Summary

Episialin/CA15-3: its structure and involvement in breast cancer progression. Hilkens J, Wesseling J, Vos HL, Storm J, Boer M, Valk SW van der and Maas MCE. Ned Tijdschr Klin Chem 1995; 20: 293-298.

Episialin, also designated EMA, PEM, CA 15-3 antigen etc., is a highly polymorphic epithelial sialomucin, which is encoded by the MUC1 gene. Episialin is present at the apical side of glandular epithelial cells. Its mucin-like extracellular domain protrudes high above the cell surface. It is often present at increased levels in breast carcinomas relative to normal breast epithelial cells. In patients with breast and other carcinomas, episialin is released from the cancer cells into the circulation and can be detected by the CA 15-3 assay. The assay is useful for monitoring the course of the disease and for early detection of recurrent breast cancer.

The episialin molecule is heavily O-glycosylated. As a result of differential glycosylation, there are many different glycoforms of episialin. Some glycoforms are preferentially present on carcinoma cells and others are preferentially present on one or a limited number of tissues. Monoclonal antibodies directed against episialin often specifically bind to one or a subset of glycoforms and thus can have a tumor or tissue preference, which explains the differences between the various serum assays that measure episialin.

We have shown that overexpression of episialin prevents cell-cell and cell-extracellular matrix adhesion by shielding the adhesion receptors. As a result, episialin augments the invasive properties of tumor cells *in vitro*, the resistance of the tumor cells to immune destruction, and the formation of experimental lung metastases following intravenous injection of cells that overexpress episialin into nude mice. Preliminary evidence suggests that a certain pattern of episialin expression in primary breast cancers correlates with lymph node involvement.

Key-words: episialin, CA 15-3, mucin, glycoforms, cell adhesion, metastasis.

Ned Tijdschr Klin Chem 1995; 20: 298-300

Application of tumour markers in mammary carcinoma

C.M.G. THOMAS

Of all malignant tumours in women, breast cancer is the first cause of death in the Netherlands. Early diagnosis by means of mammographic screening is one of the few possibilities to increase the prognosis of breast cancer. Despite the limitations of this type of examination, mammographic screening has made it possible to reduce the mortality of this lethal disease by 30% in the group of women aged 50-70 years.

A tumour marker detectable in serum would be helpful to contribute to the early diagnosis, provided the test were sensitive and specific enough to already mirror minimum amounts of one particular malignant tumour at the initial stage of the disease concerned.

Specific serum tumour marker tests would also be valuable at those stages of disease where there is a need for independent information to evaluate the condition of the patient. Tumour marker determinations in serum could also be used as prognostic factors to predict impending relapse following primary treatment, or as a reason for starting adjuvant or palliative therapy. These results could also help to evaluate the efficacy of that therapy. In addition, if serum tumour marker determinations conducted on a regular basis could identify preclinical relapses in bone or organs, this information could be used to initiate new curative therapies. In the case of metastatic breast cancer, serum tumour marker testing during therapy could play a part in assessing treatment effects. It could be an advantage to simply recognize ineffective treatment at an early stage because this would allow a quicker change to possibly more effective therapy. Finally, the simplicity of the assessment itself might comfort the patient.

Department of Obstetrics and Gynaecology and Laboratory of Endocrinology and Reproduction, University Hospital "St-Radboud", Nijmegen

Address correspondence to: Dr C.M.G. Thomas, Department of Obstetrics and Gynaecology, University Hospital Nijmegen "St-Radboud", P.O. Box 9101, 6500 HB Nijmegen, the Netherlands.

Serum tumour markers for mammary carcinoma

The various categories of potentially useful tumour markers for serodiagnostic use in breast cancer comprise Cancer Antigen 15-3 (CA 15-3), Carcinoembryonic Antigen (CEA), and Tissue Polypeptide Antigen (TPA).

CA 15-3 has been identified on the apical side of alveoli and ducts of mammary glands and as a circulating antigen. Distinct epitopes of this high molecular-weight mucine-like glycoprotein of 300-450 kD (also known as polymorphic epithelial sialomucines, episialin) are identified by monoclonal antibodies DF3 (1) and 115D8 (2).

