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Diagnostic power of VEGF, MMP-9 and TIMP-1 in patients with breast cancer. A multivariate statistical analysis with ROC curve

Monika Zajkowska[a,*], Ewa Gacuta[b], Sylwia Kozłowska[c], Emilia Lubowicka[d], Edyta K. Głażewska[d], Lech Chrostek[a], Maciej Szmitkowski[a], Przemysław Pawłowski[e], Monika Zbucka-Krętowska[f], Sławomir Ławicki[a]

[a] Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok, Poland
[b] Department of Perinatology, Medical University of Bialystok, Bialystok, Poland
[c] Department of Laboratory Diagnostics, Public Health Care Hospital, Bialystok, Poland
[d] Department of Esthetic Medicine, Medical University of Bialystok, Bialystok, Poland
[e] Department of Medical Pathomorphology, Medical University of Bialystok, Bialystok, Poland
[f] Department of Department of Re-productivness and Gynecological Endocrinology, Medical University of Bialystok, Bialystok, Poland

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ABSTRACT

Purpose: Vascular endothelial growth factor is an important factor in promoting angiogenesis in malignant processes, matrix metalloproteinase-9 in the degradation of extracellular matrix, which enhances metastasis, and tissue inhibitor of metalloproteinase-1 is its inhibitor. The aim of this study was to investigate the diagnostic power of these parameters in comparison to CA15-3 in breast cancer patients and in relation to the control group.

Materials/methods: The study included 120 breast cancer patients, 60 patients with benign breast tumors and 60 healthy women. Plasma levels of tested parameters were determined by enzyme-linked immunosorbent assay, CA15-3 by chemiluminescent microparticle immuno assay.

Results: Tissue inhibitor of metalloproteinase-1 showed the highest value of sensitivity in breast cancer group (86.25%) and, more importantly, highest value in breast cancer stage I (85%). Vascular endothelial growth factor also showed high sensitivity (stage I and II–75%, III–85%, IV–70% and 76.25% in total breast cancer group) and the highest specificity (85%) from all tested parameters. It was also the only parameter which had statistically significant area under curve in all stages. In the total breast cancer group all tested parameters showed statistically significant area under curve, but the maximum range was obtained for combination: 'vascular endothelial growth factor + CA15-3'. Vascular endothelial growth factor seems to be the best candidate for diagnosing breast cancer stage I and for differentiating between breast cancer and non-carcinoma cases.

Conclusions: The combined analysis of tested parameters and CA15-3 resulted in an increase in sensitivity and area under curve values, which provides hope for developing new panel of biomarkers that may be used in diagnosing breast cancer in the future.

1. Introduction

Breast cancer (BC) is the most common malignancy in women and the second leading cause of their death in the world [1]. The most effective way to combat cancer is through prevention and early detection. Therefore, finding markers that would detect malignant cell transformation as early as possible is vital [2,3].

At present, biomarkers used in the detection of BC include CA 15-3, CEA and CYFRA 21.1 [4,5]. Although their prognostic relevance is supported by a number of studies, these markers show low sensitivity and specificity at less-advanced stages of cancer. Hence, a search for new markers that would present higher diagnostic performance continues [6].

New candidates for tumor markers may include cytokines, for example vascular endothelial growth factor (VEGF), macrophage-colony stimulating factor (M-CSF), metalloproteinases (MMPs) and their inhibitors (TIMPs).

In cancer patients (including BC patients) there is uncontrolled tumor angiogenesis, loss of stability of the extracellular matrix, local and remote metastasis. During angiogenesis which accompanies

* Corresponding author at: Department of Biochemical Diagnostics, Medical University of Bialystok, Waszyngtona 15A, 15-269 Bialystok, Poland.
E-mail address: monika@zajkowska.com (M. Zajkowska).

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carcinogenesis, intensive production of cytokines by the tumor cells is observed. Among the many factors that stimulate this process, the key role is played by the VEGF [7]. High expression of VEGF mRNA has been found in BC cells [8,9].

Matrix metalloproteinase-9 (MMP-9, gelatinase B) is mainly produced by neutrophils, (e.g. tumor infiltrating neutrophils) which is probably the most important aspect to understand the role of MMP-9 in BC biology, but also other cells - keratinocytes, monocytes and leukocytes [10–12]. It is involved in the degradation of the extracellular matrix (ECM), which enhances metastasis and can also stimulate angiogenesis [13,14]. In tumors, MMP-9 destroys collagen (type IV) in the vascular basal membrane in the vicinity of tumor cells which invade the surrounding tissues and contributes to metastasis [15,16].

Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a naturally occurring glycoprotein found in several types of cancer, including breast tumors [17–19]. High levels of TIMP-1 in primary tumor tissue are associated with decreased objective response to chemotherapy [20] and endocrine therapy in metastatic BC patients. TIMP-1 stimulates cancer invasion by inhibiting apoptosis, promoting tumor cell growth, and regulating angiogenesis [21,22].

