Advances in Medical Sciences 64 (2019) 1–8

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

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

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ARTICLE INFO

Keywords:
Tumor markers
Area under curve
New biomarkers panel
ROC

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

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https://doi.org/10.1016/j.advms.2018.07.002
Received 1 August 2017; Accepted 31 July 2018
Available online 15 September 2018

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

Table 2
Plasma levels of tested parameters and CA 15-3 in patients with breast cancer and in control group.

Groups tested		MMP-9 (ng/ml)	TIMP-1 (ng/ml)	VEGF (pg/ml)	CA 15-3 (U/ml)
Breast cancer	I stage	267.64	98.76	108.76*	20.00
Median		46.12–737.16	44.32–335.09	12.56–702.18	7.12–34.44
Range	II stage	274.65	128.72**	115.19*	23.38*
		93.55–830.86	33.00–347.25	44.09–753.12	7.77–32.76
	III stage	361.20*	198.22*,**	219.00*,**	34.12*
		50.91–844.45	87.00–441.09	37.14–1054.65	17.64–168.12
	IV stage	273.04*	161.88**	161.23*	73.64*,**
		52.81–800.09	4.27–438.54	9.02–469.98	18.50–253.00
	Total group	278.45*	147.22**	130.34*,**	25.23*
		46.08–840.01	5.32–440.87	9.12-1051.12	7.08–251.04
Control group	Benign breast tumor	209.17	74.68	82.23	25.43
Median		36.10–840.87	6.54–157.98	9.02–972.45	12.01–49.00
Range	Healthy women	181.34	122.65	36.88	15.12
		65.14–422.57	33.98–333.12	4.43–180.89	6.07-26.22

* Statistically significant when compared with healthy women ($p < 0.05$).
** Statistically significant when compared with benign breast tumor ($p < 0.05$).

accuracy and to compare AUC for all the tested parameters separately and in combination with the commonly used tumor marker (CA 15-3). Statistically significant differences were defined as comparisons resulting in $p < 0.05$. The *cut off* values were calculated using Youden's index (as a criterion for selecting the optimum *cut-off* point) [24–26] and they were: VEGF – 70.25 pg/mL; MMP-9 –169.10 ng/mL; TIMP-1 – 84.54 ng/mL; CA 15-3 – 18.30 U/mL.

2.4. Ethics approval

The study was approved by the local Ethics Committee of the Medical University of Bialystok (R-I-002/239/2014).

All the patients gave their informed consent for study participation.

3. Results

Table 3 shows the sensitivity (SE) and specificity (SP) of the investigated parameters and CA 15-3. We indicated that the SE of the tested parameters in the total cancer group was the highest for TIMP-1 (86.25%) and was higher than for CA 15-3 (83.75%), MMP-9 (82.5%), and VEGF (76.25%). Among the parameters, the highest SE in BC stage I was also observed for TIMP-1 (85%), in stage II – MMP-9 (90%), in stage III – TIMP-1 (100%) and in stage IV – CA 15-3 (100%). The combined analysis of the tested parameters and CA 15-3 resulted in an increase in SE in all cases. The highest SE was found for the combination of TIMP-1 and CA 15-3 (98.75%) in the total BC group.

The diagnostic SP of the tested parameters was the highest for VEGF (85%) and was higher than for CA 15-3 (75%), MMP-9 (55%), and TIMP-1 (37.5%). Lower values were also obtained for the combination of the tested parameters with CA 15-3.

The relationship between the diagnostic SE and SP is illustrated by the ROC curve. The AUC indicates the clinical usefulness of a tumor marker and its diagnostic power. All data regarding AUCs in different stages of BC (I–IV) are included in Table 4. Graphical representations of the ROC curves for all the tested parameters and their combinations with the commonly used tumor marker (CA 15-3) both in the whole BC group as well as the distribution in all stages (I–IV) of BC advancement are shown in Figs. 1–5.

