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Clinical evaluation of the simultaneous determination of CA 15-3, CA 125 and sHER2 in breast cancer

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Abstract

Objective We investigated serum levels of CA 15-3, sHER2 and CA 125, and their usefulness in the detection of metastatic disease in breast cancer patients.

Methods The levels of CA 15-3, sHER2 and CA 125 tumour markers were determined in 60 patients, 40 with localized and 20 with metastatic breast carcinoma. The control group consisted of 10 healthy women.

Results We found that, at the time of diagnosis, serum levels of all three tumour markers were elevated in patients with distant metastases, but of minute importance in the detection of any breast cancer. When the data for the individual markers were combined the overall sensitivity of metastases detection with all three markers improved. In this regard, 90% of patients with distant metastases had an increase in serum level of at least one of tested tumour markers. Similar results were obtained using receiver operating characteristic curve (ROC). Moreover, using ROC we defined cut-off values for metastasis detection for each of the tested markers.

Conclusion Our findings indicate that measurement of CA 15-3 serum values in conjunction with sHER2 and CA 15-3 can increase sensitivity in metastasis detection.

Keywords: *Breast cancer, CA 15-3, sHER2, CA 125, serum levels*

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Introduction

One of the major aims in cancer research has been to develop biochemical tests for aiding screening and early diagnosis, assessing prognosis, predicting response to therapy and monitoring patients. All of these tasks, as well as treatment decisions for individual breast cancer patients were frequently (virtually obligatorily) based on traditional pathological parameters or other tissue-based assays. However, all of these methods require tumour tissue and thus invasive procedures. Consequently, the possibility of using the circulating markers as a way to predict patients' outcomes is more desirable.

To date, no tumour marker has demonstrated significant benefits in randomized controlled trials of screening and early diagnosis in the general population.

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Nevertheless, tumour markers can play a crucial role in diagnosing and monitoring of metastatic disease or assessing response to therapy in selected groups of patients.

As for the many cancer markers in routine use, no consensus exists regarding the reference cut-off for markers extensively used in monitoring of breast cancer. The difficulties in establishing the reference tumour marker values originate from appliance of different detection methodologies and different assays in the frame of one methodology. Thus, numerous studies have been performed in order of method and assay standardization (Henry & Hayes 2006). In one of the few studies describing the performance of tumour markers in external quality schemes, Pilo et al. (1995) reported the average between-laboratory and between-kit coefficient of variation for CA 15-3, CA 125 and CA 19-9. On the basis of these results we concluded that the reliability of current CA 15-3 and CA 125 assays was satisfactory. In other studies, the most reliable values of tumour markers were taken for CA 15-3, CA 125 and sHER-2, to be 20–40 U ml^{-1}, <35 IU ml^{-1} and 6.5–10.2 ng ml^{-1}, respectively, which are similar to the reference values used in our study (Sugano et al. 2000, Canizares et al. 2001, Kong et al. 2006, Tampellini et al. 2006).

It seems that the origin of these differences for CA 15-3, sHER-2 and CA 125 is not due to ethnicity or variation across different population groups (Weiss et al. 1995, Doroudchi et al. 2005, Nichols et al. 2005). On the contrary, the structure of these markers is well defined and it is known that genetic variation does not exist in human populations for any of them (Sommer et al. 1992, Duffy 1999).

The assay for CA 15-3 is currently the most widely used tumour marker in routine monitoring of breast cancer patients. Many years of investigation confirmed its central role in monitoring patients with breast carcinoma; thus, it became the 'gold standard' for other tumour markers. The CA 15-3 molecule is a mucin, a product of the *MUC1* gene (Gendler & Spicer 1995). While the physiological function of the MUC1 protein is unknown, some evidence supports the important role that MUC1 may play in the adhesion of epithelial cells; MUC1 appears to reduce both cell–cell and cell–extracelular matrix interactions (Wesseling et al. 1996). However, there is a little value in it for the detection of early disease (Safi et al. 1991). CA 15-3 concentration at initial presentation can provide useful prognostic information in patients with apparently localized disease (Shering et al. 1998). High values of CA 15-3 are indicative of the possibility of metastases (Geraghty et al. 1992). Moreover, serial determination of CA 15-3 has the potential to detect both, preclinical recurrences and to monitor the treatment of metastatic breast cancer (Anonymous 1996).

