Evaluation of Tumour Markers as Differential Diagnostic Tool in Patients with Suspicion of Liver Metastases from Breast Cancer

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Abstract. *Aim: The liver is the site of breast cancer metastasis in 50% of patients with advanced disease. Tumour markers have been demonstrated as being useful in follow-up of patients with breast cancer, in early detection of recurrence of breast cancer after radical surgical treatments, and in assessing oncologic therapy effect, but no study has been carried out on their usefullness in distinguishing benign liver lesions from breast cancer metastases. The aim of this study was therefore to evaluate the importance of tumour markers carcinoembryonic antigen (CEA), carbohydrate antigen CA19-9 (CA19-9), thymidine kinase (TK), tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and cytokeratin 19 fragment (CYFRA 21-1) in differential diagnosis between benign liver lesions and liver metastases of breast cancer. Patients and Methods: The study includes 3 groups: 22 patients with liver metastases of breast cancer; 39 patients with benign liver lesions (hemangioma, focal nodular hyperplasia, liver cyst, hepatocellular adenoma); and 21 patients without any liver disease or lesion that were operated on for benign extrahepatic diseases (groin hernia, varices of lower limbs) as a control group. The serum levels of tumour markers were assessed by means of immunoanalytical methods. Results: Preoperative serum levels of CYFRA 21-1, TPA, TPS and CEA were significantly higher in patients with liver metastases of breast cancer in contrast to healthy controls and patients with benign liver lesions (p-value<0.05). Serum levels of CA19-9 and TK were higher in patients with malignancy in comparison with benign liver disease and healthy controls but these differences were not statistically significant. Conclusion: Tumour markers CEA, CYFRA 21-1, TPA and TPS can be recommended as a good tool for differential diagnosis between liver metastases of breast cancer and benign liver lesions.*

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Breast cancer is characterized by systemic dissemination. The liver is the site of breast cancer metastasis in 50% of patients with advanced disease (1, 32). Downstaging or disease control of metastasizing breast carcinoma is the starting point for resection of macroscopic residual liver. Modern liver surgery is indicated for patients with liver metastases of breast cancer in cases of good response to chemotherapy, exclusion of extrahepatic metastases, in whom a complete and safe surgical procedure is feasible (2-6, 33). Such patients could profit from liver surgery.

The incidence of liver lesions during follow-up of patients after breast cancer treatment is high. The origin of such lesions may be benign (liver cyst, adenoma, focal nodular hyperplasia, hemangioma, *etc*.) or malignant, above all as liver metastases of breast cancer. The indication for liver surgery (resection or destruction for example by radiofrequency ablation) is sometimes complicated by the difficulty in making the decision between benign and malignant lesions (7). The former are approached mainly by conservative treatment or long-term follow-up of the lesion. On the contrary, liver metastases of breast cancer are indicative of surgical treatment. Radiodiagnostic methods only aid in the decision between malignant and benign lesions in the minority of patients.

Tumour markers were demonstrated as being useful in follow-up of patients with breast cancer, in early detection of recurrence of breast cancer after radical surgery and in assessing oncologic therapy effect (8). No study on tumour markers has been performed to distinguish benign liver lesions from breast cancer metastases.

The aim of this study was to evaluate the importance of the tumour markers carcinoembryonic antigen (CEA), carbohydrate antigen CA19-9 (CA 19-9), proliferative marker thymidine kinase (TK), and the cytokeratins tissue polypeptide antigen (TPA), tissue polypeptide-specific antigen (TPS) and cytokeratin 19 fragment (CYFRA 21-1) in differential diagnosis between benign liver lesions and liver metastases of breast cancer.

Patients and Methods

Patient groups. The study includes three groups of patients: 22 patients with liver metastases of breast cancer; 39 patients with benign liver lesions (hemangioma, focal nodular hyperplasia, liver cyst, hepatocellular adenoma); and 21 patients without any liver disease or lesion but with benign extrahepatic diseases (groin hernia, varices of lower limbs), operated on at the Department of Surgery, Teaching Hospital and Medical School in Pilsen, Charles University Prague between 2002 and 2009. Only women were included in the cohort and the study was performed retrospectively. This study also had the approval of the local Ethical Committee.

