After the three induction cycles, thrombocytopenia and leucopenia were observed in 58.7 and 44.9% of cases, respectively, with 37.8 and 24.3% at grade III–IV. Neutrophil nadir occurred at a median of 43 days and platelet nadir at a median of 37 days. In 2 patients, treatment was interrupted because of grade IV thrombocytopenia.

Comments

Fotemustine, administered according to our schedule, had no activity in patients with advanced or recurrent soft tissue sarcomas. In contrast, the only drugs with known activity in this disease were also found to have activity in second-line chemotherapy [3]. This underlines the justification of further drug testing as second-line. Of the other nitrosourea derivatives, lomustine has no activity [4] and recently we found nimustine to have minor activity. Our study also confirmed the mainly haematological toxicity of fotemustine.

The lack of responses added to the haematological toxicity mean that fotemustine must not be used for further studies in soft tissue sarcomas.

1. Khayat D, Lokiec F, Bizzari JP, *et al.* Phase I clinical study of the new aminoacid linked nitrosourea S 10036 administered on a weekly schedule. *Cancer Res.* 1987, **47**, 6782–6785.
2. Jacquillat CL, Khayat D, Banzet P, *et al.* Final Report of the French multicenter phase II study of the nitrosourea fotemustine in 153 evaluable patients with disseminated malignant melanoma including patients with cerebral metastasis. *Cancer* 1990, **66**, 1873–1878.
3. Van Oosterom, Santoro A, Rouesse J, *et al.* Chemotherapy in advanced soft tissue sarcomas. The EORTC experience. In: Ryan JR and Baker L, eds. *Recent Concepts in Sarcoma Treatment.* The Netherlands, Deventer, Kluwer Academic Publishers, 1987, 183–190.
4. Rosenberg SA, Suit HD, Baker LH. Sarcomas of soft tissue. In DeVita VT, Hellnan S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*, 2nd edition. Philadelphia, JB Lippincott, 1989, 1243.

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CA 15.3 Determination in Patients with Breast Cancer: Clinical Utility for the Detection of Distant Metastases

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In 81 healthy women, 26 pregnant women, 25 patients with fibrocystic disease and 144 breast cancer patients, the overall diagnostic sensitivity and specificity of the CA 15.3 test was 27 and 97%, respectively. The positive and negative predictive values were 93 and 43%. In 150 node-negative patients taking part in a chemoprevention trial CA 15.3 was assayed at baseline and every 4 months for a median follow-up of 24 months (range 4–48). In these patients, 5 had local recurrences, 1 had a regional recurrence, 9 had distant metastases and 3 developed cancer in the contralateral breast. Among the patients with recurrences, those with distant metastases showed the highest ratio of CA 15.3 increase (8/9); in local and regional recurrences, this ratio was lower (2/6). The patients with contralateral breast cancer had no significant increase in CA 15.3. Patients in whom metastases were detected showed an increase in CA 15.3 4–48 months before clinical or instrumental detection of the metastases.
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INTRODUCTION

SEVERAL CIRCULATING tumour markers for breast cancer have been discovered [1]. However, despite extensive research in this field, no specific markers for breast cancer are available as yet. Nevertheless, many clinical oncologists still use various tumour markers, especially carcinoembryonic antigen (CEA) [2–6] and tissue polypeptide antigen (TPA) [7–9]. Unfortunately, these tumour markers do not have sufficient specificity. The development of monoclonal antibodies (Mabs) against human mammary carcinomas has improved the specificity of the reaction with breast cancer-associated antigens and has stimulated the search for new tumour markers for breast cancer [10]. One of these markers is CA 15.3; it can be measured by an immunoradiometric method (IRMA) based on Mab DF 3 raised against a membrane fraction of liver metastases from breast cancer, and Mab 115 D8 raised against milk fat globule membranes [11, 12]. Although the diagnostic sensitivity of CA 15.3 seems to be superior to that of CEA, CA 15.3 determination does not have much validity in the early detection of primary tumours [13, 14]. Its main clinical indication is in the monitoring of response to treatment and in the follow-up of tumour-free patients. In some studies, CA 15.3 serum elevations appeared to anticipate

the clinical and instrumental discovery of distant metastases [15–18].