The oncofoetal protein CEA (3) was one of the first tumour markers tested in breast cancer, but has become well-known by its occurrence in carcinomas of the gastrointestinal tract and lung.

Serum elevations of TPA occur in breast, lung, gastrointestinal, urological and gynaecological cancers. Already described in 1957 (4), this oncofoetal antigen is found in the cytoplasm of epithelial cells and has been reported to be related to the cytoplasmic intermediate filaments.

Clinical utilities of serum tumour markers for mammary carcinoma

Among the numerous reports dealing with the specificity and sensitivity of serum tumour marker measurements in breast cancer, we assessed the percentages of increased CEA, CA 15-3 and other mucin-like markers in preoperatively collected blood samples from patients with benign breast diseases serving as controls and in UICC-staged mammary carcinomas (5). At 95% specificity, the cutoff serum concentration of CA 15-3 was 25 kU/l, and 6.4 µg/l in the case of CEA. Above these cutoffs, stage I through stage IV breast cancer showed sensitivities of CA 15-3 levels ranging from 5% to 67%, and from 5% to 38% in the case of CEA. Also, serum specimens from several gynaecologic carcinomas (ovarian, cervix, endometrium) were above these cutoff levels in 36% to 68% of cases with CA15-3, and 14% to 18% with CEA. Conversely, several gastro-intestinal carcinomas (gastric, pancreas, colorectal) were strongly positive with CEA and minimally increased in the case of CA 15-3. These data confirm the generally accepted appreciation that these serum tumour markers, like many other tumour-associated antigenic serum markers, have a rather low degree of sensitivity and are neither tumour-specific nor specific for the particular malignant breast tumour concerned.

The diagnostic accuracy of the serum test for CA 15-3 was calculated with a Receiver Operating Characteristic (ROC) curve for mammary carcinoma (n=250) versus benign breast disease (n=104, figure 1). With increasing cutoff serum concentrations of CA 15-3, the ROC curve provides a dynamic relationship between sensitivity and specificity of the serum marker test. As reported previously (6), recently suggested guidelines for interpretation of the area under the curve (AUC) of ROC curves are: 0.5-0.7, low accuracy; 0.7-0.9, accuracies useful for some purposes; and >0.9, high accuracy. Thus, the observed AUCs of the various stages of mammary carcinoma (range: AUC=0.56, stage II, n=95; AUC=0.89, stage IV, n=18; AUC=0.60 with all stages, n=250) indicate that CA 15-3 appears to be accurate enough to be useful for some purposes in the case of advanced, metastatic breast cancer. An example of this is depicted from figure 2.

The potential utility of serum tumour markers to be prognostic of outcome (recurrence-free time and total survival time) has scarcely been reported. Colomer et al (7) reported median survival of 173 metastatic breast cancer patients. Increased CA 15-3 serum levels >40 kU/l were found at study entry in 130 of these patients (75%) resulting in a significantly shorter median survival time of 10.1 months as compared with the 43 patients having lower CA 15-3 serum concentrations and a median survival time of 18.0 months (p=0.04). No such differences were calculated in the case of CEA (10.2 versus 12.2 months).

As regards the property of an ideal biological marker of tumour activity to predict recurrence, Hayes (8) recently summarized several studies dealing with the prediction of relapse by increasing CA 15-3 after primary therapy of breast cancer. Depending on the study, specificity of the serum marker CA 15-3 (i.e., normal CA15-3 and absence of metastases) is very high (range: 94-98%). Sensitivity of CA 15-3 (i.e., increased CA 15-3 and metastases present) varies from 45% to 77%. Combining specificity and sensitivity allows the determination of positive predictive values (PPV) which are based on increased CA 15-3 serum levels in patients after primary therapy who subsequently develop clinically or radiographically documented metastases during follow-up. PPVs of the reported studies range between 41% and 92%. In general, approximately 40% to 50% of patients who will develop metastases will have a preceding rise in