Our study, commenced in 2001, focused on identifying new biochemical parameters which could become biochemical markers in different types of tumors, for example breast, ovarian, cervical and endometrial cancer. Nowadays, imaging diagnostics remain the most commonly used diagnostic method in BC. Results are quite difficult to interpret and, moreover, frequently inaccurate. Hence, new methods are sought that would contribute to the development of cancer diagnostics. Genetic tests become very popular, however, they are very time-consuming and expensive, which is why we decided to focus on the biochemical methods that are fast and relatively cheap to perform.

The aim of the present study was to investigate the diagnostic power (ROC curve analysis) of the selected cytokine (VEGF), matrix metalloproteinase (MMP-9), its inhibitor (TIMP-1), and a comparative marker CA 15-3 in detecting BC. In this study, healthy volunteers and women with benign breast lesions constituted one control group, which provided a more accurate reflection of the current female population. The data obtained in this study may prove the usefulness of the analyzed parameters (separately and together) in detecting BC as a new diagnostic panel.

2. Materials and methods

2.1. Patients

Table 1 shows the studied groups. The study included 120 BCE patients diagnosed by the oncology group. The patients were treated in the Department of Oncology, Medical University of Bialystok (Poland). Tumor classification and staging were conducted in accordance with the International Union Against Cancer Tumor-Node-Metastasis (UICC-TNM) classification. BC histopathology was established in all cases by tissue biopsy of the mammary tumor or following surgery from tumor tissues (all patients with *adenocarcinoma ductale)*. The pre-treatment staging procedures included: physical and blood examinations, mammography, mammary ultrasound scanning, breast core biopsies and chest X-rays.

In addition, radio isotopic bone scans, the examination of bone marrow aspirates, and brain and chest CT scans were performed when necessary. None of the patients had received chemo- or radiotherapy prior to blood sample collection.

The control group included 60 patients with benign breast tumors (*adenoma, papilloma intraductale, fibroadenoma, mastopatia*) and 60 healthy, untreated women who underwent mammary gland examination performed by a gynecologist prior to blood sample collection. In addition, mammary ultrasound scanning was performed in all cases. Benign breast tumor histopathology was established in all cases by tissue biopsy of the mammary tumor or after surgery. Table 2 shows the actual protein levels in all studied groups.

Table 1
Characteristics of breast cancer patients and control groups: benign breast tumor and healthy women.

Study group			Number of patients
TESTED GROUP	**Breast cancer patients**	*adenocarcinoma ductale*	120
	Median age (range)		54 (34–72)
	Tumor stage	I	29
		II	30
		III	31
		IV	30
	Menopausal status:		
	- premenopausal		51
	- postmenopausal		69
CONTROL GROUP	**Benign breast tumor patients**		60
		adenoma	21
		papilloma intraductale	18
		fibroadenoma	11
		mastopatia	10
	Median age (range)		48 (26–71)
	Menopausal status:		
	- premenopausal		29
	- postmenopausal		31
	Healthy women		60
	Median age (range)		44 (23–73)
	Menopausal status:		
	- premenopausal		26
	- postmenopausal		34

The exclusion criteria for the patients qualified for the control group, were: active infections and symptoms of an infection (both bacterial and viral), other comorbidities which can affect cytokine concentrations (respiratory diseases, digestive tract diseases) or systemic diseases such as lupus or rheumatoid arthritis, or collagenosis.

2.2. Biochemical analyses

Venous blood samples were collected from each patient into a heparin sodium tube, centrifuged 1000 rpm for 15 min to obtain plasma samples and stored at −85 °C until assayed. The tested parameters were measured with the enzyme-linked immunosorbent assay (ELISA) (VEGF, MMP-9, and TIMP-1 - Quantikine Human Immunoassay, R&D Systems Inc., Minneapolis, MN, USA) and chemiluminescent microparticle immunoassay (CMIA) (CA 15-3 - Abbott, Chicago, IL, USA) according to the manufacturer's protocols. In ELISA, duplicate samples were assessed for each patient.

The intra-assay coefficient of variation (CV%) [23] of CA 15-3 is reported to be 2.2% at a mean concentration of 27.0 U/mL, SD = 0.6. VEGF is reported to be 4.5% at a mean concentration of 235 pg/mL, SD = 10.6. MMP-9 is reported to be 1.9% at a mean concentration of 2.04 ng/mL, SD = 0.039, TIMP-1 to be 3.9% at a mean concentration of 1.27 ng/mL, SD = 0.05.

The inter-assay coefficient of variation (CV%) [23] of CA 15-3 is reported to be 2.6% at a mean concentration of 27.0 U/ml, SD = 0.7. VEGF to be 7.0% at a mean concentration of250 pg/mL, SD = 17.4. MMP-9 to be 7.8% at a mean concentration of 2.35 ng/mL, SD = 0.184, TIMP-1 to be 3.9% at a mean concentration of 1.28 ng/mL, SD = 0.05.

2.3. Statistical analysis

Statistical analysis was performed using STATISTICA 12.0. We defined the receiver-operating characteristics (ROC) curve for all the tested parameters and CA 15-3. The construction of the ROC curves was performed using the GraphRoc program for Windows and the areas under ROC curve (AUC) were calculated to evaluate the diagnostic