VIA MEDICA

ORIGINAL PAPER / GYNECOLOGY

Ginekologia Polska
2018, vol. 89, no. 10, 568–572

ISSN 0017–0011

DOI: 10.5603/GP.a2018.0097

Assessment of diagnostic utility of multivariate diagnostic models in differential diagnosis of ovarian tumors

Agnieszka Horała[1], Agata Swiatly[2], Jakub Lorek[1], Zenon J. Kokot[2], Jan Matysiak[2], Ewa Nowak-Markwitz[1]

[1]Gynecologic Oncology Department, Poznan University of Medical Sciences, Poland
[2]Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction: Ovarian cancer (OC) diagnosis remains a clinical challenge due to lack of early symptoms and insufficient accuracy of the available diagnostic methods. The purpose of this study was to determine whether osteopontin could be useful in differential diagnosis of ovarian tumors.

Material and methods: Serum samples from 92 patients qualified for surgical treatment due to ovarian mass were divided into 2 groups according to the histopathological result: OC including borderline ovarian tumors (n = 39) and benign ovarian tumors (BOTs) (n = 53). CA125, HE4 and osteopontin concentrations were measured in all patients. Areas under the receiver operating characteristic curves (AUC of ROC) were used to compare the discriminative ability of the univariate and multivariate diagnostic models.

Results: The addition of osteopontin to ROMA significantly improved the diagnostic performance of the test in 3 of the 5 analyses: 1) in the OC vs BOT group (from AUC of 0.955 to 0.975), 2) in premenopausal women OC vs BOT (from AUC of 0.828 to 0.892) and 3) in the FIGO I-II stage OC vs BOT (from AUC of 0.865 to 0.895). It did not alter the diagnostic performance of multifactor tests in the group of postmenopausal women nor in OC FIGO III-IV stage group. Osteopontin was also the best single marker to differentiate between early stage OC and BOTs (AUC of 0.863).

Conclusions: Osteopontin improves the diagnostic performance of a multimarker OC diagnostic test and could be useful in differential diagnosis of ovarian tumors, especially in pre-menopausal women and for early stage OC.

Keywords: ovarian cancer, ovarian neoplasms, biomarkers, osteopontin

Ginekologia Polska 2018; 89, 10: 568–572

INTRODUCTION

Early and effective detection methods of ovarian cancer (OC) are still lacking and the disease continues to be one of the most deadly women's neoplasms. The incidence rate in Europe is 9.9 and the age-standardised mortality rate is 5.4 [1]. The biggest challenge in OC diagnosis is the detection of the disease in its early stages when the prognosis is favourable – for stage I OC (according to classification by International Federation of Gynaecology and Obstetrics – FIGO) the 5-year survival rate is around 90% whereas for advanced stages (FIGO III-IV) it decreases to 30% or less [2].

Current clinical practice involves serum cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) testing and performing a transvaginal ultrasound. Several algorithms that combine clinical information, ultrasound features and serum biomarker levels were developed to provide correct ovarian tumor differential diagnosis and achieved promising diagnostic accuracy for classic discrimination OC versus BOT (Tab. 1) but proved less effective for diagnosing early stage disease. As for the ultrasound models, most of the evidence derives from specialized ultrasound centers with particular expertise in this field and it is not

Corresponding author:
Agnieszka Horała
Gynecologic Oncology Department, Poznan University of Medical Sciences, Poland
e-mail: agnieszka0lemanska@gmail.com

Table 1. Selected models and their performance in discrimination between benign and malignant tumors

Model	Features	Specificity [%]	sensitivity [%]	Reported AUC	Reference
Risk of Ovarian Malignancy Algorithm (ROMA)	Menopausal status, CA125, HE4	76.5	94.4	0.897	[4]
Risk of Malignancy Index (RMI)	Menopausal status, CA125, ultrasound score	81.5	94.4	0.860–0.883	[5]
Assessment of Different NEoplasias in the adneXa (ADNEX)	Clinical variables, CA125, ultrasound features	96.5	71.3	0.954	[6]

clear whether the same efficacy could be achieved in other centers [3]. Moreover, none of these methods is applicable for OC screening.