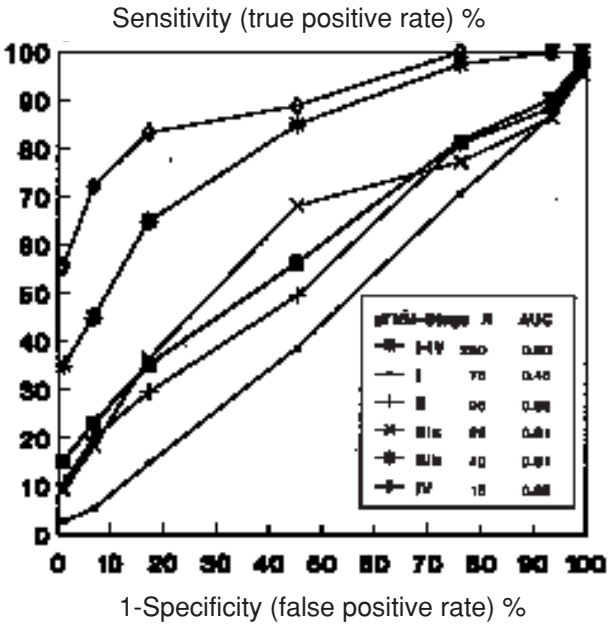

Figure 1. Receiver operating characteristic curve of CA 15-3 for mammary carcinoma (n=250) versus benign breast disease (n=104).

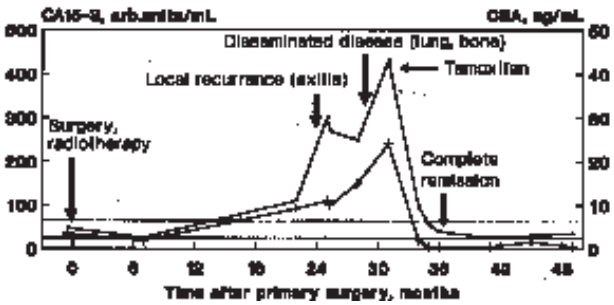

Figure 2. A 75-year-old woman was admitted for a $pT_2N_1M_0$ invasive ductal carcinoma. As she refused a regular mastectomy and a lymphadenectomy, only a tumorectomy and an excision of a large metastatic axillary lymph node were performed. The surgical treatment was followed by radiotherapy. Two years later, she developed an axillary recurrence without distant metastases which was treated with a regional lymphadenectomy. Five months later, lung and bone metastases were detected. Hormonal therapy with tamoxifen resulted in an objective remission and a steep decline of pathological high levels of both tumour markers. After 48 months, a rise in both markers was observed without clinical signs of progression. At 56 months, she died of a cerebrovascular accident. No postmortem was performed.

—•— CA 15-3, kU/l; - - - - - cutoff CA15-3; —+— CEA, µg/l; ····· cutoff CEA.

either CEA or CA 15-3, with lead times (time from first elevation of CA 15-3 until clinically or radiographically documented metastases are identified) ranging from 3 to 18 months (median: 4-7 months).
The sensitivity of serum tumour markers to monitor clinical course in metastatic breast cancer has extensively been studied. Tondini et al (9) reported tumour marker changes correlating with clinical course (progressive, responsive and stable disease) in 75%, 38%, and 75% of patients in the case of CA 15-3, and in 58%, 24%, and 25% in the case of CEA, respectively. Van Dalen et al (10) reported results of TPS of 56%, 40% and 46%, respectively. Overall results indicate that CA 15-3, CEA, and TPS correlate correctly with disease course in respectively 60%, 40% and 47% of cases.
The usefulness of the CA15-3 assay remains an issue of further study in the therapeutic decision-making process. Tondini et al (9) calculated (positive and negative) correlative values (PCV, NCV) of variations in CA 15-3 and CEA levels at the time of assessment of clinical course according to different arbitrary probabilities of progression and regression, respectively. As their calculations are based on retrospective data, these PCVs and NCVs are theoretical and optimistic (over)estimates of the real predictive values (PVs) which can be determined using Bayes' theorem on the basis of the sensitivity and specificity of the test and the prevalence of the predicted outcome. PCV and NCV reflect the clinical relevance of a test and provide an estimate of the probability that a positive (or negative) test truly predicts the presence (or absence) of the outcome in consideration. Their retrospective analysis can be exemplified as follows. If a patient who starts a new treatment for metastatic disease has a 70% probability of responding or remaining stable, the probability of progression will remain 30%. If the serum marker increases by more than 25%, the probability of progressive disease will increase from 30% to 76% in the case of CA 15-3, or to 51% in the case of CEA. Conversely, if the marker does not increase, this observation will raise the probability that the treatment is effective from 70% to 89% with CA 15-3, or to 81% with CEA. Thus, prospective studies to be initiated should evaluate this type of decision-making processes to establish the precise clinical role for serum tumour markers in monitoring clinical course.