We noticed that the VEGF area under the ROC curve (0.729) in the total BC group was higher than the area of CA 15-3 (0.698), MMP-9 (0.651), and TIMP-1 (0.692). Moreover, the AUCs for the tested parameters, similarly as for CA 15-3, were statistically significantly larger in comparison to AUC = 0.5 (borderline of the diagnostic usefulness of the test) ($p < 0.001$ in all cases). The combined analysis of AUC for MMP-9 or TIMP-1 and CA 15-3 in the total group resulted in a marginal increase in the AUCs in both cases (0.738; 0.719, respectively), but a maximum range in the total BC group was obtained for the combination of VEGF and CA 15-3 (0.753) ($p < 0.001$ in all cases).

Table 3
Diagnostic sensitivity (SE) and specificity (SP) of tested parameters and in combined analysis with CA 15-3 in breast cancer patients.

Tested parameters	Diagnostic criteria (%)	Breast cancer				
		Total group	stage I	stage II	stage III	stage IV
VEGF	SE	76.25*	75*	75*	85*	70*
	SP	85	85	85	85	85
MMP-9	SE	82.5*	80	90*	80*	80
	SP	55	55	55	55	55
TIMP-1	SE	86.25*	85	75*	100*	85*
	SP	37.5	37.5	37.5	37.5	37.5
CA 15-3	SE	83.75*	65	75	95*	100*
	SP	75	75	75	75	75
VEGF +	SE	96*	90	95*	100*	100*
CA 15-3	SP	65	65	65	65	65
MMP-9 + CA 15-3	SE	96.25*	90	95*	100*	100*
	SP	45	45	45	45	45
TIMP-1 + CA 15-3	SE	98.75*	95	100	100*	100*
	SP	32.5	32.5	32.5	32.5	32.5

SE – sensitivity.
SP – specificity.
* $p < 0.05$.

Table 4
Diagnostic criteria of ROC curve for tested parameters and CA 15-3.

	Tested parameters	ROC criteria in breast cancer			
		AUC	SE	95% C.I. (AUC)	*p* (AUC = 0.5) (AUC = 0.5)
Total group	VEGF	0.729	0.0400	0.650–0.807	< **0.001**
	MMP-9	0.6513	0.0435	0.566–0.736	< **0.001**
	TIMP-1	0.6916	0.0416	0.610–0.773	< **0.001**
	CA 15-3	0.698	0.0410	0.618–0.779	< **0.001**
	VEGF + CA 15-3	0.753	0.0377	0.679–0.826	< **0.001**
	MMP-9 + CA 15-3	0.7375	0.0392	0.661–0.814	< **0.001**
	TIMP-1 + CA 15-3	0.7194	0.0400	0.641–0.798	< **0.001**
stage I	VEGF	0.691	0.0616	0.570–0.811	**0.002**
	MMP-9	0.602	0.0738	0.457–0.746	0.169
	TIMP-1	0.566	0.0597	0.449–0.683	0.269
	CA 15-3	0.494	0.0647	0.367–0.621	1.073
	VEGF + CA 15-3	0.595	0.0680	0.462–0.729	0.161
	MMP-9 + CA 15-3	0.544	0.0762	0.394–0.693	0.566
	TIMP-1 + CA 15-3	0.460	0.0699	0.323–0.597	1.430
stage II	VEGF	0.716	0.0524	0.613–0.818	< **0.001**
	MMP-9	0.678	0.0606	0.559–0.796	**0.003**
	TIMP-1	0.640	0.0691	0.504–0.775	**0.043**
	CA 15-3	0.586	0.0665	0.456–0.716	0.196
	VEGF + CA 15-3	0.629	0.0637	0.504–0.754	**0.043**
	MMP-9 + CA 15-3	0.663	0.0633	0.539–0.787	**0.010**
	TIMP-1 + CA 15-3	0.594	0.0628	0.471–0.717	0.133
stage III	VEGF	0.818	0.0483	0.724–0.913	< **0.001**
	MMP-9	0.692	0.0709	0.553–0.831	**0.007**
	TIMP-1	0.854	0.0414	0.773–0.935	< **0.001**
	CA 15-3	0.819	0.0490	0.723–0.915	< **0.001**
	VEGF + CA 15-3	0.878	0.0376	0.804–0.952	< **0.001**
	MMP-9 + CA 15-3	0.831	0.0479	0.737–0.925	< **0.001**
	TIMP-1 + CA 15-3	0.899	0.0414	0.818–0.980	< **0.001**
stage IV	VEGF	0.690	0.0717	0.549–0.831	**0.008**
	MMP-9	0.634	0.0713	0.494–0.774	0.060
	TIMP-1	0.707	0.0683	0.573–0.841	**0.002**
	CA 15-3	0.893	0.0450	0.805–0.982	< **0.001**
	VEGF + CA 15-3	0.908	0.0390	0.832–0.985	< **0.001**
	MMP-9 + CA 15-3	0.912	0.0436	0.826–0.997	< **0.001**
	TIMP-1 + CA 15-3	0.924	0.0400	0.845–1.002	< 0.001

