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Evaluation of CA-125, CA72-4, HE-4, and M-CSF as early diagnostic markers for ovarian cancer in Egyptian women

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Summary

Objective: To evaluate the diagnostic performance of cancer antigen CA-125, CA72-4, human epididymis protein 4 (HE-4), and macrophage colony stimulating factor (M-CSF), separately or in combination in the diagnosis of early-stage ovarian cancer. *Materials and Methods:* This was a prospective case control study conducted in a University Gynecology hospital. A total of 127 patients with ovarian cancer, in Stage I (n=40) and Stage II (n=87), and 128 age-matched healthy women (controls) were included. CA-125, CA72-4, HE-4, and M-CSF serum levels were assessed and compared in patients *vs*. controls. *Results:* Seventy-six patients were menopausal (59.8%). A significant difference between cases and controls were detected in terms of each of the serum level of the studied markers.The diagnostic performance for the studied markers in patients in Stage I, namely ; CA 125, CA 72-4, HE-4, and M.CSF, showed sensitivities of 50%, 7%, 68.42%, and 32.14%, respectively. Applying the logistic regression model for all studied markers significantly increased the sensitivity and confirmed a better diagnostic performance in Stage I, when combining the four markers together, with area under the curve (AUC) of 0.978, and a sensitivity of 91.07, at a fixed specificity of 90%. *Conclusions:* Using non-invasive and simple markers' panel for early detection of ovarian cancer is the ultimate goal for the best clinical practice. Based on the present results, the authors suggest using the four studied markers in combination for satisfactory and reliable diagnostic performance.

Key words: Ovarian cancer; Markers; CA-125; CA72-4; HE-4; M-CSF.

Introduction

Having the highest mortality among gynecological malignancies, and being mostly asymptomatic at early stages, ovarian cancer has been investigated thoroughly to work on increasing survival rates from the disease. Ovarian cancer mortality, which is fifth in cancer-related deaths in US women, has not changed much over the past few decades [1]. Screening and early detection are by far the most reliable way to reduce mortality from this cancer, however, no definite screening test could be recommended to the population at risk. Non-specific symptoms, if any, make early detection a difficult task for clinicians. Biomarkers were used for early detection of ovarian cancer, but increasing their sensitivity without affecting the specificity, always remained an issue [2, 3].

CA-125 is a classic tumor biomarker, with a preclinical detection of more than 12 months in epithelial ovarian tumors. It also has a prognostic value in monitoring disease progression and response to therapy. However, CA-125 is not expressed in about one-fifth of ovarian cancers, and a false positive high levels could be related to most types of advanced adenocarcinomas, e.g. breast, lung, and colon, as well as with some benign conditions, like endometriosis [4].

According to a recent statement by the European group on tumor markers, CA-125 may lack the sensitivity and specificity to be recommended as a screening marker for early ovarian cancer, however they recommended CA-125 for monitoring of primary therapy and post-therapy surveillance [5]. HE-4 serum concentrations are higher in serous ovarian cancer, as well as some benign conditions such as renal failure [5]. A meta-analysis including 12 publications, confirmed a better diagnostic role of HE4 than CA125 in ovarian cancer in terms of sensitivity and specificity [6]. M-CSF is a cytokine implicated in the pathogenesis of ovarian cancer, acting as a growth factor [7]. CA72-4 is a glycoprotein, which becomes elevated in ovarian adenocarcinomas, and is less sensitive than CA125 for epithelial ovarian tumors. Being not affected by pregnancy or the menstrual cycle, it might be of value in combination with CA125 for the screening of early-stage disease [8].

In this study, the diagnostic performance of preoperative serum levels of CA-125, CA72-4, HE-4, and M-CSF separately or in combination for early-stage ovarian cancer will

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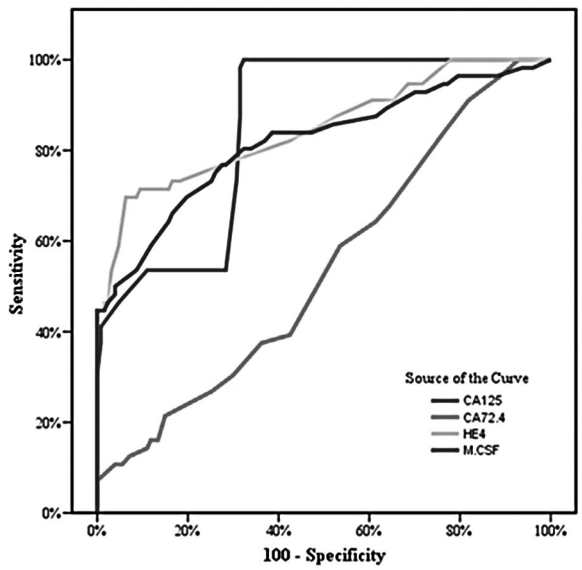

Figure 1. — ROC curve for all markers according to Stage.

be evaluated.

