Growth Factors and Breast Tumors, Comparison of Selected Growth Factors with Traditional Tumor Markers

RADEK KUCERA[1], MONIKA CERNA[3], ANDREA NARSANSKA[3], SARKA SVOBODOVA[2,4], MARKETA STRAKOVA[2], JINDRA VRZALOVA[2], RADKA FUCHSOVA[2], INKA TRESKOVA[3], TOMAS KYDLICEK[3], VLADISLAV TRESKA[3], LADISLAV PECEN[5], ONDREJ TOPOLCAN[2] and PETR PAZDIORA[6]

[1]*Department of Nuclear Medicine, Hospital Klatovy, Czech Republic;*
[2]*Laboratory of Immunoanalysis, Charles University in Prague, Faculty of Medicine, Pilsen, Czech Republic;*
[3]*Department of Surgery, Faculty Hospital in Pilsen, Pilsen, Czech Republic;*
[4]*Third Internal Medicine Department and First Medical Faculty, Charles University, Prague, Czech Republic;*
[5]*Institute of Computer Science, Academy of Sciences of the Czech Republic, Prague, Czech Republic;*
[6]*Institute of Epidemiology, Charles University in Prague, Faculty of Medicine, Pilsen, Czech Republic*

Abstract. *Background: The first aim of this project was to study new possibilities for distinguishing benign from malignant tumors using growth factors and to compare them with the traditional tumor markers Carcinoembryonic antigen (CEA) and Cancer antigen 15-3 (CA15-3) for breast tumors. The second aim was to make a comparison of CEA, CA 15-3, Insulin-like growth factor I (IGF1), Insulin-like growth factor-binding protein 3 (IGFBP3), Hepatocyte growth factor (HGF) and Epidermal growth factor (EGF) for individual stages of cancer. Patients and Methods: Our group of patients consisted of 110 females, 89 with breast cancer and 21 with benign breast tumors (fibroadenomas). Serum levels of CEA and CA 15-3 were measured using a DxI instrument. Serum levels of IGF1 and IGFBP3 were measured using IRMA radioisotope assay kits. HGF and EGF were measured using an xMAP Luminex multiplex panel. Serum samples were collected prior to surgery and those of the two groups of patients were compared (malign vs. benign). Patients with diabetes mellitus were excluded from this project. Results and Discussion: Comparing the individual parameters of serum levels between the two groups of patients (malignant vs. benign) only HGF was found to show a statistically significant difference. The mean of HGF in patients with malignant diseases prior to surgery was 3370 pg/ml compared to 1799 pg/ml in benign tumors with p=0.0016. We found significantly lower serum values of IGF1 at stage III in comparison to stages I and II: mean values: at stage I=181 ng/ml, at stage II=182 ng/ml and at stage III=70 ng/ml; stage III vs. stage II, p=0.0167. Conclusion: Tumor markers are currently used for therapy monitoring in cancer patients as one of the indicators of successful therapy. Our findings correspond to existing literature. IGF1 and its binding protein IGFBP3 cannot be used to distinguish between malignant and benign tumor. HGF is considered to be a marker of progression and of the aggressiveness of breast cancer; our data fully corresponds to this. Based on our data, this marker could potentially be used as an additional tool for the differentiation between benign and malignant tumor.*

Correspondence to: Professor Ondrej Topolcan, MD, Ph.D., Immunoanalytic Laboratory, Department of Nuclear Medicine, Faculty Hospital in Pilsen, Dr. E. Benese 13, CZ-305 99 Pilsen, Czech Republic. E-mail: topolcan@fnplzen.cz

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Breast cancer is one of the most frequent cancer types among females worldwide. Based on WHO statistical data it affects up to 20% of females. In several epidemiological studies performed in Europe, is a breast cancer with colorectal cancer in first place in terms of type of cancer in the population (1-3).

The first aim of this project was to study new possibilities for distinguishing benign from malignant tumors using growth factors and to compare them with the traditional tumor markers Carcinoembryonic antigen (CEA) and Cancer antigen 15-3 (CA15-3) for breast tumors. There are currently no reliable noninvasive methods for distinguishing between benign and malign tumors. The only method is through biopsy and subsequent histological investigation.

The second aim was the correlation of tumor markers and growth factors with individual stages of cancer.

