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Human Plasma Levels of Vascular Endothelial Growth Factor, Matrix Metalloproteinase 9, and Tissue Inhibitor of Matrix Metalloproteinase 1 and Their Applicability as Tumor Markers in Diagnoses of Cervical Cancer Based on ROC Analysis

Monika Zajkowska, MS[1], Monika Zbucka-Krętowska, MD[2], Iwona Sidorkiewicz, MS[2], Emilia Lubowicka, MS[3], Grażyna Ewa Będkowska, PhD[4], Ewa Gacuta, MD[5], Maciej Szmitkowski, MD[1], and Sławomir Ławicki, MD[1]

Abstract

Cervical cancer (CC) remains a major diagnostic problem. The introduction of human papillomavirus vaccination significantly reduced the number of new cases; however, the search for new methods that would earlier indicate the development of cancerous changes is vital. The aim of this study was to investigate the diagnostic power of those parameters in comparison to Cancer Antigen 125 (CA 125) and Squamous Cell Carcinoma Antigen (SCC-Ag) in patients with CC and in relation to the control group. The study included 100 patients with CC and 50 healthy women. Plasma levels of tested parameters were determined by enzyme-linked immunosorbent assay, CA 125, and SCC-Ag by chemiluminescent microparticle immunoassay. Plasma levels of all parameters in the total cancer group showed statistical significance (in all cases $P < .05$). In stage I cancer, only vascular endothelial growth factor (VEGF) and tissue inhibitors of metalloproteinase 1; in stage II, all the tested parameters and CA 125; and in stage III + IV, VEGF, matrix metalloproteinase 9 (MMP-9), and CA 125 showed statistical significance when compared to the healthy volunteers group. Vascular endothelial growth factor showed the highest value of sensitivity from all tested parameters (I: 75%, II: 76%, III + IV: 94%, and 82% in total CC group). The highest specificity was obtained by MMP-9 (94%). In the total CC, stage I, and stage II groups, all tested parameters showed statistically significant area under the receiver operating characteristics curve (AUC), but maximum range was obtained for the combination VEGF + SCC-Ag (I: 0.9146, II: 0.8941, III + IV: 0.9139, total CC group: 0.9347). The combined analysis of tested parameters and tumor markers resulted in an increase in sensitivity and AUC values, which provides hope for developing new panel of biomarkers that may be used in the diagnosis of CC in the future.

Keywords

diagnostic utility, CA 125, SCC-Ag, VEGF, MMP-9, TIMP-1

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[1] Department of Biochemical Diagnostics, Medical University of Bialystok, Bialystok, Poland
[2] Department of Reproduction and Gynecological Endocrinology, Medical University of Bialystok, Bialystok, Poland
[3] Department of Esthetic Medicine, Medical University of Bialystok, Bialystok, Poland
[4] Department of Haematological Diagnostics, Medical University of Bialystok, Bialystok, Poland
[5] Department of Perinatology, Medical University of Bialystok, Bialystok, Poland

Corresponding Author:
Monika Zajkowska, Department of Biochemical Diagnostics, Medical University of Bialystok, Waszyngtona 15A, 15-269 Bialystok, Poland.
Email: monika@zajkowska.com

Introduction

Cervical cancer (CC) is the fourth most common cancer affecting women worldwide. The use of the cytological test (the Pap smear) in CC screening programs has led to a reduction in the incidence of CC death in developed countries.[1] Despite improvements in technology and implementation of screening programs designed to detect cancer in its earliest stages of formation, detection at the microscopic level is often too late for successful intervention.[2] It has been estimated that more than half a million women develop CC every year, and therefore, cancer detection, especially at the early stages of the disease, and early detection of cancer recurrence are of utmost importance.[3]

Vaccination against the human papillomavirus (HPV) became a major advance—it offers avoidance of the infectious agent that remains the main cause of the disease. In the United States, more than 65% of CC cases were determinable to HPV types 16 and 18. Nevertheless, CC incidence has decreased more than 50% based on the success of cytological screening. The epidemiological evidence clearly demonstrates that HPV infection is the cause of cervical intraepithelial neoplasia (CIN) (of all grades) and adenocarcinoma in situ. Moreover infection with a high-risk HPV is the major risk factor for the subsequent development of both squamous cell carcinoma and adenocarcinoma of the cervix. The determination of HPV as the etiology for CC has also some implications for screening—HPV testing has been shown consistently to be superior to cytology in terms of sensitivity (SE) and positive predictive value in developed countries. The real-world effectiveness data showed cross-sectional reduction in the prevalence/incidence of vaccine-related HPV types, genital warts, and precancerous cervical lesions in countries and regions with HPV vaccination coverage.[4-6]