p - statistically significantly larger AUC's compared to AUC = 0.5.
AUC – Area Under Curve.
SE – Standard Error.
95% C.I. – 95% Confidence Interval.

The maximum diagnostic power was achieved for VEGF in BC stage I and II (0.691; 0.716, respectively) alone and for the combination of VEGF with antigen CA 15-3 in these stages of cancer (0.595; 0.629, respectively). In BC stage II, the AUC of all the tested parameters (with the exception of CA 15-3) was statistically significantly larger in comparison to AUC = 0.5.

In BC stage III, the highest AUC of all the tested parameters was observed in TIMP-1 (0.854) and the maximum range in BC stage III was obtained for the combination of TIMP-1 and CA 15-3 (0.899).

In BC stage IV, the maximum diagnostic power was obtained for CA 15-3 (0.893) and for the combination of TIMP-1 with CA 15-3 (0.924). In stages III and IV all the tested parameters were statistically significantly larger in comparison to AUC = 0.5.

4. Discussion

The formation of blood vessels (angiogenesis) is crucial for tumor progression and nutrition. VEGF has a direct effect on vascular endothelial cell proliferation, migration and is a potent stimulatory factor of angiogenesis. Statistically significant plasma over expression of VEGF was detected in patients suffering from many types of tumors, including gastric [27] or colorectal cancer [28] as well as gynecological malignancies such as ovarian [29] or cervical cancer [30]. High plasma levels of VEGF were also found in BC [31,32].

Matrix metalloproteinases and their tissue inhibitors play a crucial role in ECM and basement membrane homeostasis [33]. MMPs are a family of human, zinc-dependent peptidases. MMP-9 plays an important role in cancer growth and metastasis thanks to its powerful, unique ability to destroy collagen type IV and other ECM components [34]. Furthermore, the over expression of this factor is correlated with poor outcome [35]. Moreover, scientists who examined this factor have demonstrated that MMP-9 is a good candidate to be a marker of breast [36], endometrial [37], ovarian [38] or lung [39] cancers.

TIMPs are natural inhibitors of MMPs. In fact, only 4 proteins are included in this group [40]. TIMP-1 was detected in various body fluids and tissues [41]. This factor stimulates cancer invasion by inhibiting apoptosis, promoting tumor cells growth and regulating angiogenesis [42,43]. The relationship between high levels of TIMP-1 and poor BC patients prognosis has also been previously confirmed [44]. Due to many reports regarding the usefulness of tumor markers not only in BC, it is very important that the diagnosis is not limited to diagnostic imaging [29–32].