The aims of this study were (a) to confirm the diagnostic efficacy of CA 15.3 determination in patients with breast cancer; and (b) to evaluate serial determinations of CA 15.3 in the follow-up of patients treated for breast cancer in order to detect cancer relapse.

MATERIALS AND METHODS

Patient selection

(*a*) *Use of the CA* 15.3 *test in various breast pathologies.* Single determinations of CA 15.3 levels were performed in 81 healthy women (age range 18–68 years), in 26 pregnant women (age range 21–38 years), in 25 patients with fibrocystic disease (age range 21–67 years) and in 144 patients with breast cancer (age range 29–77 years). All diagnoses of breast pathology were histologically confirmed; 40 patients had no axillary lymph node involvement (N−), 54 had axillary lymph node involvement (N+) and 50 patients had distant metastases.

(*b*) *Prospective study of CA* 15.3 *serum levels during follow-up of breast cancer.* CA 15.3 serum levels were determined in a group of 150 N− patients taking part in a chemoprevention trial. The patients had undergone a modified mastectomy or quadrantectomy plus axillary dissection and radiotherapy prior to the start of the trial. The diagnosis of breast cancer was based on histological examination of the surgical specimen and nodes. The age ranged from 35 to 65 years and patients had no detectable relapse of cancer when they entered the study. Physical examination and blood tests including renal, hematologic and hepatic parameters were performed at baseline and once every 4 months. Chest and skeleton X-rays, liver ultrasonography and mammography were performed at baseline and once every year. CA 15.3 serum levels were determined at baseline and once every 4 months, for an average follow-up period of 24 months (range 4–48 months).

CA 15.3 *determination*

The test for CA 15.3 was performed on the serum by an immunoradiometric assay (IRMA), a solid-phase assay based on the 'sandwich' principle (CIS centocor). In this study CA 15.3 concentrations were expressed as U/ml. The range of normality was established by taking concentrations under the 95th centile as normal CA 15.3 values. The coefficient of variation (CV) was evaluated on a panel consisting of three groups of samples with low, medium and high CA 15.3 concentrations. The intra-assay and inter-assay CVs were: for low levels, 7.1–9.3%; for medium levels, 5.4–8.2%; for high levels, 6.5–9.7%.

RESULTS

Single determinations revealed significantly higher CA 15.3 serum levels in breast cancer patients than in controls, pregnant women and subjects with benign breast pathology (ANOVA $P<0.02$). Among breast cancer patients, the highest CA 15.3 concentrations were found in patients with distant metastases. CA 15.3 serum levels (U/ml) were: in the control group, mean = 18, S.D. = 9.4, range 7–32; in pregnant women, mean = 29, S.D. = 14.9, range 14–80; in benign disease, mean = 12, S.D. = 6.3, range 4–30; in breast cancer patients, mean = 53.6, S.D. = 129.8, range 4–600. The overall diagnostic sensitivity and specificity of the CA 15.3 assay were 27 and 97%, respectively. The predictive value of the positive and negative results was 93 and 49%.

Table 1. *CA* 15.3 *serum levels in the follow-up of patients treated for breast cancer and evidence of relapse or contralateral breast cancer*

Evidence of disease	Increased CA 15.3 levels (n = 10)	Normal CA 15.3 levels (n = 8)
Patients with recurrences (n = 14)		
Local		
Chest wall and surgical scar	2	
Ipsilateral breast after quadrantectomy		3
Regional		1
Distant metastases	8 2 liver 2 lung 1 bone 2 lung and bone 1 bone and skin	1 1 bone
Patients with contralateral breast cancer (n = 3)		3

In the prospective study of serial CA 15.3 determinations, breast cancer patients in follow-up had five local recurrences, one regional recurrence, nine distant metastases, while 3 of them developed cancer in the contralateral breast. The distribution of increased CA 15.3 levels is shown in Table 1. Among patients with cancer recurrence, those with distant metastases had the highest ratio of CA 15.3 increase (8/9). For patients with local and regional recurrences, the ratio was lower (2/6). Contralateral breast cancer cases did not show any significant increase in CA 15.3 levels.

The 8 patients who developed cancer showed low CA 15.3 levels in serial determinations during follow-up. These levels were stable and showed non-significant negative or positive oscillations.