Several methods are currently available for recurrence detection, which normally occurs when the disease is advanced with accompanying clinical symptoms. Serum tumor markers are useful in screening for cancers, assessing a response to therapies, and monitoring cancer recurrence as supportive tests.[7] However, there are still no specific tumor markers for CC. The discovery of useful serum biomarkers for the early detection of gynecological cancers is therefore an urgent priority. An ideal tumor marker should have a high SE and a high specificity (SP) in order to discriminate between patients with cancer and those with benign conditions or healthy controls and should also provide information related to tumor burden and activity.[7]

One of the most extensively studied stages of tumor progression is stromal invasion and subsequent metastasis which involve the degradation and remodeling of the extracellular matrix (ECM). During these processes, tumor cells are firmly attached to the basement membrane and ECM. Three steps have been suggested to describe the sequence of events during tumor cell invasion of the ECM: attachment, matrix dissolution, and migration.[8] During local degradation of the matrix by tumor cell-associated metalloproteinases (MMPs), tumor cells directly secrete enzymes to degrade the ECM. Matrix metalloproteinases are classified as gelatinases, collagenases, membrane-type, stromelysins, and matrilysins, based mainly on the in vivo substrate SP and sequence homology of individual MMPs.[9] To date, more than 20 MMPs have been identified, among which MMP-2 and MMP-9 (72 kDa gelatinase and 92 kDa gelatinase) are primarily responsible for basement membrane ECM protein degradation which facilitates tumor cell migration to blood vessels.[10,11] Elevated levels of MMP-9 are found in breast, brain, ovarian, pancreatic, colorectal, bladder, prostate and lung cancers, and melanoma.[12-15] Matrix metalloproteinase 9 is considered to be a powerful factor stimulating the secretion of proangiogenic factors such as vascular endothelial growth factor (VEGF), which is widely regarded as one of the most important growth and survival factors affecting the vascular endothelium.[16,17] The activities of MMPs are controlled in vivo by tissue inhibitors of metalloproteinases (TIMPs) and currently there are 4 known inhibitors referred to as TIMP-1, TIMP-2, TIMP-3, and TIMP-4.[18] Tissue inhibitors of metalloproteinase 1, for instance, inhibits MMP-9 with a high affinity. Disruption of the MMP-TIMP balance can result in a number of pathogenic processes including tumor invasion, metastasis, angiogenesis, and wound healing.[19] Tissue inhibitors of metalloproteinase 1 levels have been demonstrated to be elevated in primary tumor in non-small cell lung cancer and advanced breast carcinoma, and high TIMP-1 levels would therefore be associated with a worse prognosis.[20-22]

The aim of the present study was to determine plasma levels of VEGF, MMP-9, TIMP-1, and the commonly accepted tumor markers (Cancer Antigen 125 and Squamous Cell Carcinoma Antigen [CA 125 and SCC-Ag]) in patients with CC in relation to healthy controls. Additionally, comparisons between plasma levels of the tested parameters and cancer stage were performed. Diagnostic criteria (SE, SP) and receiver operating characteristics (ROC) curve for the tested parameters alone and in combination with both tumor markers were defined.

Material and Methods

Human Participants

Table 1 shows the tested groups. The study comprised 100 patients with invasive primary carcinoma of the uterine cervix who were referred to the Department of Gynaecology, Bialystok Medical University Teaching Hospital, Poland. Clinical stages and histological classification based on the criteria of the International Federation of Gynecology and Obstetrics were established in all cases. Written consent including the participants' own statements regarding their medical history (ie, data related to reproductive history, personal or family history of cancer, general health issues—hospitalization or surgery, use of medication) and lifestyle habits including smoking was obtained from all the participants. None of the patients had received chemo- or radiotherapy prior to blood sample collection. Pretreatment staging procedures included physical and blood examinations, ultrasound scanning, and chest X-rays. In addition, computed tomography scans or magnetic

Table 1. Characteristics of Patients With Cervical Cancer and Control Group.