CA 125 is a glycoprotein expressed in normal tissues originally derived from coelomic epithelia such as peritoneum or pleura. However, it is now believed that derivates of all three layers of embryonic development may harbour the antigen (Hardardottir et al. 1990). Elevated CA 125 values are most often associated with epithelial ovarian cancer (Meyer & Rustin 2000), although CA 125 levels can also be elevated in other tumours and non-malignant disease (Buamah 2000). Although CA 125 production has been demonstrated in the healthy breast, the significance of CA 125 elevation in breast cancer is uncertain. It has been reported that higher serum levels of CA 125 are associated with increasing bulk of disease and worse prognosis mainly related to metastasis development (Norum et al. 2001).

HER2 (from Human Epidermal growth factor Receptor) is a component of a four-member family of closely related growth factor receptors, including HER1, HER2,

HER3 and HER4 (Maguire & Greene 1989). This molecule is regularly expressed on normal cells and overexpressed on malignant cells. The overexpression of this molecule has been observed in 20–30% of breast carcinoma cases and associated with poor clinical outcome (Slamon et al. 1989). The full-length HER2 protein undergoes proteolytic cleavage by metalloproteases (Codony-Servat et al. 1999), and its extracellular domain is shed into the blood as a circulating antigen (Pupa et al. 1993). The currently published literature suggests that the circulating HER2 antigen concentration lacks sensitivity for the detection of early disease (Carney et al. 2003, Kong et al. 2006). However, it has been shown that increasing concentration correlates with extensive tumour burden, which, in turn, correlates with progressive disease and worse prognosis (Jensen et al. 2003, Pichon et al. 2004).

In the present study, we investigated serum CA 15-3, CA 125 and sHER2 concentration and evaluated the usefulness of their combined determination in metastasis detection in breast carcinoma patients.

Patients and methods

Patients

Tumour markers were studied prospectively in 60 consecutive patients admitted to the Surgical Clinic at the Kragujevac University Hospital, 40 with localized and 20 with metastatic breast cancer. Patients with localized tumours were included in the study before surgical treatment, whereas the group with metastatic carcinoma was enrolled in the investigation after the final decision about the type of treatment, but before any treatment. In this way, any possible therapy effects (operative, chemo or hormonal), which could influence the results of the study were avoided. Patients with positive biohumoral markers of inflammation, as well as patients with clinical or biochemical evidence of co-existing chronic diseases such as endocrine and autoimmune diseases were excluded from the study. All cases with localized disease were histologically confirmed with breast cancer TNM (tumor-node-metastasis) staging of the American Joint Committee on Cancer (AJCC) and the International Union Against Cancer (UICC) (Sobin & Wittekind 1997). The control group consisted of 10 healthy women chosen from the general population. A control group was matched with the cancer group on the basis of age (within 5 years), sex and menopausal status.

Methods

Blood samples were obtained from 70 different Serbian women, 60 of whom were hospitalized at the Kragujevac University Hospital. The same serum samples were used to quantitate the serum levels of CA 15-3, CA 125 and sHER2. Tested markers were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Breast Cancer Antigen CA 15-3 Enzyme Immunoassay Test Kit and Antigen CA 125 Enzyme Immunoassay Test Kit, DRG International, Inc., USA; Human $sp185^{HER\text{-}2}$ ELISA, Bender MedSystems, Vienna, Austria). Cut-off values were suggested by the manufacturer: 36 U ml^{-1} for CA 15-3, 36 IU ml^{-1} for CA 125 and 6.4 ng ml^{-1} for sHER2.

Statistical analysis

The data were evaluated using the SPSS commercial program package (version 10.0 SPSS Inc., Chicago, IL, USA). The normal data distribution was evaluated by the Kolmogorov–Smirnov test. The non-parametric data of two or more groups were compared with the Mann–Whitney's *U*-test and the Kruskal–Wallis test. Receiver operating characteristic (ROC) curves were constructed for the individual tumour markers and the differences in the area under the curve (AUC) values were determined.

Results

Table I shows tumour marker results subdivided according to the disease extent. At the time of diagnosis, the serum levels of CA 15-3 and CA 125 were significantly higher in patients with metastatic breast cancer than either in the patients with localized disease (regardless of nodal involvement) or in the control group (CA 15-3: $p<0.001$; CA 125: $p=0.01$). No significant difference could be found in serum levels of sHER2 ($p>0.05$). Although CA 15-3 serum values showed some gradual increase depending on the disease stage, there were no statistically significant differences between lymph node-negative and lymph node-positive breast cancer ($p=0.1$) (Table I).