Conclusions

From this and a previous review (5) it is concluded that none of the currently available serum tumour markers for breast cancer is either organ-specific, or tumour-specific, or sensitive enough to have potential clinical utilities for determining risk. None of these markers can be used for the primary diagnosis by screening for breast cancer at an early stage. Also, the serum tumour markers cannot be used for the differential diagnosis, prognosis, or prediction of response to therapy. In the prediction of impending relapse, some role can be attributed to serum tumour markers, although not as independent absolute indicators. Established clinical use of these markers can be found in monitoring the disease course. As mentioned above, well designed prospective studies have to be conducted to establish the precise clinical role for serum tumour markers in monitoring the clinical course.

We are grateful to Ing. M.F.G. Segers and Dr Th. Wobbes for their helpful discussion.

Literature

1. Kufe DF, Inghirami M, Abe D, Hayes H, Justi-Wheeler H, Schlom H. Differential reactivity of a novel monoclonal antibody (DF3) with human malignant versus benign breast tumors. Hybridoma 1984; 3: 223-232.
2. Hilkens J, Buijs F, Hilgers J, et al. Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors. Int J Cancer 1984; 34: 197-206.
3. Gold P, Freedman SO. Demonstration of tumor specific antigens in human colonic carcinoma by immunological tolerance and absorption techniques. J Exp Med 1965; 121: 439-462.
4. Björklund B, Björklund V. Antigenicity of pooled human malignant and normal tissues by cito-immunological technique: Presence of an insoluble, heat-labile tumor antigen. Int Arch Allergy 1957; 10: 153-184.
5. Thomas CMG, Wobbes Th, Bonfrer JMG, Peterse JL, Nap M, Beex LVAM. Tumormerkstoffen bij opsporing, diagnostiek en behandeling van patiënten met mammacarcinoom. Ned Tijdschr Geneeskd 1993; 137: 2354-2357.
6. Thomas CMG, Massuger LFAG, Segers MFG, Schijf CPT, Doesburg WH, Wobbes Th. Analytical and clinical performance of improved Abbott IMx CA-125 assay: Comparison with Abbott CA-125 RIA. Clin Chem 1995; 41: 211-216.
7. Colomer R, Ruibal A, Salvador L. Circulating tumor marker levels in advanced breast carcinoma correlate with the extent of metastatic disease. Cancer 1989; 64: 1674-81.
8. Hayes DF, Tumor markers for breast cancer: Current utilities and future prospects. Hematol Oncol Clin N Amer 1994; 8: 485-506.
9. Tondini C, Hayes DF, Gelman R, Henderson IC, Kufe DW. Comparison of CA 15-3 and carcinoembryonic antigen in monitoring the clinical course of patients with metastatic breast cancer. Cancer Res 1988; 48: 4107-12.
10. Dalen A van, Linde DL van der, Heering KJ, Blusse Van Oudalblas A. How can treatment response be measured in breast cancer patients? Anticancer Res 1993; 13: 1901-1904.

Summary

Application of tumour markers in mammary carcinoma. Thomas CMG. Ned Tijdschr Klin Chem 1995; 20: 298-300.
The determination of tumour marker concentrations in serum of patients with mammary carcinoma has limited clinical utility. Mainly due to the lack of sufficient specificity and sensitivity, serum tumour markers of the present generation are not useful to determine risk and, as a screening method they do not contribute to the early diagnosis of breast cancer. Also, circulating tumour markers have no place in the differential diagnosis, prognosis, or prediction of response to therapy. At present, some current clinical utility can be attributed to their use in the prediction of impending relapse, while established clinical utility exists in monitoring disease course.
In conclusion, well-designed prospective randomized studies must be conducted to assess the role of serum tumour markers as independent, absolute indicators a) in predicting relapse of patients after primary therapy of breast cancer, and b) in contributing to the management of newly diagnosed metastatic disease so as to ultimately improve survival time.
Key-words: tumormarkers, mammary carcinoma, prediction, relapse, follow-up.