Study Group		Number of Patients
Tested group	Patients with cervical cancer	
	Squamous cell carcinoma	85
	Adenocarcinoma	15
	Median age (range)	46 (25-61)
	Tumor stage	
	I	32
	II	33
	III + IV	35
	Menopausal status	
	Premenopausal	78
	Postmenopausal	22
Control group	Healthy women	50
	Median age (range)	42 (22-61)
	Menopausal status	
	Premenopausal	39
	Postmenopausal	11

resonance imaging were performed where necessary. The control group included 50 healthy, untreated women. In these women, prior to blood collection, cervical smears had been examined by a gynecologist. The controls were not referred from other medical centers. All participants had undergone annual checkups (laboratory tests, chest X-ray, cervical cytology screening, mammography). The study was approved by the local ethics committee (R-I-002/239/2014) and all the patients gave their informed consent for participation in the study.

Plasma Collection and Storage

Venous blood samples were collected from each patient. Blood was collected into heparin sodium tubes, centrifuged 3500 rpm for 20 minutes to obtain plasma samples, and stored at −85°C until assayed.

Measurements (VEGF, MMP-9, TIMP-1, CA 125, and SCC-Ag)

The tested cytokines (VEGF, MMP-9, and TIMP-1) were measured with enzyme-linked immunosorbent assay (Quantikine Human M-CSF Immunoassay; R&D Systems Inc, Minneapolis, Minnesota) and the commonly used tumor markers were determined by chemiluminescent microparticle immunoassay (CA 125 and SCC-Ag; Abbott, Chicago, Illinois) according to the manufacturer's protocols. The intra-assay coefficient of variation (CV) of VEGF is reported to be 4.5% at a mean concentration of 235 pg/mL, standard deviation (SD) = 10.6; MMP-9: 1.9% at a mean concentration of 2.04 ng/mL, SD = 0.039; TIMP-1: 3.9% at a mean concentration of 1.27 ng/mL, SD = 0.05; CA 125: 2.4% at a mean concentration of 43.5 U/mL, SD = 1.10; SCC-Ag: 4.3% at a mean concentration of 1.97 ng/mL, SD = 0.085. The interassay CV of VEGF amounted to 7.0% at a mean concentration of 250 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; CA 125 to be 3.9% at a mean concentration of 43.5 U/mL, SD = 1.7; SCC-Ag to be 5.1% at a mean concentration of 1.97 ng/mL, SD = 0.1. The value of intra- and interassay CVs were calculated by the manufacturer and enclosed in the reagent kits. The assay did not exhibit cross-reactivity or interference with numerous human cytokines and other growth factors. Duplicate samples were assessed for each patient.

Statistical Analysis

Statistical analysis was performed using STATISTICA version 12.0 (StatSoft, Tulsa, Oklahoma). Diagnostic SE and SP were calculated using the *cutoff* values, which were calculated by Youden index (as a criterion for selecting the optimum *cutoff* point), and for each of the tested parameters: VEGF: 78.85 ng/mL; MMP-9: 316.80 ng/mL; TIMP-1: 104.23 pg/mL; CA 125; 13.40 U/mL; and SCC-Ag: 0.85 ng/mL. We defined the ROC curve for all the tested parameters and tumor markers. The construction of the ROC curves was performed using the GraphROC program for Windows (Windows, Royal, Arkansas), and the areas under the ROC curve (AUC) were calculated to evaluate the diagnostic accuracy and to compare AUC for all tested parameters separately and in combination with the commonly used tumor markers (CA 125 and SCC-Ag). Statistically significant differences were defined as comparisons resulting in $P < .05$.

Results

Table 2 shows plasma levels of the tested parameters and tumor markers in patients with CC and in the control group. Plasma levels of all the parameters in the total cancer group were statistically significantly higher (only in the case of TIMP-1 were they statistically significantly lower) when compared with the healthy women group (in all cases $P < .05$). In stage I cancer, only VEGF and TIMP-1; in stage II, all the tested parameters and only one of the commonly used tumor markers (CA 125); in stage III + IV, two of the tested parameters (VEGF and MMP-9) and CA 125 showed statistical significance when compared to the healthy volunteers group (in all cases $P < .05$).