Patients and Methods

Group of patients. The patients consisted of 110 females with a histological confirmation of breast tumors. They were all indicated for surgery. We divided the patients into two groups. The first

consisted of 89 females with breast cancer and the second consisted of 21 females with benign tumors (fibroadenomas). The detailed characteristics of these groups are shown in Table I. The following serum parameters were measured in both groups: tumor markers CEA and CA 15-3; and growth factors Insulin-like growth factor I (IGF1), Hepatocyte growth factor (HGF), Epidermal growth factor (EGF) and Insulin-like growth factor-binding protein 3 (IGFBP3).

The group with malignant tumors was divided into three subgroups according to cancer stage. There were 82 females in total at stages I and II and 7 at stage III.

The serum samples were analyzed at the Laboratory of Immunoanalysis, Faculty of Medicine in Pilsen, (Czech Republic) from the year 2008 to 2010. Patients with diabetes mellitus were excluded from this project due to the fact that this condition may affect the serum levels of some growth factors, particularly of IGF1 (4).

Serum samples. Serum samples were collected prior to surgery. Samples of venous blood were collected using the VACUETTE blood collection system (Greiner Bio-one Company, Austria). Blood was centrifuged for 10 minutes at 1700× g. Serum samples were immediately frozen to –80˚C. Samples were thawed once just prior to analyses.

Methods used. CEA and CA 15-3 serum levels were measured using the DxI system (Beckman Coulter, USA). Serum levels of IGF1 were measured using an IRMA radioisotope IGF1 assay kit (IMMUNOTECH, France). Serum levels of IGFBP3 were measured using an IRMA radioisotope IGFBP3 assay kit (DiaSource, Belgium). HGF and EGF were measured using an xMAP Luminex multiplex panel (MERCK, USA). Serum samples were collected prior to surgery and those of the two subgroups of patients were compared (malignant *vs*. benign).

Statistical methods. SAS 9.2 (Statistical Analysis Software release 9.2, SAS Institute Inc., Cary, North Carolina, USA) was used for all statistical analysis. The summary of statistical findings for age and serum levels of each of the analytes was presented. The Wilcoxon test is used to compare distributions of values between benign and malignant tumors. The Kruskal-Wallis test was used in the comparison of individual tumor stages.

Results

With regards to our first aim, the differences in serum levels between benign and malignant tumors were recorded. All females had been indicated for an operation. The benign diagnosis was fibroadenoma, the malignant diagnosis was tumors at stages I, II and III. The age distribution of both groups of patients is shown in Table I. The age difference between the two groups of females was found to be significant ($p<0.0001$). The group of patients with benign breast disease was significantly younger than the group of patients with breast cancer. This age difference was considered when evaluating the data because it is well known that serum levels of IGF1 decrease with age.

Results are summarized in Table II, showing results for both groups, including statistical evaluation.

Table I. *Age characteristics of the patient groups*.

Diagnosis	N	Age			
		Mean	Median	Min.	Max.
Breast cancer	89	61.8	65	28	84
Benign breast tumor	21	42.2	42	17	71

Secondly, we divided the results found into three subgroups of malignant tumors according to the stage of cancer. The serum levels of each analyte and statistical evaluation are shown in Table III.

Discussion

In evaluating data we have focused on the differences between the two subgroups of patients regarding the individual serum levels because distinguishing between malignant and benign tumors using the traditional tumor markers has always been difficult. The presence of most benign lesions can slightly increase the traditional serum tumor marker levels. But there are some other clinical conditions that may also increase serum levels, *e.g*. liver cirrhosis, acute and chronic hepatitis, chronic renal insufficiency, chronic bronchitis, pneumonia, smoking (5). Therefore there have always been limitations to the clinical use of tumor markers for distinguishing between malignant and benign tumors at early stages (6). Our data fully correlates with published data.

IGF1 serum levels were statistically at their lowest at cancer stage III. We did not confirm any use for IGF1 in distinguishing between malignant and benign tumors. IGF1 is produced by the liver and so its elevated serum level is not a direct result of tumor development (7, 8). It is more likely one of the causes of tumor development. Paracrine production of IGF1 may also contribute to tumor development (9-11). But this source of production does not significantly affect the total serum level. It was, however, very important to adjust the serum levels of IGF1 to the age of patients, as it is well known that with increasing age, the serum level of IGF1 decreases. Without adjustment for age, we would have obtained data with statistically significant differences ($p=0.0110$) between the two groups, but this would have been an incorrect evaluation because our groups of patients were significantly different in terms of age distribution. After adjustments due to age distribution, we found no statistically significant difference ($p=0.6292$).