The proportions of elevated marker levels are shown in Table II. Ten patients (25.0%) with localized and 12 patients (60%) with metastatic breast cancer had CA 15-3 values above the recommended cut-off value of 36 U ml^{-1}. A similar proportion of patients, nine in the localized group (22.5%) and 11 in the metastatic group (55%), showed elevation of the sHER2 levels higher than the suggested cut-off of 6.4 ng ml^{-1}. Of the 40 patients with localized breast cancer, only two (5%) had increased serum CA 125 levels, whereas nine of 20 (45.0%) of metastatic patients had CA 125 values greater than cut-off at 36 IU ml^{-1}. Distributions of individual data for all three tumour markers are shown in Figure 1. All together, at the time of diagnosis, 15 patients (37.5%) with localized and 18 (90%) with metastatic tumours had elevated levels of either one, or several, tumour markers. Table III shows tumour marker sensitivity and specificity determined according to cut-off values suggested by manufacturer (36 U ml^{-1} for CA 15-3, 6.4 ng ml^{-1} for sHER2 and 36 IU ml^{-1} for CA 125). The sensitivity of CA 15-3, sHER2 and CA 125 was very low in the detection of localized tumour. However, combined use of all markers showed that

Table I. Stratifying CA 15-3, CA 125 and sHER2 serum levels to different stages of disease. CA 15-3, CA 125 and sHER2 were determined in the serum samples from 40 patients with localized (26 lymph node negative, 14 lymph node positive) and 20 patients with metastatic breast cancer. Data are presented as median value (range) for each group.

	n	CA 15-3 (U ml^{-1})	CA 125 (IU ml^{-1})	sHER2 (ng ml^{-1})
Control	10	22.5 (15.4–31.1)	8.3 (3.4–24.2)	5.9 (4.8–8.0)
Localized nodus	26	21.1 (9.1–94.0)	8.2 (1.3–79.5)	5.8 (3.3–16.6)
Localized nodus	14	29.7 (6.0–69.0)	8.9 (4.6–73.9)	4.8 (3.7–33.0)
Metastatic	20	70.9 (16.3–240.0)*	31.1 (3.0–400.0)*	12.1 (3.8–28.9)

*Significant difference between metastatic and other groups (Kruskal-Wallis $p<0.05$).

Table II. The proportions of serum CA 15-3, sHER2 and CA 125 levels elevated above the recommended cut-offs (36 U ml^{-1}, 6.4 ng ml^{-1} and 36 IU ml^{-1}, respectively) in 60 breast cancer patients. Values are shown as elevated/total (%).

	Control	Localized	Metastasis
CA 15-3	0/10 (0)	10/40 (25.0)	12/20 (60.0)
sHER2	1/10 (10)	9/40 (22.5)	11/20 (55.0)
CA 125	0/10 (0)	2/40 (5.0)	9/20 (45.0)
Any	1/10 (10)	15/40 (37.5)	18/20 (90.0)

elevation of any tumour marker increased sensitivity to 0.40 (Table III). In the group with metastatic disease all markers showed a higher sensitivity than in localized group, especially CA 15-3 whose sensitivity was the highest (0.60 with a specificity of 1.00). When combination of all markers was used, elevation of any tumour marker was indicative of metastases in 90% of patients with increased sensitivity of 0.90 and specificity of 0.90 (Table III). In addition, we showed that combined use of all markers increased sensitivity in differentiating between local and metastatic disease, as well as, between any and no disease (Table III).

Similar results were obtained when the data were compared with the receiver operating characteristic curve (ROC) (Figure 2). Of the individual markers, CA 15-3 had the largest AUC (AUC 0.85; $p < 0.0001$) for metastasis detection. Moreover, using ROC we defined cut-off values for metastasis detection. The cut-off point for the CA 15-3 tumour marker was 30 U ml^{-1}, with a sensitivity and specificity of 0.88 and 0.71, respectively; for CA 125 the cut-off was 18 U ml^{-1} with a sensitivity of 0.65 and a specificity of 0.89, respectively; for sHER2 the cut-off was 17 ng ml^{-1} with a sensitivity and specificity of 0.41 and 0.96, respectively. When cut-offs obtained using ROC were used for metastasis detection overall sensitivity with all three markers further improved to 0.95.