Table 3 shows the SE and SP of the investigated parameters and tumor markers. We indicated that the SE of all the tested parameters in the total cancer group was highest for VEGF (82%), higher than the SE of the routinely used tumor markers, CA 125 (78%), SCC-Ag (77%), and other tested parameters (TIMP-1: 30% and MMP-9: 52%). Among all the parameters, the highest SE in stage I cancer was observed also for VEGF (75%), in stage II for VEGF (76%) and commonly used markers (CA 125 and SCC-Ag, both 79%), in stage III + IV, VEGF (94%) followed by CA 125 (91%). The diagnostic SP of the tested parameters was highest for MMP-9 (94%) in all groups of patients with CC. Combined analysis of the tested parameters and CA 125 or SCC-Ag resulted in an increase in SE in all cases. The best combination in the total group of CC proved to be VEGF + SCC-Ag (SE: 97%; SP; 60%).

Table 2. Plasma Levels of Tested Parameters, CA 125, and SCC-Ag in Patients With Cervical Cancer and in Control Group.

Groups Tested	VEGF (pg/mL)	MMP-9 (ng/mL)	TIMP-1 (ng/mL)	CA 125 (U/mL)	SCC-Ag (ng/mL)
Cervical cancer, median (range)					
Stage I	132.40[a] (11.80-615.50)	273.12 (44.00-815.60)	77.61[a] (7.17-264.26)	14.95 (6.60-49.60)	0.74 (0.38-1.10)
Stage II	136.90[a] (28.92-395.60)	302.14[a] (76.40-740.00)	73.40[a] (27.53-262.30)	17.70[a] (4.40-77.41)	0.70 (0.45-1.10)
Stage III + IV	199.00[a/b] (44.50-598.50)	344.44[a] (36.00-1099.40)	112.35[b,c] (21.30-733.19)	25.60[a,b] (6.34-120.10)	0.85 (0.30-5.20)
Total group	141.10[a] (11.80-615.50)	325.80[a] (36.00-1099.40)	87.41[a] (7.17-733.19)	17.65[a] (4.40-120.10)	0.80[a] (0.30-5.20)
Control group, median (range)					
Healthy women	45.80 (11.20-194.50)	166.00 (18.00-420.00)	119.05 (23.38-266.09)	11.70 (3.50-36.60)	0.75 (0.40-1.60)

Abbreviations: MMP-9, matrix metalloproteinase 9, TIMP-1, tissue inhibitors of metalloproteinase 1; VEGF, vascular endothelial growth factor
[a]Statistically significant when patients with CC compared with healthy women.
[b]Statistically significant when patients with stage III or IV CC compared with patients with stage I CC.
[c]Statistically significant when patients with stage III or IV CC compared with patients with stage II CC.

Table 3. Diagnostic Criteria of Tested Parameters, CA 125, and SCC-Ag in Patients With Cervical Cancer.

		Cervical Cancer			
Tested Parameters	Diagnostic Criteria (%)	Stage I	Stage II	Stage III/IV	Total Group
VEGF	SE	75	76	94	82
	SP	76	76	76	76
MMP-9	SE	44	48	63	52
	SP	94	94	94	94
TIMP-1	SE	16	21	51	30
	SP	36	36	36	36
CA 125	SE	63	79	91	78
	SP	68	68	68	68
SCC-Ag	SE	75	79	77	77
	SP	74	74	74	74
VEGF + CA 125	SE	88	97	100	95
	SP	52	52	52	52
MMP-9 + CA 125	SE	84	88	97	90
	SP	64	64	64	64
TIMP-1 + CA 125	SE	69	85	97	84
	SP	26	26	26	26
VEGF + SCC-Ag	SE	97	97	94	96
	SP	60	60	60	60
MMP-9 + SCC-Ag	SE	78	88	89	85
	SP	72	72	72	72
TIMP-1 + SCC-Ag	SE	81	85	91	86
	SP	26	26	26	26

Abbreviations: MMP-9, matrix metalloproteinase 9; SE, sensitivity; SP, specificity; TIMP-1, tissue inhibitors of metalloproteinase 1; VEGF, vascular endothelial growth factor

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 relating to AUCs in different stages of CC (I-IV) are included in Table 4. Graphical versions of the ROC curves for all the tested parameters and their combinations with the commonly used tumor markers (CA 125 and SCC-Ag), both in the whole group of CC, are presented in Figure 1. Additional data, the distributions in all CC stages (I-IV), are presented as Supplemental Files. We noticed that the VEGF AUC (0.8623) in the total group of CC was highest from all the single parameters. In stage I, SCC-Ag demonstrated the highest value (0.8041), but VEGF demonstrated almost the same value (0.7925). In stages II and III + IV, it was VEGF as well that demonstrated the highest values. Combined analysis of the tested parameters and CA 125 or SCC-Ag resulted in an increase in AUC in all cases. The best combination in the total cancer group and cancer stages I, II, and III + IV proved to be VEGF + SCC-Ag (AUC = 0.9146, 0.8941, 0.9139, 0.9347, respectively). The AUCs for the tested parameters, similarly to the ones for commonly used tumor markers, were statistically significantly larger in comparison to AUC = 0.5 (borderline of the diagnostic usefulness of the test; $P < .05$ in all cases).

