ELSEVIER

European Journal of Cancer 40 (2004) 481–486

European Journal of Cancer

www.ejconline.com

Monitoring different stages of breast cancer using tumour markers CA 15-3, CEA and TPA

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Received 22 May 2003; received in revised form 19 June 2003; accepted 22 October 2003

Abstract

The ability of the tumour markers Cancer Antigen 15-3 (CA 15-3), Carcinoembryonic Antigen (CEA), and Tissue Polypeptide Antigen (TPA) to signal progression in breast cancer patients was investigated in this study. Marker interpretation considered the analytical variation, intra-individual biological variation, and the rate of increase. Patient cohorts were as follows: (A) 90 stage II breast cancer patients who were monitored postoperatively, (B) 204 recurrent breast cancer patients who were monitored during first-line chemotherapy, and (C) 112 patients who were monitored during the time period after first-line chemotherapy. The sensitivity for progression was 44% (cohort A), 69% (cohort B), and 68% (cohort C) without any false progression signals. Marker lead-times exceeded 3 months in 20% (cohort A) and 27% (cohort C) of patients. Marker lead-times were 1–6 months among 33% of the patients receiving first-line chemotherapy (cohort B). Trials are necessary to determine whether tumour marker-guided therapy has any prognostic impact. The data suggest that tumour marker information may be used to stop ineffective treatments and reduce unnecessary adverse effects.

Keywords: Tumour markers; Biological; CA-15-3 antigen; Carcinoembryonic antigen; Tissue polypeptide antigen; Breast neoplasms

1. Introduction

For decades, disease activity in breast cancer has been monitored clinically, as well as by biochemistry and imaging. Routine laboratory tests lack the sensitivity to be of major clinical value [1]. Imaging techniques lack the sensitivity to detect only a few tumour cells and small changes in the tumour burden [2]. Consequently, there is a need for reliable and easily performed quantitative diagnostic tests that may predict an increasing tumour burden in the individual patient.

Several studies have investigated whether serological tumour markers: Cancer Antigen 15-3 (CA 15-3), Carcinoembryonic Antigen (CEA), and Tissue Polypeptide Antigen (TPA), may fulfill this need in breast cancer. However, their precise clinical utility is not known as the predictive value of increasing tumour marker concentrations is uncertain. This is because there are no generally accepted guidelines for interpreting sequential concentrations obtained during therapy and follow-up [3]. The purpose of our study was to investigate the usefulness of CA 15-3, CEA, and TPA in detecting progressive disease among patient groups with different stages of breast cancer and the following were considered: (i) the cut-off value for the assay system, (ii) the random analytical variation associated with the applied assay system, (iii) the random normal background intra-individual biological variation of the considered marker and, (iv) the rate of marker increase.

2. Patients and methods

2.1. Patients

Cohort A consisted of 90 consecutive patients with stage II breast cancer who were monitored post-operatively after a total mastectomy. The patients had a

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doi:10.1016/j.ejca.2003.10.015

high risk of recurrence with a primary tumour $\geqslant 5$ cm in diameter or positive axillary lymph nodes or tumour invasion into the deep resection line. Following adjuvant loco-regional radiotherapy, they were randomised to different forms of systemic adjuvant therapy [4]. The clinical follow-up included (a) physical examination every 3 months for 18 months, then every 6 months for the next 3 and a half years, and then once a year for a total of 10 years, (b) chest X-ray examination every 6 months for the first year and then annually for the next four years, (c) X-ray examination of the axial skeleton or bone scintigraphy twice during the first postoperative year, (d) liver function tests and calcium monthly for the first year, then every second month for the following 2 years. Recurrent disease was confirmed by biopsy or ultrasound scanning or X-ray in instances of liver, pulmonary or osseous metastases. Biopsy was omitted if metastases developed at more sites.

Cohort B consisted of 204 consecutive patients with histologically-proven recurrent breast cancer with measurable or evaluable disease (stage III/IV) during receipt of first-line chemotherapy for metastatic disease, with epirubicin or epirubicin and cisplatin in a randomised trial [5]. History-taking, physical examination, blood cell counts, liver function tests, and calcium were repeated monthly before each treatment cycle. Evaluable or measurable indicators were evaluated every second month, except for bone lesions that were evaluated every third month.

Cohort C consisted of 112 consecutive patients with metastatic breast cancer (stage III/IV) monitored during the time period after response to first-line chemotherapy. Physical examination and re-evaluation was performed every 3 months in the first year of follow-up and biochemistry (blood cell counts, liver function tests, and calcium) every 6 weeks. The programme was repeated every 3 months in the second year, and every 6 months thereafter.