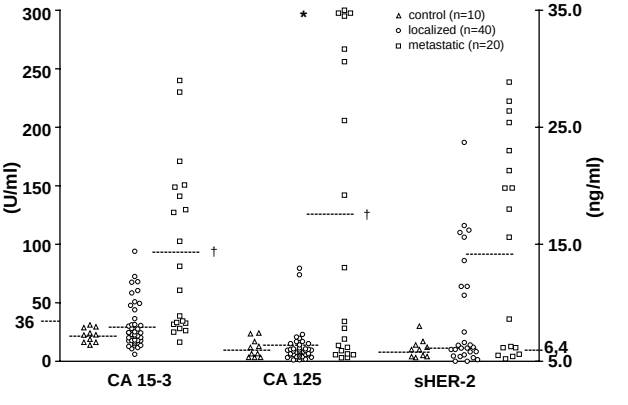

Figure 1. Concentration of CA 15-3, CA 125 and sHER2 serum levels in patients with localized and metastatic breast cancer. Data are presented as individual values. Each dot represents the data from a given individual and the horizontal bars represent the overall mean values for all individuals evaluated. Primary x-axis at 36 U ml^{-1} corresponds to recommended cut-off levels (according to manufacturer's manual) for CA 15-3 and CA 125. Secondary x-axis at 6.4 ng ml^{-1} corresponds to sHER2 cut-off level recommended by the manufacturer. †Statistical analysis using Kruskal–Wallis ANOVA showed a significant difference between groups (CA 15-3, $p = 0.0002$; CA 125, $p = 0.0116$). *Values above 300 IU ml^{-1}.

Table III. Tumour marker sensitivity and specificity.

	CA 15-3	CA 125	HER2	Any
Healthy vs localized				
Sen	0.25	0.05	0.23	0.40
Spec	1.00	1.00	0.90	0.90
PPV	1.00	1.00	0.90	0.94
NPV	0.25	0.21	0.23	0.27
Healthy vs metastatic				
Sen	0.60	0.45	0.55	0.90
Spec	1.00	1.00	0.90	0.90
PPV	1.00	1.00	0.92	0.95
NPV	0.56	0.48	0.50	0.82
Localized vs metastatic				
Sen	0.60	0.45	0.55	0.90
Spec	0.75	0.95	0.78	0.60
PPV	0.54	0.82	0.55	0.53
NPV	0.79	0.77	0.78	0.92
Any disease vs no disease				
Sen	0.37	0.18	0.33	0.57
Spec	1.00	1.00	0.90	0.90
PPV	1.00	1.00	0.95	0.97
NPV	0.21	0.17	0.18	0.26

Sen, sensitivity; Spec, specificity; PPV, postitive predictive value; NPV, negative predictive value.

Discussion

Serum tumour markers are circulating tumour-associated indicators of a tumour's biological and 'structural' behaviour. Measurement of serum tumour markers appears to reflect the total tumour burden in the body and represents the summation of numerous sub-clinical metastases. As such they are not suitable for screening and early diagnosis of primary breast cancer since the tumour burden is small in these circumstances. However, they are valuable as adjuncts for the medical follow-up care of breast cancer patients, where alterations of their serum level may anticipate tumour behaviour and provide valuable prognostic and predictive information.

In order to establish a normal, adult reference interval for analyte using a particular assay, one must calculate the mean plus or minus two standard deviations (95% confidence interval) of the assay results from a population set of adults known to be in good health. Subsequently, any patient's result, which falls within this interval, is considered to be 'normal', or healthy. However, results which fall outside (above or below) the limits of this interval are considered to be either abnormally elevated or decreased, respectively. A low result for tumour markers would not be of clinical significance. Therefore, one establishes the cut-off between normal (presumed negative for disease) and abnormal (presumed positive for disease) results by using the mean plus two standard deviations. The cut-off values for tumour markers used in our study were taken from the manufacturer's description of the assay.