Discussion

Despite widespread availability of HPV vaccines, CC is one of the major causes of cancer-related death in women

Table 4. Diagnostic Criteria of ROC Curve for Tested Parameters, CA 125, and SCC-Ag in Total Group and All Stages of CC.

	ROC Criteria in Cervical Cancer (Total Group)			
Tested Parameters	AUC	SE	95% CI (AUC)	*P* (AUC = 0.5)[a]
VEGF	0.8623	0.0306	0.802-0.922	<.001
MMP-9	0.7397	0.0398	0.662-0.818	<.001
TIMP-1	0.6884	0.0451	0.600-0.777	<.001
CA 125	0.7225	0.0456	0.633-0.812	<.001
SCC-Ag	0.7883	0.0369	0.716-0.861	<.001
VEGF + CA 125	0.8637	0.0307	0.804-0.924	<.001
MMP-9 + CA 125	0.7394	0.0397	0.662-0.817	<.001
TIMP-1 + CA 125	0.6957	0.0454	0.607-0.785	<.001
VEGF + SCC-Ag	0.9146	0.0237	0.868-0.961	<.001
MMP-9 + SCC-Ag	0.8343	0.0324	0.771-0.898	<.001
TIMP-1 + SCC-Ag	0.8103	0.0356	0.741-0880	<.001
	ROC Criteria in Cervical Cancer (Stage I)			
Tested Parameters	AUC	SE	95% CI (AUC)	*P* (AUC = 0.5)
VEGF	0.7925	0.0540	0.687-0.898	<.001
MMP-9	0.6944	0.0641	0.569-0.820	.0024
TIMP-1	0.7625	0.0561	0.653-0.872	<.001
CA 125	0.6369	0.0614	0.517-0.757	.0258
SCC-Ag	0.8041	00506	0.705-0.903	<.001
VEGF + CA 125	0.7997	0.0533	0.695-0.904	<.001
MMP-9 + CA 125	0.6938	0.0642	0.568-0.820	.0025
TIMP-1 + CA 125	0.7591	0.0567	0.648-0.870	<.001
VEGF + SCC-Ag	0.8941	0.0365	0.822-0.966	<.001
MMP-9 + SCC-Ag	0.8163	0.0528	0.713-0.920	<.001
TIMP-1 + SCC-Ag	0.8444	0.0433	0.760-0.929	<.001
	ROC Criteria in Cervical Cancer (Stage II)			
Tested Parameters	AUC	SE	95% CI (AUC)	*P* (AUC = 0.5)
VEGF	0.8542	0.0408	0.774-0.934	<.001
MMP-9	0.7485	0.0567	0.637-0.860	<.001
TIMP-1	0.7636	0.0551	0.656-0.872	<.001
CA 125	0.7203	0.0567	0.609-0.832	<.001
SCC-Ag	0.7979	0.0519	0.696-0.900	<.001
VEGF + CA 125	0.8558	0.0404	0.777-0.935	<.001
MMP-9 + CA 125	0.7473	0.0574	0.635-0.860	<.001
TIMP-1 + CA 125	0.7697	0.0550	0.662-0.878	<.001
VEGF + SCC-Ag	0.9139	0.0343	0.847-0.981	<.001
MMP-9 + SCC-Ag	0.8630	0.0460	0.773-0.953	<.001
TIMP-1 + SCC-Ag	0.8370	0.0465	0.746-0.928	<.001
	ROC Criteria in Cervical Cancer (Stage III + IV)			
Tested Parameters	AUC	SE	95% CI (AUC)	*P* (AUC = 0.5)
VEGF	0.9359	0.0246	0.888-0.984	<.001
MMP-9	0.7738	0.0594	0.657-0.890	<.001
TIMP-1	0.5456	0.0656	0.417-0.674	.4871
CA 125	0.8053	0.0481	0.711-0.899	<.001
SCC-Ag	0.7641	0.0559	0.655-0.874	<.001
VEGF + CA 125	0.9318	0.0255	0.882-0.982	<.001
MMP-9 + CA 125	0.7747	0.0595	0.658-0.891	<.001
TIMP-1 + CA 125	0.5641	0.0658	0.435-0.693	.3296
VEGF + SCC-Ag	0.9347	0.0282	0.880-0.990	<.001
MMP-9 + SCC-Ag	0.8235	0.0537	0.718-0.929	<.001
TIMP-1 + SCC-Ag	0.7524	0.0560	0.643-0.862	<.001