Osteopontin has been recently intensively investigated as a potential OC biomarker. The meta-analysis by Hu et al. concluded that it could be a useful OC biomarker but nevertheless emphasized the need for future studies to confirm its diagnostic potential in OC [7]. We have also previously investigated osteopontin as part of a biomarker panel of 16 factors related to angiogenesis where it was identified as the best single marker for OC detection [8].

The purpose of this study was to investigate the diagnostic potential of selected multifactor tests in differential diagnosis of ovarian tumors with special emphasis on the role of osteopontin and early OC detection. To the best of our knowledge, this is the first study to provide such a broad analysis of the potential use of osteopontin as a part of multifactor diagnostic tests.

MATHERIAL AND METHODS

Serum samples were collected from 122 patients qualified for surgical treatment due to ovarian tumor in Gynecologic Oncology Department in 2014 and 2015. Blood samples were collected on the day before the surgery, incubated for 30 minutes in room temperature to clot, and centrifuged for 15 minutes at 4000 rpm. Serum was then transferred to vials and stored at -80°C until analysis. 30 patients were disqualified due to meeting the exclusion criteria (non-epithelial OC or presence of any other malignancy currently or in anamnesis; $n = 16$) or incomplete clinical data recorded ($n = 14$). The remaining 92 patients were divided into 2 groups according to the histopathological result: OC and borderline ovarian tumors ($n = 35 + 4$) and BOT ($n = 53$). Table 2 presents the study group characteristics. The age and BMI did not differ significantly between the groups. All patients were Caucasian females. Additionally, the menopausal status was recorded. In women with previous hysterectomy, the menopausal age was set at the Polish average, i.e. 51 years old.

Considering the fact that most sources recommend complete staging procedure if a borderline ovarian tumor is found, for the purpose of this study borderline ovarian tumors were combined with the OC group. Borderline ovarian tumors constitute about 15% of all epithelial ovarian tumors and occur in women approximately 10 years younger than OC. Although they are considered a distinct clinical entity than invasive OC, they can give implants in the *omentum* and *peritoneum* and share the staging system. The risk of recurrence after conservative surgery is significant (up to 27%) and invasive recurrences appear in up to 6% [9].

Table 2. Study group characteristics

	Ovarian cancer	Borderline tumors	Benign tumors
Number of samples [%]	35 (38.0)	4 (4.3)	53 (57.6)
Age [years] median (range)	59 (32–78)	48 (42–52)	41 (17–72)
BMI median (range)	25.2 (18.5–38.4)	28.5 (26.8–31.6)	24.2 (17.8–39.9)
% of postmenopausal	**77**	**25**	**28**
FIGO stage, n [%]			
I	8 (22.9)	4 (100)	N/A
II	1 (2.9)	0	N/A
III	25 (71.4)	0	N/A
IV	1 (2.9)	0	N/A
Histopathological type, n [%]			
Serous	15 (42.9)	2 (50.0)	10 (18.9)
Endometrioid	2 (5.7)	1 (25.0)	14 (26.4)
Mucinous	1 (2.9)	1 (25.0)	1 (1.9)
Clear cell	3 (8.6)	0	N/A
Undifferentiated	10 (28.6)	0	N/A
Non identified	4 (11.4)	0	N/A
Teratoma	N/A	N/A	10 (18.9)
Other	N/A	N/A	18 (34.0)

CA125 and HE4 concentrations were measured by ECLIA on Roche Cobas System in the Central Hospital Laboratory according to the manufacturer's instructions. The level of osteopontin was measured as part of the immunoassay Bio-Plex Pro Human Cancer Biomarker Panel 1 (Bio-Rad, Hercules, CA, USA) using the flow cytometer Bio-Plex MAGPIX