SE measures the proportion of correctly identified positives. In this study, TIMP-1 displayed the highest SE in the total group of BC patients. The same parameter had the highest SE in BC stage I which is very important since it may be useful to confirm the occurrence of BC in its earliest stage while contributing to an increase in detecting cancer, whose course is often asymptomatic. An early diagnosis is associated with a greater chance of survival as well as a better quality of life and improved life expectancy for BC patients.

In the study by Niewiarowska et al. [45], the SE for serum TIMP-1 was marginally lower in the total group of patients (67.4%), which might be associated with the fact that a different type of cancer was investigated. Similar data were also obtained in the study by Kozłowski et al. [46] in esophageal cancer where the SE for TIMP-1 was very similar to the value obtained in our study (80%), but in both cases, the study groups were smaller and there were no additional control groups consisting of benign tumor patients. In our previous studies on BC [31,47] we obtained very low SE for TIMP-1, both in stage I and in the total group of BC, but statistical analyses in both cases were performed only in reference to healthy women. Other parameters tested also had high SE when compared to the mixed control group (healthy women and benign breast tumor patients), but as in the case of TIMP-1, in our previous studies [31,47] their SE was markedly lower when compared to only healthy women.

SP measures the proportion of correctly identified negatives. In this study, VEGF had the highest SP in all groups of BC patients. Similar data were obtained in our previous study on BC [31], where SP for this parameter was 96%. However, in contrast to this paper, statistical analysis in the previous publication was conducted in a group of 'BC patients vs. healthy women' only. We also observed similar data in other types of cancer, for example esophageal cancer [46], where SP for VEGF was marginally lower than in our case. This might be associated with different types of cancer investigated, a different number of patients or reference only to healthy patients. Other parameters studied in the present study had a lower SP and the values were lower than those obtained for the same parameters in our previous studies [31,47].

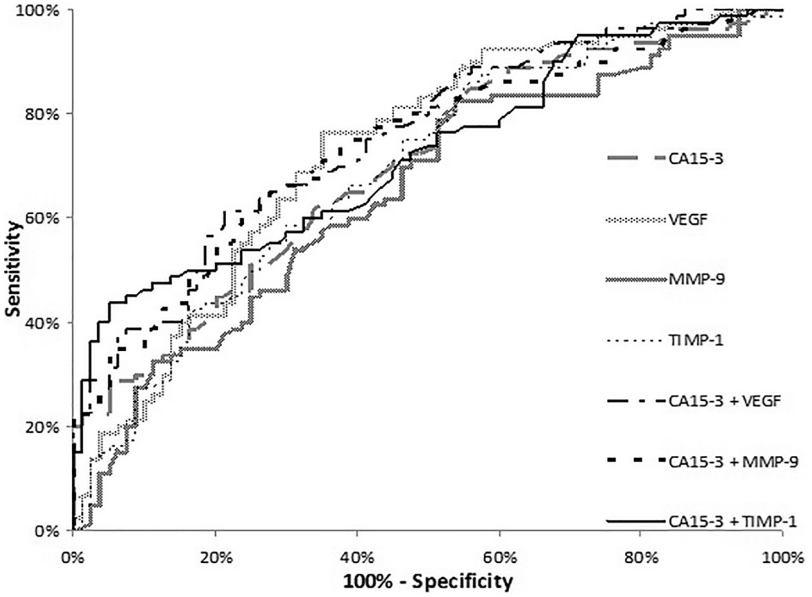

Fig. 1. Diagnostic criteria of ROC curve for tested parameters and in combination with CA 15-3 in total BC group.

However, this might be related to a different composition of control groups.