Methods. Blood for the tumour marker assessment was always collected from the cubital vein in the morning hours before surgery, with the serum obtained from centrifugation stored at a temperature of –20˚C until processing. The serum levels of routine tumour markers (CEA, CA 19-9) and proliferative and cytokeratine tumour markers (TK, CYFRA 21-1, TPA and TPS) were assessed by using the following immunoanalytical methods: CEA- LIA, Beckman Coulter (μg/l); CA 19-9- LIA, Beckman Coulter (kIU/l); CYFRA 21-1 - TRACE, Brahms (μg/l); TPA- IRMA, DiaSorin (IU/l); TPS-IRMA, IDL Biotech AB (IU/l); TK- REA, Immunotech (IU/l).

Statistical analysis. Statistical analysis was performed by the software packages CRAN 2.4.0 and STATISTICA 98 edition. The purpose was to describe the dependency of the serum level of tumour markers upon the type of liver lesion (benign *vs*. liver metastases of breast cancer). Wilcoxon two-sample test and Spearman rank correlation coefficient were used for statistical evaluation.

Results

Table I provides the basic descriptive statistics (median, 25%-75% percentile) of preoperative serum levels of conventional tumour markers (CEA and CA 19-9), proliferative tumour marker TK and cytokeratins (CYFRA 21-1, TPA and TPS) in the three patient groups. Differences in preoperative serum levels of all studied cytokeratins (CYFRA 21-1, TPA, TPS) between patients with liver metastases of breast cancer and the other two groups were statistically significant (p-value<0.05) (Table II). On the contrary, differences in serum levels of TK, as a marker of proliferation, between patients with liver metastases of breast cancer and the other two groups were not statistically significant (p-value>0.05) (Table II). The preoperative serum level of conventional tumour marker CA19-9 was higher in patients with liver metastases of breast cancer in contrast to patients with benign liver lesions and the healthy controls, but these differences were also not statistically significant (p-value>0.05). The second conventional tumour marker, CEA, was proven a good marker for malignancy compared to groups of healthy controls and patients with benign liver lesions (p-value<0.05) (Table II).

Table III presents correlations between particular tumour markers within the frame of individual groups of patients. In the group of patients with liver metastases, the best correlations were achieved between cytokeratins TPA and TPS (r=0.84; p-value<0.0001) and between cytokeratin CYFRA 21-1 and proliferative marker TK (r=0.61; p-value<0.0051). Correlations between other tumour markers in this group of patients were lower or statistically insignificant. In the group of patients with benign liver lesions, the best correlation was achieved between cytokeratins TPA and CYFRA 21-1 (r=0.46; p-value<0.0099). In the group of healthy controls, no statistically significant correlations between tumour markers were found.

Discussion

A large number of tumour markers have been validated for use in routine clinical practice. These include MUC-1 (*e.g.* CA 15-3), CEA, oncoproteins, carbohydrate antigens, cytokeratins and proliferative markers. Of these, CEA and CA 15-3 are most commonly used (9-12). In primary diagnostics of breast cancer, tumour marker sensitivity in patients with early-stage disease is low (up to 35%) (13). It is still not clear if these tumour markers are independent prognostic factors (14). Serial CEA and CA 15-3 serum determination is useful in supervising the course of cancer, especially during treatment. Monitoring these tumour markers for the early detection of recurrence following the radical treatment of the tumour is the second most useful application (15-17, 29). Tumour marker sensitivity in patients with advanced disease is significantly higher than for these with locoregional disease (18, 19, 28, 30). Using a combination of several markers can increase the sensitivity up to 95%, especially in patients with distant metastases (10, 18, 31). The main clinical application of tumour markers in advanced disease is in therapy monitoring. However, whether this monitoring leads to enhanced survival or better quality of life remains to be determined (9, 18, 34).