Abbreviations: AUC, areas under the ROC curve; MMP-9, matrix metalloproteinase 9; TIMP-1, tissue inhibitors of metalloproteinase 1; SE, sensitivity; VEGF, vascular endothelial growth factor

[a]*P* statistically significantly larger AUCs compared to AUC = 0.5.

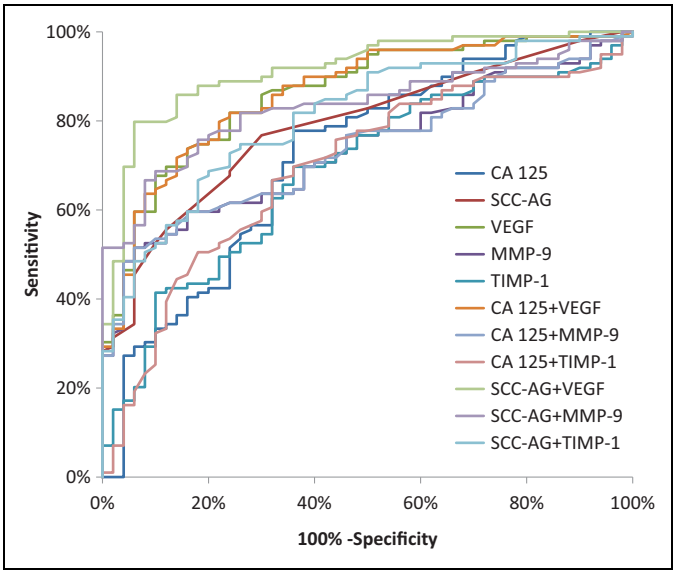

Figure 1. Diagnostic criteria of ROC curve for tested parameters and in combination with commonly used tumor markers in total group of CC. CC indicates cervical cancer; ROC, receiver operating characteristics.

worldwide.[23,24] It has been proven that the enhanced activity of the VEGF, MMPs, and their tissue inhibitors is strongly linked to a number of tumors.[25-29] Vascular endothelial growth factor is considered to be an important factor in blood vessel formation (angiogenesis), which is closely connected with tumor progression and metastasis.[29] It has been indicated recently that MMP-9 and its tissue inhibitor (TIMP-1) may be potential markers of ovarian and breast cancers.[30-32]

In the present study, we investigated the usefulness of VEGF, MMP-9, and TIMP-1 separately and in combination with CA 125 and SCC-Ag (commonly used tumor markers) in patients with CC, not only in the total group of patients but also in particular cancer stage groups (stages I, II, and III + IV).

Our results showed statistically significantly higher concentrations of VEGF and MMP-9 (tested parameters) and statistically significantly lower concentrations of TIMP-1 when the total group of patients with CC was compared to the healthy participants. We found comparable results in the studies of Li et al,[23] but those authors observed significantly higher expression of MMP-9 in CC tissues. Similar results were demonstrated by Guo et al,[33] where MMP-9 expression was associated with lymph node metastasis and suggested an invasive potential in early CC. Some researchers have demonstrated the statistical significance of VEGF expression in CC tissues.[34,35] Our previous studies regarding breast cancer,[29,30] in which the same parameters and the tumor marker commonly used in this type of cancer were investigated, also showed that VEGF, MMP-9, and TIMP-1 had high statistical significance.

Interestingly, low concentrations of TIMP-1 as an inhibitor of MMP-9 indicate a dependence—an increase in metalloproteinase concentration and a decrease in inhibitor concentration, which further confirms the hypothesis that the production of TIMP-1 in low (physiological) amounts by healthy cells is insufficient to inhibit large amounts of metalloproteinases produced by cancer cells which decompose type IV collagen and contribute to the degradation of the ECM.