Table 3. Discriminatory value of different serum markers and multivariate models in differential diagnosis of ovarian tumors. The best AUC values obtained for single markers and for multimarker models are bolded

Marker Group		CA125 [U/mL]	HE4 [pmol/l]	osteopontin [pg/mL]	ROMA	CA125 + HE4	CA125 + HE4 + osteopontin	ROMA + osteopontin	CA125 + HE4 + osteopontin + age
OC (incl. borderline) vs BOT — total	p-value	< 0.001	< 0.001	< 0.001	< 0.001				
	AUC	0.933	0.939	0.825	0.955	0.946	0.957	0.975	0.951
	Cut-off	71.4	87.6	44500.0	28.0				
	Sens/spec	0.85/0.91	0.87/0.96	0.72/0.79	0.90/0.96				
OC (incl. borderline) vs BOT — pre-menopausal women	p-value	< 0.001	< 0.001	0.016	0.001				
	AUC	0.832	0.866	0.741	0.828	0.788	0.823	0.892	0.800
	Cut-off	71.4	59.7	41446.8	11.3				
	Sens/spec	0.73/0.87	0.83/0.79	0.64/0.76	0.82/0.79				
OC (incl. borderline) vs BOT— post-menopausal women	p-value	< 0.001	< 0.001	< 0.001	< 0.001				
	AUC	0.995	0.962	0.848	0.998	0.995	0.978	0.997	0.971
	Cut-off	53.85	104.9	44842.0	45.6				
	Sens/spec	0.96/1.0	0.89/0.93	0.79/0.80	0.96/1.0				
FIGO stage I-II (incl. borderline) OC vs BOT	p-value	< 0.001	< 0.001	< 0.001	< 0.001				
	AUC	0.833	0.840	0.863	0.865	0.783	0.872	0.895	0.870
	Cut-off	63.16	59.67	41435.1	15.1				
	Sens/spec	0.75/0.87	0.83/0.75	0.92/0.70	0.83/0.83				
FIGO stage III-IV (incl. borderline) OC vs BOT	p-value	< 0.001	< 0.001	< 0.001	< 0.001				
	AUC	0.977	0.985	0.808	0.988	0.998	0.992	0.990	0.976
	Cut-off	107.55	104.9	45300.9	56.5				
	Sens/spec	0.93/0.94	0.96/0.98	0.70/0.81	0.96/0.98				

(Bio-Rad, Hercules, CA, USA). The detailed methodology is described in our previous publication [8].

Data analysis was performed using Statistica version 13.1 (StatSoft Inc., Tulsa, OK, USA) and MetaboAnalyst version 4.0, Biomarker Analysis module (www.metaboanalyst.ca). Normality of data distribution was examined with the Shapiro-Wilk test. Differences in markers concentrations between the studied groups were evaluated using a t-test or Mann-Whitney U test. P-values ≤ 0.05 were considered statistically significant. In order to calculate the discriminative ability of the investigated models, the univariate and multivariate receiver operating characteristic (ROC) curves were used. AUC (area under curve) of ROC were used to compare the diagnostic performance of the single markers (CA125, HE4 and osteopontin) and selected multifactor tests (ROMA, CA125 + HE4, ROMA + osteopontin, CA125 + HE4 + osteopontin and CA125 + HE4 + osteopontin + age).

RESULTS

The results of the performed statistical analyses are summarized in Table 3.

As expected, all analysed single markers (CA125, HE4 and osteopontin) and multifactor tests (ROMA, CA125 + HE4, ROMA + osteopontin, CA125 + HE4 + osteopontin and CA125 + HE4 + osteopontin + age) significantly differentiated the analysed groups.