CA 15-3 and, to a lesser extent carcinoembryonic antigen (CEA), are the most commonly used serum tumour markers in breast cancer. However, as a marker for breast cancer, CEA is a non-specific and it lacks sensitivity for the detection of early disease. On the contrary, CA 15-3 represents the 'gold standard' for the circulating

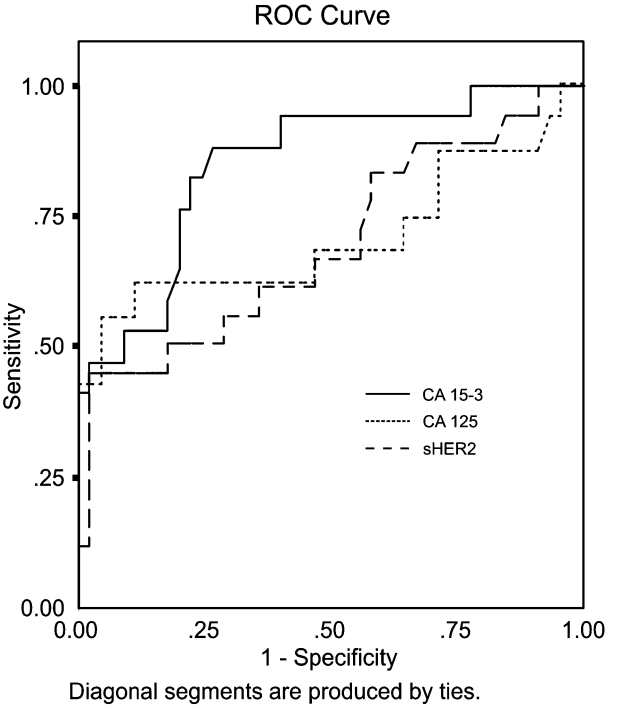

Figure 2. Comparison of serum CA 15-3, CA 125 and sHER2 with ROC curve analysis. The serum samples from 20 patients with metastatic breast cancer were used. ROC curves for each tumour marker in serum: solid line = CA 15-3; dotted line = CA 125; discontinuous line = sHER2. AUC (95% confidence interval): CA 15-3 = 0.85 (cut-off 30 U ml^{-1}, $p = 0.000$); sHER2 = 0.69 (cut-off 17 ng ml^{-1}, $p = 0.017$); CA 125 = 0.73 (cut-off 18 U ml^{-1}, $p = 0.006$). The difference in the AUC value of CA 15-3 compared with the AUC of the other markers was highly significant ($p = 0.000$).

markers in breast cancer, Moreover, new biochemical tests for this disease should be judged against this marker.

In several studies, CA 15-3 levels were compared between different population groups. One study found that concentrations of CA 15-3 were similar in both healthy men and women (Hayes et al. 1986). Further, Bon et al. (1997) found that CA 15-3 level was significantly higher in healthy postmenopausal women than in healthy premenopausal women. Patients with benign liver disease had higher CA 15-3 level than those with benign tumour of the breast (Hayes et al. 1986). There was no evidence about ethnic differences in concentrations of this marker (Weiss et al. 1995, Doroudchi et al. 2005, Nichols et al. 2005). CA 15-3 is not an organ-specific marker and therefore it is of a little value in identifying unknown primary cancer (Hayes et al. 1986).

Although CA 15-3 is of a little value in the early diagnosis of breast cancer, its pretreatment level is a recognized prognostic factor. An initially high level of CA 15-3 is more often observed in patients with advanced cancer than in those with localized cancer, where the concentration is correlated with the disease stage and therefore high levels indicate a worse outcome (Duffy et al. 1996, Shering et al. 1998). CA 15-3 levels that are initially high and remain high, despite applied treatment, indicate a failure to respond to the treatment and a very poor prognosis. Summarizing the data

from 11 studies, the authors concluded that measurement of CA 15-3 levels during treatment follow-up in patients with metastatic disease is useful in the evaluation of the treatment response (Anonymous 1996). The sensitivity of CA 15-3 in the diagnosis of local recurrence is poor, but it is clearly useful in the early diagnosis of breast cancer metastases (Anonymous 1996).

Similarly to current literature data we found that CA 15-3 is of little importance in the diagnosis of primary breast cancer, but is very useful in the diagnosis of metastatic disease. Although its serum values showed a gradual increase in patients with lymph node-positive breast cancer, its efficacy in the diagnosis of local metastasis is poor.

Regarding soluble HER2, numerous reports have shown that the prevalence of an increased sHER2 concentration is highly variable in breast cancer. A review of 24 references used to evaluate sHER2 concentration in primary breast cancer showed that only 18.5% (out of a total of 1923 patients) had circulating sHER2 above the control cut-off values described for each publication. In contrast, a review of 45 references and 4622 patients with metastatic breast cancer showed that 43% of patients had sHER2 levels above the cut-off for the control group in each study (Molina et al. 1996).