In the present pilot study, the preoperative serum level of the studied cytokeratins CYFRA 21-1, TPA and TPS and the conventional tumour marker CEA were statistical significantly higher (p-value<0.05) in patients with liver metastases of breast cancer in contrast to healthy controls and patients with benign liver lesions. On the other hand, serum levels of CA19-9 and TK were higher in malignancy in comparison with benign controls and healthy patients, but

Table I. *Descriptive statistics for patients with liver metastases from breast cancer (n=22), with benign liver lesions (n=39) and healthy controls (n=21).*

	Breast cancer (n=22)		Benign lesions (n=39)		Controls (n=21)	
Tumour marker	Median	25%-75% Percentile	Median	25%-75% Percentile	Median	25%-75% Percentile
CEA	2.0	1.30-8.20	1.2	0.60-2.30	0.8	0.60-1.60
CA 19-9	14.3	6.30-50.40	1.1	5.40-22.90	7.7	5.20-11.30
TK	8.0	5.50-10.20	6.1	3.60-8.30	4.9	2.60-7.80
CYFRA 21-1	1.4	0.70-3.00	0.6	0.40-0.90	0.4	0.20-1.00
TPA	59.0	33.00-226.00	16.0	10.00-37.00	28.5	18.00-46.00
TPS	66.0	32.00-153.00	30.0	30.00-51.00	30.0	12.00-59.00

Table II. *Stastistical differences (p-values) between individual patient groups.*

Wilcoxon test	*p*-Values					
	CEA	CA 19-9	TK	CYFRA 21-1	TPA	TPS
*G1 vs. G2	0.022	**ns.	ns.	0.010	0.001	0.006
G1 vs. G3	0.005	ns.	0.050	0.040	0.020	0.020
G2 vs. G3	0.020	ns.	ns.	0.010	0.001	0.006

*G1=group 1 patients with breast cancer liver metastases (n=22); G2=group 2 patients with benign liver leasions (n=39); G3= group 3 healthy controls (n=21). **ns, Not significant.

Table III. *Mutual correlations between particular tumour markers within the frame of individual groups of patients*

Markers		
	Spearman rank correlation coefficient	*p*-value
Group I. Liver metastases (n=22)		
TPA *vs*. TPS	0.83	0.0001
TPA *vs*. CYFRA 21-1	0.46	0.0432
CEA *vs*. TK	0.46	0.0430
TK *vs*. CYFRA 21-1	0.61	0.0051
Group II. Benign liver lesions (n=39)		
TPA *vs*. TPS	0.41	0.0234
TPA *vs*. CYFRA 21-1	0.46	0.0099
Group III. Healthy controls (n=21)		
All markers	* ns.	ns.

*ns, Not significant.

these differences were not statistically significant. Such use of tumour markers was not found in any previous study. Only one published paper was found that considered the alteration of genes in liver metastases of breast cancer but not tumour markers (25).

This pilot study has no intention to present definitive conclusions about tumour markers in relation to differential diagnosis between liver metastases of breast cancer and benign liver leasions due to the small size of studied cohorts of patients. We only want to demonstrate the meaning of these tumour markers in borderline diagnosis and show the directions of future research in this field. The conclusions of large studies of tumour markers in patients with breast cancer were presented in recommendations of many oncological societies (20-24), but the problem of tumour markers in patients with liver metastases of breast cancer is still neglected, in spite of one half of patients with extensive breast cancer being affected by malignant liver lesions (2). We did not compare liver metastases of breast cancer with those of colorectal cancer because we tried to

fulfill the demands of clinical praxis on the use of tumour markers in differential diagnosis in this study when radiodiagnostic methods are not conclusive regarding the malignant or benign properties of liver lesions in patients with anamnesis of breast cancer. The clinical situation for recognition of colorectal liver metastases from liver metastases of breast cancer is rare and is possible only in double malignancy. This clinical situation is only theoretical because in both cases liver surgery is indicated and so this question does not arise practically. Recognition of the clinical situation for which assessment of tumour markers is crucial for the validity of results could enable patients to profit from tumour marker measurement. The interpretation of tumour markers depends on the art of processing, which should be chosen with regard to "the clinical question asked" (23, 25-27).

The classical recommendations for clinical usage of tumour markers as EGTM 2003, 2007 and ASCO 2006 refer only to the basic malignancies and give no advice as to the meaning of tumour markers for malignancies with lower incidence or in extreme clinical situations (22-24), despite the fact that tumour markers could play a key role in the prognosis of these malignancies. Knowledge of the properties of liver leasions in patients with a history of breast cancer could indicate surgical therapy for these patients with higher certainty and could spare some of them from surgical operation in cases of benign lesion.

We have demonstrated that tumour markers CEA, CYFRA21-1, TPA and TPS may be an excellent tool for differential diagnosis of liver metastases of breast cancer from benign liver lesions.

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