In the whole cohort, the best single marker to distinguish OC and BOT (benign ovarian tumors) was HE4 (AUC of 0.939). Interestingly, the optimal cut off value was 87.6 pmol/l whereas the laboratory cut off recommended by the manufacturer is 70 pmol/l for premenopausal women and 140 pmol/l for postmenopausal women. Among the multifactor tests, the highest AUC was obtained by ROMA + osteopontin. The addition of osteopontin to ROMA significantly improved the diagnostic performance of the test (AUC of 0.955 and 0.975, respectively).

HE4 was identified as the best single marker in the group of premenopausal women and again the model ROMA + osteopontin obtained the highest AUC of 0.892 among the multifactor models. In postmenopausal women, the discriminatory performance of all tests was clearly superior to that in premenopausal group. CA125 and the multifactor model ROMA obtained the best results (AUC of 0.995 and 0.998, respectively). The model ROMA + osteopontin also achieved a comparably high AUC of 0.997.

In the analysis based on the OC stage, the best single marker to differentiate between early stage OC and BOTs was osteopontin (AUC of 0.863) whereas for advanced stage

disease HE4 obtained the highest AUC of 0.985. In multifactor models, the addition of osteopontin to ROMA again improved the diagnostic performance of the test in the early stage OC group (from AUC = 0.865 to AUC = 0.895). The best marker distinguishing advanced stage OC and BOTs was HE4 and, consistently, the best multifactor model in this comparison was CA125 + HE4.

DISCUSSION

Osteopontin was first described as a glycoprotein secreted by osteoblasts in bone but it was found to be involved in various other cellular processes, such as inflammation, immune response, angiogenesis and finally tumorigenesis [10]. It was confirmed to be overexpressed in several malignancies including cervical, breast, prostate, colorectal, lung and pancreatic cancer [11]. In OC osteopontin was shown to promote the OC cell growth in vitro and in vivo and to increase the survival of OC cells under stress conditions [12]. Other studies proved its role in OC cells migration and invasion [13] as well as identified osteopontin as an independent predictor of poor prognosis [14]. Consequently, osteopontin was recently intensively investigated as a potential novel OC biomarker. Nevertheless, our study provides additional information on the performance of osteopontin in the selected groups and in multimarker diagnostic tests.

Two meta-analyses focused on the role of osteopontin in OC. A meta-analysis by Lan et al. reports the overall diagnostic sensitivity and specificity of osteopontin as a diagnostic test for OC of 0.766 (95% CI 0.685–0.831) and 0.897 (95% CI 0.849–0.931), respectively. For the two-marker test based on osteopontin and CA125, the sensitivity and specificity were 0.871 (95% CI 0.788–0.924) and 0.881 (95% CI 0.837–0.914), respectively. The meta-analysis comprised studies comparing OC, BOTs and healthy controls and concludes that osteopontin is a useful biomarker to be applied in OC screening tests and a promising adjunct to CA125 [15]. The second meta-analysis by Hu et al. reports the overall sensitivity and specificity of osteopontin to be 0.66 (95% CI, 0.51–0.78) and 0.88 (95% CI, 0.78–0.93), respectively, and the AUC of ROC of 0.85 (95% CI, 0.81–0.88) [7]. This study also sees osteopontin as a useful biomarker in OC diagnosis, however emphasizes the need for a rigorously designed study to confirm it. Finally, a study similar to ours on 114 women analysing the role of osteopontin in differential diagnosis of ovarian tumors reports its overall sensitivity of 0.72, specificity of 0.89 and AUC of 0.812 at a cut-off level of 28000 pg/mL [16]. All the above-mentioned results correspond with our findings. None of these studies, however, conducted such a broad analysis of the potential use of osteopontin as a part of multifactor diagnostic tests.