The diagnostic power (AUC) represents the overall accuracy of a test, with the value approaching 1.0 indicating a perfect SE and SP. Our results show that VEGF had the highest AUC of all the tested parameters in the total group of BC patients. It was marginally lower than the AUC obtained by Zhang et al. [48]. The discrepancy between our research results and the results obtained in that study may be related to a different type of measurement used (ELISA vs. Luminex System) or the number of patients comprising study groups and their classification (in the case of the study by Zhang et al. [48] cancer patients were pre- and post- operative while in our study all patients were preoperative). With regards to other types of tumors e.g. esophageal cancer [46] the authors also obtained a marginally higher AUC value for VEGF, but the control group in their study comprised only healthy subjects. Since VEGF was found to present high diagnostic performance in those tumors, it may prove to be a good biomarker for a variety of other cancer types.

In the case of other parameters for which AUC was marginally lower than for VEGF, e.g. TIMP-1, Kozłowski et al. [46] in their research on esophageal cancer, or Holten-Andersen et al. [49] in their studies on colorectal cancer obtained very similar AUCs but in those cases control groups consisted only of healthy subjects. In the case of MMP-9, other authors such as Zhang et al. [48] demonstrated higher AUC than that obtained in our study, but in their case the cancer group included both pre- and post- operative patients.

According to this study, the ROC area of VEGF was the largest of all the tested parameters (even higher than CA 15-3, which is currently commonly used in diagnosing BC) and is the only parameter for which AUC was statistically significantly larger in comparison to AUC = 0.5 in BC stage I. This is of vital importance since it indicates higher usefulness of VEGF than CA 15-3 in the differentiation between BC patients and non-carcinoma patients, especially in the earliest stages of this type of cancer.

In BC stage I, VEGF was the only parameter for which AUC was

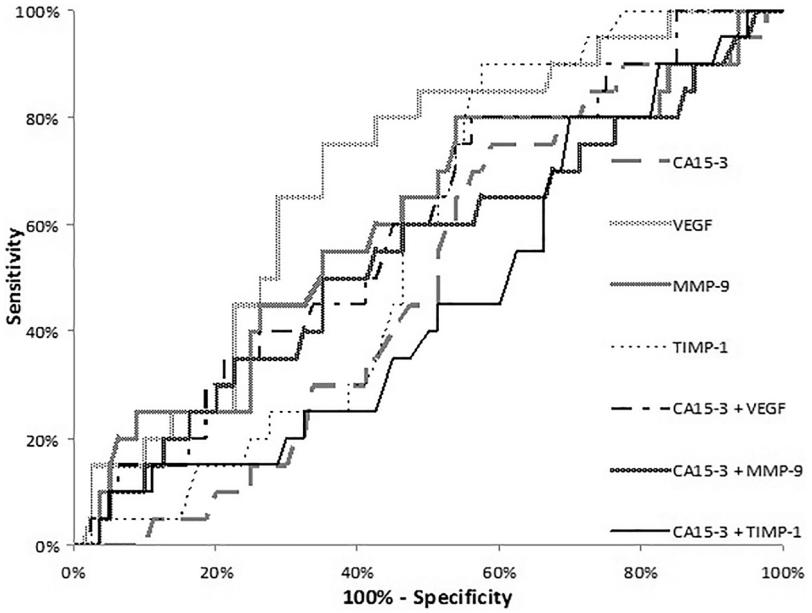

Fig. 2. Diagnostic criteria of ROC curve for tested parameters and in combination with CA 15-3 in BC stage I.