Clinical evaluation was based on criteria of the World Health Organization (WHO) [6] and performed by one investigator without knowledge of the tumour marker data. The studies were conducted according to the requirements of the Scientific Ethics Committee of Copenhagen County, Denmark.

2.2. Marker sampling, analysis, and quality control

- Cohort A: The sampling frequency was monthly for the first year, and every third month during the next 24 months.
- Cohort B: Samples were collected every four weeks prior to each treatment cycle.
- Cohort C: Samples were collected every 6 weeks during the first year of the control period, every third month the second year, and every 6 months thereafter.

Additional specimens were sampled whenever routine biochemistry was requested outside of the scheduled time points. Each blood sample was analysed in duplicate for CA 15-3, CEA, and TPA levels. The applied cut-off values were 30 kU/l, 7.5 μg/l, and 357 U/l, for CA 15-3, CEA and TPA, respectively. Three quality control samples with different concentrations of the analytes were included in each assay run and a multirule combination was used to accept or reject runs (1_{3s}, 2 of 3_{2s}, and R_{4s}) [7]. The results of each lot of control material were used to calculate the total analytical variation for that concentration level of the analyte and the precision profiles were plotted.

2.3. Assessment of single markers

Previous kinetic studies have suggested that the rates of increase of CA 15-3 and CEA are different from those of TPA. For CA 15-3 and CEA, the rates of increase can be characterised by a slow or fast pattern, whereas the rates for increases in TPA are usually homogeneous. Assessment of slow-rise CA 15-3–CEA increments and TPA increments involved estimation of the significance of a change between a baseline concentration and a following measurement. A change, expressed as a percentage of their mean value, was statistically significant at $P<0.05$, if the difference exceeded the random variation inherent in the two test results, the Reference Change Value (RCV). The $RCV = \sqrt{2}\times Z\times\sqrt{(CV_a^2+CV_i^2)}$ [8]. $\sqrt{2}$ is a constant (two measurements). Z is the Z-statistic; the value is 1.65 if the expected change is unidirectional (either an increment or a decrement). CV_a, the analytical imprecision corresponding to the baseline concentration, was read from the precision profile of the total analytical variation of the considered marker. CV_i is the average intra-individual biological variation, 6.2% (CA 15-3), 9.3% (CEA), and 28.3% (TPA) [9,10].

The criterion for slow-rise CA 15-3 or CEA increments starting below the cut-off point was that there were at least two consecutive increments and that the latest and second latest concentrations were above the cut-off point. The second latest concentration should also significantly exceed any previous concentration below the cut-off point.

The criterion for slow-rise CA 15-3 or CEA increments starting above the cut-off point was that there were at least two consecutive increments and that the latest concentration taken significantly exceeded the third latest or any previous concentration above the cut-off point.

The criterion for fast-rise CA 15-3 or CEA increments starting below the cut-off point was that the latest concentration above the cut-off point was double that of any previous concentration below the cut-off point.

The criterion for fast-rise CA 15-3 or CEA increments starting above the cut-off point was that the latest

concentration was double that of any previous concentration above the cut-off point.

The criterion for TPA increments starting below the cut-off point was that the latest concentration above the cut-off point significantly exceeded any previous concentration below the cut-off point.

The criterion for TPA increments starting above the cut-off point was that the latest concentration significantly exceeded any previous concentration above the cut-off point.

The sampling interval between measurements was $\geqslant 3$ weeks for all of the criteria.

If an increment fulfilled both the slow-rise and the fast-rise criteria, the date of marker progression was assigned to the criterion that first signalled progression.

The sequential tumour marker data were assessed by one investigator without knowledge of the clinical evaluation of the patients.

2.4. Assessment of marker combinations

Progression was based upon the marker that first fulfilled a progression criterion. Unchanged activity of disease was recorded if one marker fulfilled a progression criterion simultaneously with a significant decrease from above the cut-off level concentrations of another.

2.5. Classification of information

All patients were included for tumour marker evaluation, regardless of their CA 15-3, CEA, and TPA concentration levels.

The clinical and tumour marker evaluations in terms of progression or non-progression was matched only once for each patient. Sensitivity is the percentage of patients with progressive disease identified by a marker $(TP/TP+FN)\times 100$. The predictive value of a negative marker test is the probability of clinical non-progression at unchanged marker concentrations $(TN/TN+FN)\times 100$. The false-negative rate of a marker test is the probability of clinical progression at unchanged marker concentrations $(FN/FN+TN)\times 100$.