HGF may be considered as a marker of progression of metastatic process in breast cancer (12, 13). Our results of HGF fully correspond to this theory. HGF serum results are much higher in patients with malignant tumors (approximately double) than in those with benign tumors, with a statistical

Table II. *Comparison of individual parameters in breast cancer versus benign breast tumors.*

Parameter (units)	Diagnosis	N	Mean	Median	Minimum	Maximum	*p*-Value Wilcoxon test
CEA (µg/l)	Cancer	89	2.32	1.50	0.50	33.7	0.5753
	Benign	21	1.75	1.20	0.50	5.10	
CA15-3 (kIU/l)	Cancer	89	14.1	11.0	10.0	78.0	0.2878
	Benign	21	11.7	11.0	10.0	17.0	
IGF1 (ng/ml)	Cancer	81	175	166	24.0	384	0.6292*
	Benign	21	248	237	92.0	521	
IGFBP3 (ng/ml)	Cancer	89	3618	3683	2203	6637	0.4223
	Benign	21	3898	3739	2182	7121	
HGF (pg/ml)	Cancer	85	3370	2676	262	21838	0.0016
	Benign	21	1799	1597	248	6861	
EGF (pg/ml)	Cancer	86	327	279	33.2	2115	0.1393
	Benign	21	366	342	105	776	

*p-Value of age-adjusted IGF1 data (p=0.0110 of IGF1 data without age-adjustment).

Table III. *Serum levels of the parameters in each cancer stage.*

	Stage	N	Mean	Median	Minimum	Maximum	*p*-Value Kruskal-Wallis test
CEA (µg/l)	I	48	1.71	1.35	0.5	7.4	-
	II	34	3.19	1.55	0.5	33.7	0.1731*
	III	7	3.70	3.70	2.80	4.60	0.1004**
CA 15-3 (kIU/l)	I	48	13.3	11.5	10.0	26.0	-
	II	34	12.8	11.0	10.0	24.0	0.5611*
	III	7	42.0	30.0	18.0	78.0	0.0063**
IGF1 (ng/ml)	I	48	181	168	24	369	-
	II	34	183	168	32	384	0.9962*
	III	7	70	51	48	110	0.0167**
IGFBP3 (ng/ml)	I	48	3564	3645	2532	4864	-
	II	34	3737	3825	2203	6637	0.1512*
	III	7	3286	3142	2616	4100	0.3442**
HGF (pg/ml)	I	47	2796	2748	323	7893	-
	II	32	3623	2399	262	15855	0.7951*
	III	7	12585	12585	3331	21838	0.1243**
EGF (pg/ml)	I	47	294	237	33	1924	-
	II	32	334	343	88	738	0.0334*
	III	7	855	266	183	2115	0.9062**

* Stage I *vs*. II, ** stage II *vs*. III.

significance of p=0.0016. Regarding cancer monitoring, its usefulness is not clear. The mean serum value increased from stages I to III, but not with statistical significance. In our opinion, it is necessary to study more samples to confirm the effectiveness of HGF for cancer monitoring.

EGF growth factor is one of the most important growth factors regulating cellular growth, proliferation and differentiation. There are already drugs currently used in oncological practice to block EGF receptor. Breast cancer is one of the indications for the use of these drugs. These drugs represent the latest generation of such drugs and their use has been very promising. The development of these drugs was made possible because the whole signal pathway of EGF receptor has been successfully mapped (14, 15). Despite the benefit of EGF for cancer therapy, its role in the cancer diagnostic process has not yet been made very clear. Our data does not support any potential use either for distinguishing between malignant and benign tumors or for cancer monitoring. The maximum levels were higher in cancer groups, but the means and medians of EGF were higher in patients with benign tumors. Regarding cancer monitoring, the usefulness of EGF is comparable to that of HGF.

Conclusion

Tumor markers are currently used for therapy effect monitoring. Our findings fully correspond to this practice.

IGF1 and IGFBP3 can be used neither for distinguishing between malign and benign disease or as a marker in the monitoring of the development of cancer diseases.

HGF growth factor is considered to be a marker of disease progression and aggressiveness in breast cancer. Our findings fully support this approach. This marker could potentially be used as a marker for distinguishing between benign and malignant tumors. Regarding cancer monitoring, it is necessary to study more samples in order to confirm the usefulness of HGF for this purpose.

EGF growth factor is not useful for distinguishing between benign and malignant tumors either. Regarding cancer monitoring, as with HGF, it is necessary to study more samples.

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