In Fig. 1, cases are presented which develsoped local recurrences and distant metastases. They showed a continuous increase in CA 15.3 levels and cancer was invariably detected several (4–48) months after the initial rise in CA 15.3 at the first marker determination.

DISCUSSION

Our study confirmed that CA 15.3 serum levels are significantly higher in patients with breast carcinoma than in control subjects, pregnant women and patients with benign disease. These findings are in agreement with data reported by other authors [19, 20]. Pregnancy led to an increase in CA 15.3 levels in 46% of the observed women. This is probably due to the

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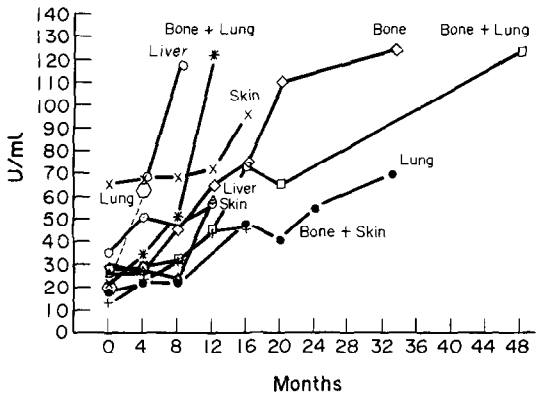

Fig. 1. Increased CA 15.3 levels in cases with evidence of disease during follow-up.

modifications of the mammary gland occurring in pregnancy and, in particular, to enhanced secretion of mucin. In our patient series, the differences in CA 15.3 levels among cancer patients were not influenced by the number of lymph nodes involved but by the presence of distant metastases.

CA 15.3 determination with the established cut-off value (95th centile) had a limited overall diagnostic sensitivity (27%), but a good diagnostic specificity (97%). This poor sensitivity is confirmed by other authors: the early stages of breast cancer are often characterised by low CA 15.3 concentrations [13, 14, 20]. In contrast, the diagnostic sensitivity was markedly enhanced in the presence of distant metastases. This was observed in many other studies which demonstrated that the sensitivity of CA 15.3 increased with the stage of the disease [13, 15, 17, 20]. This is why the occurrence of high levels may be a predictive signal of tumour relapse during the follow-up of breast cancer patients [15, 16, 20].

In our follow-up study, which included 150 breast cancer patients who underwent surgery with axillary dissection plus radiotherapy, CA 15.3 levels were tested at baseline and every 4 months. The duration of follow-up was 24 months and only in four disease-free patients who showed increased CA 15.3 levels at 24 months was CA 15.3 determination performed for another 24-month period. 15 patients relapsed: 5 had local recurrence, 1 regional and 9 distant metastases. 3 patients developed contralateral breast cancer. CA 15.3 levels increased in 10 patients with recurrences (2 local recurrences and 8 distant metastases) while we did not observe any variations in CA 15.3 levels in 3 patients with local recurrences, in 1 patient with a regional relapse and in 1 patient with distant metastases. Patients who developed contralateral breast cancer did not show any increase in CA 15.3 levels. Patients in whom metastases were found showed an increase in CA 15.3 levels from 4 to 48 months before the clinical or instrumental detection of metastases. Only 2 of them had elevated CA 15.3 levels at the first determination; also in these cases the concentrations progressively increased during the subsequent serial determinations.

The observed alterations in CA 15.3 levels seem to confirm that in nearly all cases with distant metastases (8/9), CA 15.3 is a strong indicator of tumour relapse. In contrast, CA 15.3 determinations predict only one-third of local recurrences. In contralateral breast cancer, CA 15.3 serum variations failed to reach significant concentrations.

Among patients with evidence of disease in our series, we found only 1 case with a progressive increase in CA 15.3 at 48 months of follow-up. This patient had a diagnosis of suprarenal gland adenomas, and at this stage she can be considered as a false-positive result.

In our opinion, these observations are very important for the interpretation of serum marker levels in the follow-up of breast cancer patients. In fact, high levels of CA 15.3 can be interpreted as a reliable signal of cancer only in the presence of distant metastases. Local or regional disease does not always cause positive biochemical modifications due to the small size of the tumour or perhaps to other local conditions, such as poor vascularisation, tissue fibrosis of surgical scar or limited cell proliferation. For these reasons, the monitoring of breast cancer patients by CA 15.3 determination should be combined with other clinical and/or instrumental examinations to avoid the false-negative results which are frequent, especially in patients with local and regional relapse or with contralateral cancer.