3. Results

- Cohort A: 90 patients were monitored for 747 days (median) from operation (range 46–1211 days) and 17 samples (median) were collected (range 2–26).

25 patients developed clinical recurrence. CA 15-3 detected eleven recurrences without any false-positive signals (sensitivity 44%, 95% confidence interval (CI) 24–65%) (Table 1).

CEA and TPA were of little additive value. CEA identified two recurrences, but CA 15-3 had identified both of these. TPA identified four recurrences, three of which were also identified by CA 15-3. The tumour marker CA 15-3 provided a lead-time of 3–18 months among 20% (95% CI 7–41%) of patients with clinical recurrence. The probability of clinical non-recurrence at unchanged CA 15-3 concentrations (the negative predictive value) was 82% (95% CI 72–90%). The probability of clinical recurrence at unchanged CA 15-3 concentrations (the false-negative rate) was 18% (95% CI 10–28%).

- Cohort B: 204 patients were monitored for 211 days (median) during first-line chemotherapy (range 51–592 days) and 15 samples (median) were collected (range 3–35). 58 patients developed clinical progression during therapy. CA 15-3 identified 26 of these, CA 15-3 or CEA 35 patients, and CA 15-3 or CEA or TPA 40 patients (sensitivity 69%, 95% CI 55–80%) (Table 1).

The cytotoxic effect caused two false-positive CEA progression signals and one false CA 15-3 signal within the first treatment cycles. According to our method of interpretation, simultaneously decreasing CA 15-3 concentrations eliminated the two false CEA signals and decreasing TPA concentrations eliminated the single false-positive CA 15-3 signal.

By using information from the marker which first signalled progression, the combination of CA 15-3, CEA, and TPA provided a lead-time of 3–6 months among 10% (95% CI 4–21%) and a lead-time of 1–6 months among 33% (95% CI 21–46%) of the patients with clinical progression. The probability of clinical non-progression at unchanged CA 15-3, CEA, and TPA concentrations (the negative predictive value) was 89% (95% CI 83–93%). The probability of clinical progression at unchanged CA 15-3, CEA, and TPA concentrations (the false-negative rate) was 11% (95% CI 7–17%).

- Cohort C: 112 patients were monitored for 260 days (median) during follow-up after response to first-line chemotherapy (range 49–1757 days) and 5 samples (median) were collected (range 1–30). 75 patients developed clinical progression. CA 15-3 identified 43 of these without false-positive signals; CA 15-3 or CEA identified 51 without false signals (sensitivity 68%, 95% CI 56–78%) (Table 1). TPA had little additive value because 26 of the 27 progression events were also identified by CA 15-3 or CEA.

By using information from the marker which first signalled progression, the combination of CA 15-3 and

Table 1
Classification of tumour marker information

True-positive results (TP)		False-positive results (FP)	
• Concordant clinical and marker information in terms of recurrence or progression and zero or positive lead-time:		• Discordant information with marker recurrence or progression without clinical recurrence or progression:	
Cohort A (CA 15-3):	11 TP	Cohort A (CA 15-3):	0 FP
Cohort B (CA 15-3 or CEA or TPA):	40 TP	Cohort B (CA 15-3 or CEA or TPA):	0 FP
Cohort C (CA 15-3 or CEA):	51 TP	Cohort C (CA 15-3 or CEA):	0 FP
False-negative results (FN)		True-negative results (TN)	
• Discordant clinical and marker information with clinical recurrence or progression without marker recurrence or progression or negative lead-time:		• Concordant clinical and marker information in terms of non-progression:	
Cohort A (CA 15-3):	14 FN	Cohort A (CA 15-3):	65 TN
Cohort B (CA 15-3 or CEA or TPA):	18 FN	Cohort B (CA 15-3 or CEA or TPA):TN	146 TN
Cohort C (CA 15-3 or CEA):	24 FN	Cohort C (CA 15-3 or CEA):	37 TN

CEA provided a lead-time of 3–15 months among 27% (95% CI 17–38%) of patients with clinical progression. The probability of clinical non-progression at unchanged CA 15-3 and CEA concentrations (the negative predictive value) was 61% (95% CI 47–73%). The probability of clinical recurrence at unchanged CA 15-3 and CEA concentrations (the false-negative rate) was 39% (95% CI 27–53%).