In stage I cancer, we observed statistical significance only in the concentrations of VEGF and TIMP-1 when compared to healthy participants. This finding is consistent with our previous results concerning other types of cancer.[30] What is of vital importance is that none of the commonly used tumor markers showed any significance. Our findings are in opposition to those described by Takeda et al,[36] who demonstrated that the levels of SCC-Ag and CA 125 were related to disease stage and that elevated levels of those tumor markers had predictive value for cancer prognosis. Interestingly, another research group presented findings similar to ours, which revealed that serum SCC-Ag levels showed no independent prognostic value in the early stages of CC[37] and, in another publication,[38] that in only 30% of patients both of the commonly used tumor markers gave positive results. Therefore, the establishment of new tumor markers, which would show the presence of tumor progression at an early stage, is crucial.

In stage II CC, all the tested parameters and CA 125, and in stage III + IV, two of the tested parameters (VEGF and MMP-9) and CA 125 showed statistical significance when compared to the healthy volunteers group. This indicates that the commonly used tumor markers become useful only at more advanced cancer stages, when more extensive surgery with more aggressive treatment is needed.

Sensitivity measures the proportion of positive results which are correctly identified. In the present study, VEGF demonstrated the highest SE for the total CC group (82%). We had obtained similar results in our previous study on breast cancer.[29,30] Conversely, in our research concerning ovarian cancer,[39] VEGF obtained only 48% of SE in the total cancer group. Some other researchers[40,41] have concluded that VEGF concentration in CC is strongly associated with disease stage.

In stage I cancer, the highest SE was observed also for VEGF (75%), in stage II commonly used markers and VEGF (76%), in stages III + IV VEGF (91.2%). This indicates a high diagnostic SE of VEGF not only in the total study group but also in the early stages of malignancy, when cancerous changes are particularly difficult to diagnose. We obtained far worse results for VEGF (44% in stage I and 48% in stage II) in ovarian cancer, but with the highest SE in stage I cancer from among the tested parameters.[39] We also obtained the highest values of SE for VEGF in stage I breast cancer.[30]

Specificity measures the proportion of negative results which are correctly identified. In this study, MMP-9 demonstrated the highest SP in the total CC group (94%). We obtained similar results regarding MMP-9 in our previous study concerning CC (91.67%).[42] We obtained the same results in other work concerning breast cancer (94%).[30]

The combined analysis of the tested parameters and CA 125 or SCC-Ag resulted in an increase in SE in all cases. The best combination in the total group of CC proved to be VEGF + SCC-Ag. To our knowledge, this report is the first to evaluate

such a comprehensive statistical analysis performed using not only the investigated parameters but also their combined analysis with CA 125 or SCC-Ag in both CC and other cancers.

Area under the ROC curve represents the overall accuracy of a test, with the value approaching 1.0 indicating perfect SE and SP. According to this study, the ROC area of VEGF (0.8623) was the largest of all the tested parameters (even higher than the commonly used tumor markers) in the total group of CC. In stage I, SCC-Ag showed the highest value (0.8041), although VEGF demonstrated almost the same value (0.7925). In stages II and III + IV, it was also VEGF that showed the highest values (0.8542 and 0.9359, respectively). This finding is in line with our previous results in breast cancer, where VEGF showed the highest AUC (0.729) from all the tested parameters in every stage of the tumor.[29]

The best combination in the total cancer group and all stages proved to be VEGF + SCC-Ag. This report is also the first to evaluate the combined statistical analysis of the investigated parameters and CA 125 and SCC-Ag in CC and other cancers.

Unfortunately, we could not compare our data with the findings of other authors. The majority of results in the available literature concern tissue expression or different evaluation of the parameters investigated in our study.

Conclusions

In summary, to the authors' knowledge, our report is the first to evaluate the plasma levels and, what is more important, the diagnostic usefulness of such an extensive analysis of VEGF, MMP-9, TIMP-1 in CC, not only independently but especially in combination with both established cervical tumor markers. All tested parameters showed statistical significance when compared their concentrations in patients with CC to healthy women. Almost all parameters showed high usefulness in detecting tumor development. The most important results of this study suggest that combining VEGF + SCC-Ag measurements may allow for the improved, earlier detection of CC when compared with the use of either marker alone.

Authors' Note

Data available on request, after contact with corresponding author. The study was approved by the local ethics committee of the Medical University of Bialystok (R-I-002/239/2014). Informed consent was obtained from all individual participants included in the study.

Declaration of Conflicting Interests

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ORCID iD

Emilia Lubowicka http://orcid.org/0000-0001-5002-4076

Supplemental Material

Supplemental material for this article is available online.

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