1. Saccani Jotti G, Bombardieri E. Circulating tumor markers in breast cancer (review). *Anticancer Res* 1990, **10**, 253–258.
2. Wang DY, Knyba RE, Bulbrook RD, *et al.* Serum carcinoembryonic antigen in the diagnosis and prognosis of women with breast cancer. *Eur J Cancer Clin Oncol* 1984, **20**, 25.
3. Myers RE, Sutherland DJ, Meakin JW, *et al.* Carcinoembryonic antigen in breast cancer. *Cancer* 1978, **42**, 1520–1526.
4. Lokich JJ, Zameheek N, Lowenstein M. Sequential carcinoembryonic antigen levels in therapy of metastatic breast cancer: a predictor and monitor of response and relapse. *Ann Intern Med* 1978, **89**, 902–906.
5. Lamerz R, Leonhardt A, Ehrhart H, *et al.* Serial carcinoembryonic antigen (CEA) determinations in the management of metastatic breast cancer. *Oncodev Biol Med* 1980, **1**, 123–136.
6. Horton J. Follow-up of breast cancer patients. *Cancer* 1984, **53**, 790–797.
7. Schmidt-Rhode P, Schulz KD. Tissue polypeptide antigen (TPA) in patients with breast cancer—longitudinal study. In: Peters H, ed. *Protides Biological Fluids*. Oxford, Pergamon Press, 1984, Vol. 32, 731.
8. Ruibal A, Duran Bellido P, Lafuerra A, *et al.* L'antigene polypeptide tissulaire: marquer du cancer du sein. *Int J Breast Mammary Pathol—Senologia* 1983–1984, **2**, 103–108.
9. Nemoto T, Costantine R, Chu TM. Human tissue polypeptide antigen in breast cancer. *J Natl Cancer Inst* 1979, **63**, 1347–1350.
10. Sell S. Cancer markers of the 1990s. *Clin Lab Med* 1990, **10**, 1–37.
11. Hilkens J, Kroezen V, Bonfrer J. MAM-6 antigen, a new serum marker for breast cancer monitoring. *Cancer Res* 1986, **46**, 2582–2587.
12. Kufe DG, Inghirami M, Abe D, *et al.* Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. *Hybridoma* 1984, **3**, 223–226.
13. Gion M, Mione R, Dittadi R, *et al.* Evaluation of CA 15.3 serum levels in breast cancer patients. *J Nucl Med Allied Sci* 1986, **30**, 29–36.
14. Schmidt-Rhode P, Schulz KD, Sturm G, *et al.* CA 15.3 in breast cancer: first experience with a new monoclonal test system. *Med Sci Res* 1987, **15**, 765–766.
15. Kallioniemi OP, Oksa HJ, Aaran RK, *et al.* Serum CA 15.3 assay in the diagnosis and follow-up of breast cancer. *Br J Cancer* 1988, **58**, 213–215.
16. Hoffman L, Heinzerling D, Schafer E, *et al.* CA 15.3 and CEA monitoring in the evaluation of the course of metastasizing breast cancer. *In-Vitro Diagnosis* 1987, **12**, 76–79.
17. Bieglmayer C, Szepesi T, Neunteufel W. Follow-up of metastatic breast cancer patients with a mucin-like carcinoma-associated antigen: comparison to CA 15.3 and carcinoembryonic antigen. *Cancer Lett* 1988, **42**, 199–206.
18. Tondini C, Hayes DF, Gelman R, *et al.* Comparison of CA 15.3 and carcinoembryonic antigen in monitoring the clinical course of patients with metastatic breast cancer. *Cancer Res* 1988, **48**, 4107–4112.
19. Safi F, Kohler I, Rottinger E, Suhr P, Beger HG. Comparison of CA 15.3 and CEA in diagnosis and monitoring of breast cancer. *Int J Biol Markers* 1989, **4**, 207–214.
20. Schmidt-Rhode P, Schulz KD, Sturm G, Raab-Frick A, Prinz H,