4. Discussion

The potential role of serological tumour markers to detect progressive disease in patients with breast cancer has been the subject of several studies [11,12]. However, their precise clinical role is undefined owing to uncertainty of the predictive value of increasing tumour marker concentrations [13]. It remains a paradox that tumour marker measurements are recommended for monitoring purposes by their manufacturers, but they do not provide guidelines for interpreting sequential concentrations obtained during therapy and follow-up. In addition, there are no generally accepted guidelines that describe how clinical tumour marker trials should be designed, conducted, evaluated and presented. The investigated patient populations and the applied methodology are heterogeneous and it is difficult to compare and validate the obtained results. The main impression is that it is difficult to design and conduct clinical tumour marker trials and interpretation of results is more complicated than hitherto recognised.

Most investigators have considered a marker increment to be significant if there was (i) one elevated concentration, (ii) two elevated concentrations, (iii) a concentration increment of 20%, or (iv) an increment of 25%. These criteria, although easy to use, are due to habit rather than being founded on a sound statistical analysis and the prediction of progression was low [14–21]. A different approach integrated information from three markers CA 15-3, CEA, and the erythrocyte sedimentation rate into one index score [22]. 34 of 39 patients (87%) with progressive metastatic breast cancer were detected with a lead-time of 0–3 months.

In our study, increments of CA 15-3, CEA, and TPA detected 44% of the recurrences in stage II breast cancer patients and 68–69% of the progression events in metastatic breast cancer patients. In this context, the reliability of an increment as well as the length of the lead-time was addressed.

CA 15-3 did not provide false signals of recurrence among the stage II breast cancer patients. The results suggest that CA 15-3 increments may be highly predictive for recurrence and provide a clinically relevant lead-time exceeding 3 months in 20% of patients. However, the impact of the routine use of early marker-guided treatment on survival, quality of life, and costs is, as yet, unknown. In any case, randomised trials will be necessary to determine the benefit for the individual patient.

Monitoring of tumour markers in stage III and IV breast cancer patients during first-line chemotherapy requires special attention because transient increments in the concentrations of single markers occurred during the first series of therapy and provided three false signals of tumour progression. Our findings are in accordance with those of other investigators and may be explained by tumour lysis and marker release from disintegrating cells [12]. According to the applied method for marker interpretation, the false progression signals were eliminated by simultaneous decrements in one of the other markers. The lead-time in 90% of the patients was shorter than 3 months and it is unlikely that an early new tumour marker-guided treatment will have any prognostic impact. However, 33% of the patients presented a tumour marker lead-time of 1–6 months. Even though the lead-times are short they may have a major importance with regard to the quality of life of

the patients because an ineffective therapy can be stopped earlier, thereby minimising unnecessary and sometimes irreversible adverse effects.

As regards the control period of stage III and IV breast cancer there were no false progression signals as long as the criteria were applied. The increasing marker concentrations were associated with continuous increments and not with transient fluctuations. The results suggest that CA 15-3 or CEA increments may be highly predictive of progression and may provide a clinically relevant lead-time exceeding 3 months in 27% of patients.

Overall, the investigated markers CA 15-3, CEA, and TPA were unreliable in excluding ongoing clinical recurrence and progression. The low ability to exclude tumour growth is shared by all other serological tumour markers, irrespective of malignancy.

Critics may argue that we used the same patient population to determine our tumour marker assessment criteria and to assess their performance. However, this was not the case. In a previous study on CA-549 measurements in metastatic breast cancer patients, we developed different sets of assessment criteria to interpret sequential tumour marker concentrations [23]. As the results were encouraging, the same principles were implemented to assess other markers, CA 15-3, CEA, and TPA, and other patients with different stages of disease [24,25]. By keeping the algorithms, changing the markers, and extending the number of investigated patients the bias resulting from using the same population to develop the algorithms and to assess their performance was minimised. It may also be argued that our method for tumour marker assessment is time-consuming in clinical practice because it requires a large workload if performed manually. However, assessment of sequential marker concentrations may be facilitated by use of graphical software programs designed for monitoring purposes and for calculating the significance of a change in concentrations [26].

In conclusion, CA 15-3, as a single marker, is useful in the recognition of recurrence postoperatively during the disease-free interval. The combination of CA 15-3, CEA, and TPA shows progression during first-line chemotherapy of metastatic disease (stage III/IV) and may be used to decide upon a change in therapy. The combination of CA 15-3 and CEA is useful in monitoring progression during the period following first-line chemotherapy.

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