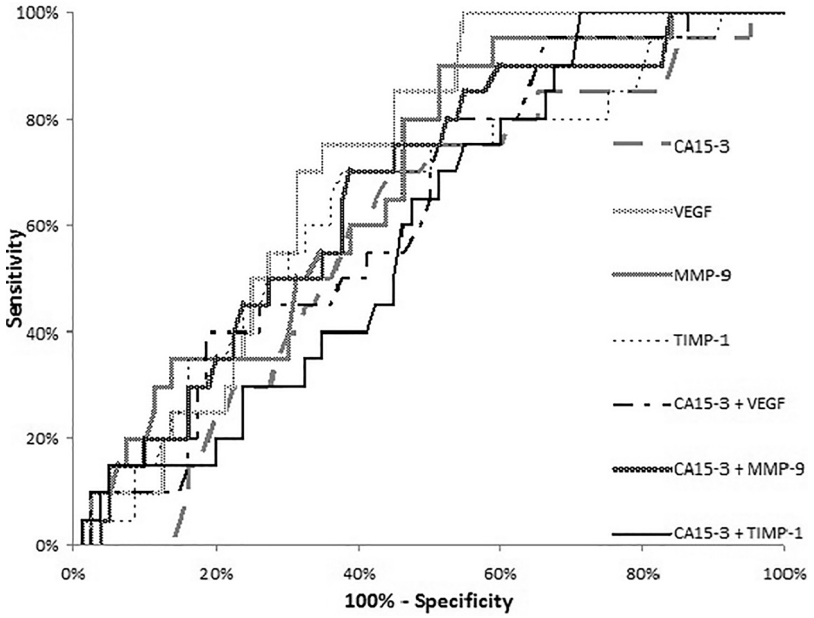

Fig. 3. Diagnostic criteria of ROC curve for tested parameters and in combination with CA 15-3 in BC stage II.

statistically significantly larger in comparison to AUC = 0.5. In our previous study in BC [50], which comprised BC patients and only healthy women as a control group, the highest AUC value was obtained for CA 15-3. The present statistical analysis with a new, combined control group revealed even better results for the tested cytokine (VEGF is a better marker than CA 15-3).

In BC stage II, the AUC of all the tested parameters (besides CA 15-3) was statistically significantly larger in comparison to AUC = 0.5. This may indicate much higher diagnostic usefulness of the tested parameters in comparison with CA 15-3, which is currently the most common parameter in BC diagnosis. The superior performance of the tested parameters may prompt one to question the diagnostic validity of CA 15-3 as its usefulness cannot be confirmed until the third stage of cancer, where patient survival is estimated at approximately 40%. In our previous study on BC [50], VEGF also had significant and similar values (however, the control group was composed of only healthy subjects).

In stage III and IV almost all the parameters were statistically significantly higher in comparison to AUC = 0.5. The best AUC was presented by TIMP-1 in BC stage III and by CA 15-3 in stage IV. In our previous study on BC [50] CA 15-3 also showed similar values of AUC.

We have not found publications that would indicate the value of AUC for MMP-9 and TIMP-1 in different stages of BC or any other types of cancer when compared to control groups.

What is important, in future diagnosis, combined analysis of tested parameters with CA 15-3 can be the most correct way to improve the detection rate of BC, because most of other parameters (not only VEGF, MMP-9 and TIMP-1, but also other cytokines, matrix metalloproteinases and their inhibitors) are non-specific and should be used only in a panel to improve the sensitivity of the available to date specific markers.