In several retrospective studies that included a significant number of patients, serum HER2 levels have demonstrated prognostic significance with respect to disease-free and overall survival. Furthermore, longitudinal changes in serum HER2 concentrations paralleled the clinical course of a patient's disease (Molina et al. 1996, Schippinger et al. 2004). Moreover, there are strong data showing an increased serum HER2 concentration as a predictor of poor response to therapy using chemotherapeutic (Harris et al. 2001) and hormonal treatment regimes (Lipton et al. 2005).

Our findings are, to a great extent, related to data in the literature. We found that 22.5% of primary breast cancer patients have soluble HER2 above the cut-off value. In contrast, 55.0% of metastatic breast cancer patients showed soluble HER2 above the cut-off recommended by the manufacturer.

The CA 125 tumour marker is commonly seen in ovarian carcinoma (Meyer & Rustin 2000). Elevation of this marker has also been observed in several advanced carcinomas of different origins (Buamah 2000, Norum et al. 2001). Unlike other established breast cancer markers such as CEA, CA 15-3 or sHER2, data related to CA 125, as a marker in breast cancer, is limited. In line with that, the cause and significance of its elevation in some metastatic breast cancer have been poorly defined. In reviewing reports evaluating CA 125 in primary breast cancer, Leonard et al. found very low detection rates, from 1% to 27%, and only one of eight studies reported CA 125 levels according to disease stage (Omar et al. 1989). In metastatic breast cancer, higher, but very variable, rates of detection are documented (18–84%). Similarly, we showed that the serum level of CA 125 was above recommended cut-off in a high proportion (45.0%) of metastatic patients, whereas only 5% of patients with localized disease had increased CA 125 values. The heterogeneity of serum CA 125 levels may be explained by the location of metastases, since high CA 125 is more common in visceral disease than in bone or soft tissue involvement (Berruti et al. 1994). In one study, authors found that 91% of cases with elevated CA 125 were due to pleural-based disease. However, only about 30% of them had direct involvement of the pleura, while other patients had lung or bone involvement adjacent to the pleura (Norum et al. 2001). In our study, we demonstrated several cases of elevated CA 125 in the

settings of advanced local disease, which could be related to the underlying inflammatory breast cancer or sub-clinical visceral metastases.

Several studies have shown that measurement of CA 15-3 serum values in conjunction with other tumour markers can increase sensitivity and specificity in metastasis detection. In their report, Colomer et al. (2000) suggested that a panel of tumour tests such as CEA, CA 15-3 and sHER2 could be used postoperatively with increased sensitivity of detecting early recurrence. The increase in sensitivity observed by Molina et al. (1996) was also supported by studies by Watanabe et al. (1994) and Schwartz et al. (2000); however, Eskelinen et al. (1997) reported only limited value in measuring serum CEA, CA 15-3 and sHER2 in conjunction with other cancer tests.

Our results showed that overall sensitivity of tumour detection with all three markers combined improved from 40% for localized to 90% for metastatic tumours. In addition, statistical analysis of our data showed that combined measurement of CA 15-3 and sHER2 was nearly as valuable as a combination of all three markers in discriminating not only between local and metastatic disease, but also between any and no disease (data not shown). Given that the sensitivity of each test alone is fairly low, these would be inadequate to use for screening in the general population or among persons already diagnosed with breast cancer. Therefore, as our study has shown that combined use of tested tumour markers was better than single marker detection, we recommended measurement of CA 15-3 serum values in conjunction with other tumour markers.

Finally, we have also demonstrated an interesting observation regarding the potential use of the combined measurement of serum tumour markers as a tool for detecting the development of metastatic disease. When cut-offs obtained using ROC were used for metastasis detection overall sensitivity with all three markers further improved to 0.95.

Although the significance of detecting tumour markers in the monitoring of primary disease after surgery, or the diagnosis and monitoring of metastatic disease is unequivocal, early detection of metastatic disease has not been always successful, and does not benefit the patient in terms of overall survival or time until the appearance of clinical signs. The value of early detection of metastasis will increase with the introduction of a variety of targeted therapies that can attack the tumour when the tumour burden is smaller, and in this way may enhance the probability of successful treatment.

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