resistance proteins, which might explain circulating tumour-cell resistance to chemotherapy.[32] These findings might explain why circulating tumour cells are often identified in patients after cytotoxic therapies that targeted proliferating cells. Furthermore, previous studies have identified discordance in oestrogen receptor[33] and HER2[34,35] positivity between the primary tumour and circulating tumour cells. These data suggest that patients harbouring occult micrometastatic disease might benefit from additional adjuvant systemic therapies targeting circulating tumour cells.

Present guidelines by the American Society of Clinical Oncology do not recommend that data on circulating tumour cells be used for staging non-metastatic breast cancer, but they do recognise the option of measuring circulating tumour cells in patients with metastatic disease.[36] Studies of patients with metastatic breast cancer used a cutoff of five circulating tumour cells as a positive result.[4] The recently released seventh edition of the American Joint Commission on Cancer Staging Manual[19] includes a new category of cM0(i+) to designate patients with microscopic disease in blood or bone marrow, although these patients will still be judged as having non-metastatic disease. These study results support the idea that information on circulating tumour cells should be included in the staging algorithms for patients with non-metastatic breast cancer, especially since it provides important biological information on the metastatic process.

Contributors

AL designed the study. AL, HMK, IB, and AEA collected the samples. AL, AKL, and AB set up the database. AL, CSH, AKL, and AB analysed the data and LX did the statistical analyses. AL, CSH, and SK wrote the report with assistance and final approval from all authors.

Conflicts of interest

We declare that we have no conflicts of interest.

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