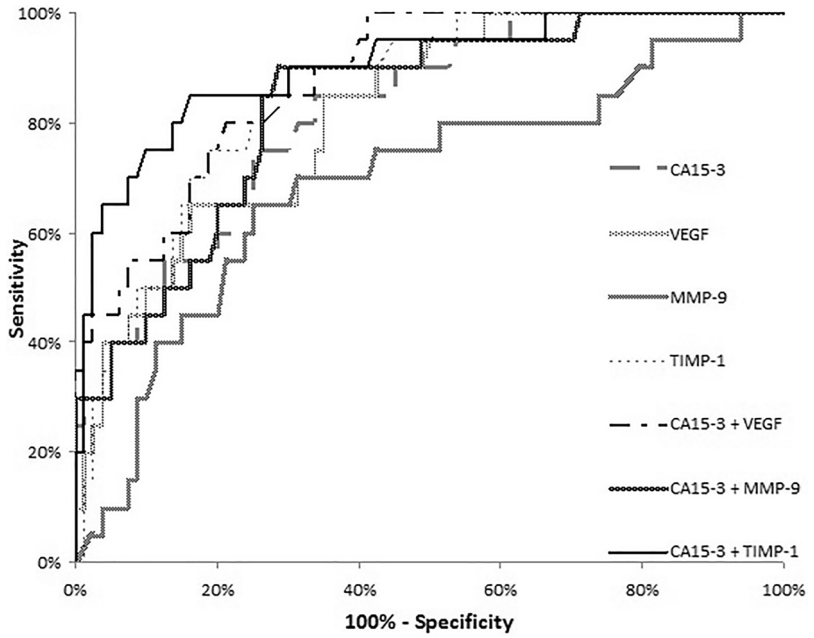

Fig. 4. Diagnostic criteria of ROC curve for tested parameters and in combination with CA 15-3 in BC stage III.

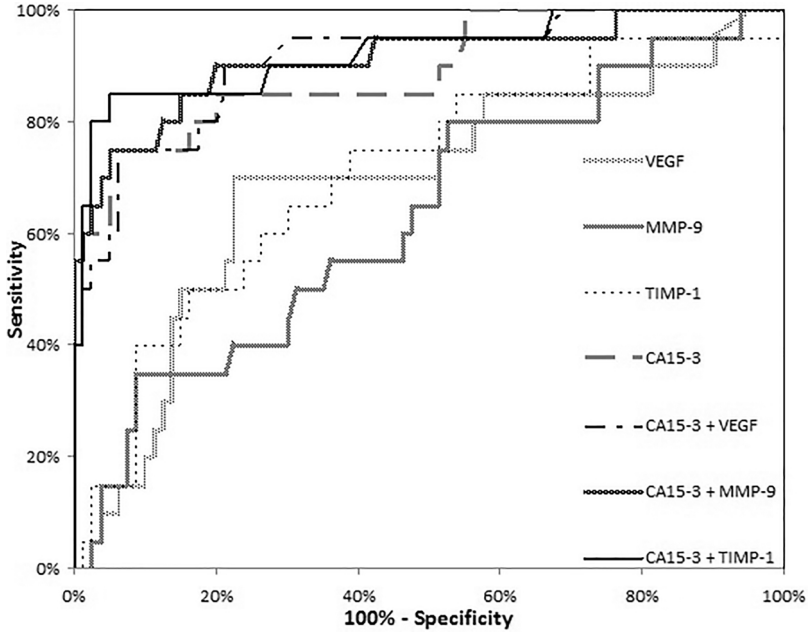

Fig. 5. Diagnostic criteria of ROC curve for tested parameters and in combination with CA 15-3 in BC stage IV.

5. Conclusions

In conclusion, our present results indicate the usefulness and high diagnostic power of all the tested parameters in detecting BC. TIMP-1 showed the highest value of sensitivity in BC group and, more importantly, the highest value in BC stage I. VEGF also showed high sensitivity in all stages of BC and the highest specificity from all the tested parameters. It was also the only parameter which had statistically significant AUC in all the stages. Among the tested parameters, VEGF appeared to be the best candidate for cancer diagnostics (superior to the commonly used tumor marker – CA 15-3) especially in BC stage I as well as in the differentiation between BC and non-carcinoma patients. The combined analysis of the tested parameters and CA 15-3 resulted in an increase in SE and AUC values. In the total BC group all the tested parameters showed statistically significant AUC, but the maximum range was obtained for the combination VEGF + CA15-3. VEGF seems to be the best candidate for diagnosing BC stage I and for differentiating between BC and non-carcinoma cases. It provides hope for developing a new panel of biomarkers that may be used in diagnosing BC in the future.

Conflict of interests

The authors declare that they have no conflict of interest.

Financial disclosure

This research was financed by grants for the Medical University of Bialystok (nos. N/ST/MN/16/001/2207 and N/ST/MN/16/005/2207) in 2016 from the Polish Ministry of Science and Higher Education granted to MSz and SŁ.

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