One of the main challenges in diagnosing OC is how to detect the disease in its early stages. In the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), in which over 200000 post-menopausal women underwent screening using annual CA125 measurements interpreted by the Risk of Ovarian Cancer Algorithm (ROCA), less than a half of the OCs detected by screening were in early stages (FIGO I-II) [17] with most women not diagnosed until the disease is in its advanced stages. The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS. Hence, there is an urgent need for new early detection strategies. In our study in the FIGO stage I-II group osteopontin was identified as the best single marker and combining it with ROMA resulted in further improvement of the diagnostic performance of the test in the early stage OC group. These results indicate that osteopontin could be a valuable early stage OC marker.

Another difficulty in diagnosing OC is the group of pre-menopausal women. The performance of most of the markers is much poorer in this age group as compared to older women. In our study, the addition of osteopontin did not alter the diagnostic performance of ROMA test in post-menopausal women whereas in pre-menopausal group the AUC was significantly improved. This leads to a conclusion that osteopontin is a promising marker principally for the population of pre-menopausal women.

Additionally, some studies point at the potential usefulness of osteopontin in OC treatment monitoring and diagnosing recurrent disease, especially in those patients with CA125 concentrations within the reference value. Those studies report that the rise in the levels of osteopontin, which correlated with the disease recurrence, preceded the rise in CA125 even by 3 months [18, 19].

Due to a limited number of samples this study did not compare the diagnostic performance of the models between OC type I and II as well as between different histopathological types of OC.

CONCLUSIONS

Osteopontin significantly improved the diagnostic performance of the ROMA test in both, pre-menopausal women and in early stage OC. These results indicate that it is a perfect candidate to become a valuable early stage OC marker, especially as a part of multimarker tests. Further research with rigorous study design and larger study groups focusing on premenopausal women and/or on borderline/early-stage OC is needed to confirm its diagnostic potential and optimal use.

Ethical statement

The study was conducted in accordance with the Declaration of Helsinki and the protocol was approved by the local Bioethical Commission of Poznan University of Medical Sciences, Poland (Decision No. 165/16). A written consent

for inclusion was obtained from all participants prior to sample collection.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgements

The project received support from the Polish National Science Centre (grant number: 2014/15/B/NZ7/00964). The funders did not participate in the study design, data collection and analysis, decision to publish and manuscript preparation.

Authors' roles

AH coordinated the study. AH, AS, JM and ENM designed research. AH, JL and ENM contributed important samples. AS performed research and statistical analyses. AH, JL and AS collected data. AH and AS analyzed data. AH and AS wrote the manuscript. JM, ZJK and ENM critically revised the manuscript. All authors have read and approved the submitted manuscript.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015; 136(5): E359–E386, doi: 10.1002/ijc.29210, indexed in Pubmed: 25220842.
2. Spaczyński M, Nowak-Markwitz E, Kędzia w, Bednarek W. Praktyczna ginekologia onkologiczna: podręcznik dla lekarzy. Wielkopolskie Towarzystwo Onkologii Ginekologicznej 2012.
3. Levine D, Asch E, Mehta TS, et al. Assessment of factors that affect the quality of performance and interpretation of sonography of adnexal masses. J Ultrasound Med. 2008; 27(5): 721–728, indexed in Pubmed: 18424647.
4. Karlsen MA, Sandhu N, Høgdall C, et al. Evaluation of HE4, CA125, risk of ovarian malignancy algorithm (ROMA) and risk of malignancy index (RMI) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2012; 127(2): 379–383, doi: 10.1016/j.ygyno.2012.07.106, indexed in Pubmed: 22835718.
5. Van Holsbeke C, Van Calster B, Valentin L, et al. International Ovarian Tumor Analysis Group. External validation of mathematical models to distinguish between benign and malignant adnexal tumors: a multicenter study by the International Ovarian Tumor Analysis Group. Clin Cancer Res. 2007; 13(15 Pt 1): 4440–4447, doi: 10.1158/1078-0432.CCR-06-2958, indexed in Pubmed: 17671128.
6. Van Calster B, Van Hoorde K, Valentin L, et al. International Ovarian Tumour Analysis Group. Evaluating the risk of ovarian cancer before surgery using the ADNEX model to differentiate between benign, borderline, early and advanced stage invasive, and secondary metastatic tumours: prospective multicentre diagnostic study. BMJ. 2014; 349: g5920, indexed in Pubmed: 25320247.
7. Hu ZD, Wei TT, Yang M, et al. Diagnostic value of osteopontin in ovarian cancer: a meta-analysis and systematic review. PLoS One. 2015; 10(5): e0126444, doi: 10.1371/journal.pone.0126444, indexed in Pubmed: 25951060.
8. Horala A, Swiatly A, Matysiak J, et al. Diagnostic Value of Serum Angiogenesis Markers in Ovarian Cancer Using Multiplex Immunoassay. Int J Mol Sci. 2017; 18(1), doi: 10.3390/ijms18010123, indexed in Pubmed: 28075407.
9. Daraï E, Fauvet R, Uzan C, et al. Fertility and borderline ovarian tumor: a systematic review of conservative management, risk of recurrence and alternative options. Hum Reprod Update. 2013; 19(2): 151–166, doi: 10.1093/humupd/dms047, indexed in Pubmed: 23242913.
10. Rittling S. Osteopontin in macrophage function. Expert Reviews in Molecular Medicine. 2011; 13, doi: 10.1017/s1462399411001839.
11. Categorical meta-analysis of Osteopontin as a clinical cancer marker. Oncology Reports. 2011; 25(2), doi: 10.3892/or.2010.1106.
12. Song G, Cai QF, Mao YB, et al. Osteopontin promotes ovarian cancer progression and cell survival and increases HIF-1α expression through the PI3-K/Akt pathway. Cancer Science. 2008, doi: 10.1111/j.1349-7006.2008.00911.x.
13. Tilli TM, Franco VF, Robbs BK, et al. Osteopontin-c splicing isoform contributes to ovarian cancer progression. Mol Cancer Res. 2011; 9(3): 280–293, doi: 10.1158/1541-7786.MCR-10-0463, indexed in Pubmed: 21263033.
14. Bao LiH, Sakaguchi H, Fujimoto J, et al. Osteopontin in metastatic lesions as a prognostic marker in ovarian cancers. J Biomed Sci. 2007; 14(3): 373–381, doi: 10.1007/s11373-006-9143-1, indexed in Pubmed: 17219251.
15. Lan Z, Fu D, Yu X, et al. Diagnostic values of osteopontin combined with CA125 for ovarian cancer: a meta-analysis. Fam Cancer. 2016; 15(2): 221–230, doi: 10.1007/s10689-015-9847-3, indexed in Pubmed: 26458935.
16. Moszynski R, Szubert S, Szpurek D, et al. Role of osteopontin in differential diagnosis of ovarian tumors. J Obstet Gynaecol Res. 2013; 39(11): 1518–1525, doi: 10.1111/jog.12097, indexed in Pubmed: 23875677.
17. Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). The Lancet Oncology. 2009; 10(4): 327–340, doi: 10.1016/s1470-2045(09)70026-9.
18. Mrochem-Kwarciak J, Mrochen-Domin I, Wojcieszek, A A, et al. Mrochem-Kwarciak, J.; Mrochen-Domin, I.; Wojcieszek, A.; Deja, R.; Chmura, A.; Masłyk, B.; Nowara, E.; Kaleta, B.; Kołosza, Z.; Bartnik, W. [Usefulness of osteopontin (OPN) determinations in ovarian cancer patients who underwent first-line chemotherapy]. Ginekol Pol. 2011; 82: 911–917.
19. Schorge JO, Drake RD, Lee H, et al. Osteopontin as an adjunct to CA125 in detecting recurrent ovarian cancer. Clin Cancer Res. 2004; 10(10): 3474–3478, doi: 10.1158/1078-0432.CCR-03-0365, indexed in Pubmed: 15161704.