Materials and Methods

The study was formally approved by the Ethical Committee of the Faculty of Medicine, Alexandria University before commencing the research. A total of 126 patients with suspected early ovarian cancer (Stages I and II) were recruited from Alexandria University-hospital's gynecology clinic, during the period of January 2014 to August 2016. One hundred twenty-seven age-matched healthy women (controls) were also recruited. A full informed consent was taken from all subjects before commencement. Full history taking and thorough physical examination as well as, imaging studies via transvaginal ultrasound (TVUS), and computed tomography were performed in all subjects. Patients were further subdivided based on the disease stage into group 1 in Stage I (n=57) and group 2 in Stage II (n=69). All enrolled subjects promptly received the appropriate management according to the standard protocols. Histopathology confirmed the disease in all subjects' ovarian specimens. Staging of the disease was also confirmed during surgery. For all subjects, a preoperative serum sample was obtained using standardized sampling, separation, and analysing process following approved internal quality control results. CA 125 and CA 27.4 were analysed using diagnostics, while HE4 and M.CSF were analysed using ELISA technique.

Data were fed to the computer and analyzed using SPSS software package version 20.0. The Kolmogorov-Smirnov test was used to verify the normality of distribution of variables. Student *t*-test was used to compare two groups for normally distributed quantitative variables while ANOVA was used for comparing the four studied groups. Receiver operating characteristic curve (ROC) was used to determine the diagnostic performance of the markers. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. Significance of the obtained results was judged at the 5% level.

The diagnostic performance of all markers for prediction of early ovarian cancer was performed using Receiver operating characteristics curve (ROC). Also the regression analysis was done for combined markers, as well as evaluation of sensitivity at certain specificities 90% and 95% to evaluate the best sensitivity for these markers to be used for early diagnosis of ovarian cancer.

Table 1. — *Comparison between patients and controls according to serum markers' levels.*

	Cases (n=126)	Control (n=127)	*p*
CA125	45.6 ± 15.8	22.3 ± 8.3	<0.001*
CA72.4	3.9 ± 1.7	2.3 ± 0.7	<0.001*
HE4	289.2 ± 152.7	113.4 ± 15.5	<0.001*
M.CSF	1483.5 ± 691.9	579.7 ± 128.6	<0.001*

*Normally quantitative data was expressed in mean ± SD and was compared using student t-test. *Statistically significant at p ≤ 0.05.*

Results

There was no difference in terms of mean age among the studied groups (patients' group: 50.8 (SD 9.13) years, and control: 49.45 (SD 8.42) years). Seventy-six patients were menopausal (59.8%). Histology of tumours in patients were serous in 64.5%, mucinous (17.3%), endometrioid (13.3%), clear cell (3.9%), and germ cell in (0.7%) A significant difference between cases and controls, were detected in terms of each of the serum level of the studied markers (Table 1).

The diagnostic performance for the studied markers in patients in Stage I, namely: CA 125, CA 72-4, HE-4, and M.CSF, showed sensitivities of 50%, 7%, 68.42%, and 32.14%, respectively, calculated at the upper limit of reference interval as a cut off. The sensitivity was also evaluated at fixed specificities of 90% and 95%, which increased all sensitivities significantly. This was also shown with a ROC curve (Figure 1, Table 2). Applying the logistic regression model for all studied markers, significantly increased the sensitivity at the same specificity level. It also confirmed a better diagnostic performance in Stage I, when combining the four markers together, with area under the curve (AUC) of 0.978, and a sensitivity of 91.07, at a fixed specificity of 90%, which did not change by adding CA 72.4 to the other three markers, due its low sensitivity (Tables 3 and 4).

Discussion

Ovarian cancer is not one of the most common malignancies, however, its high case-to-fatality rates, makes it crucial, to possibly detect the disease at an early stage, where distinctive symptoms are usually lacking [9]. One in every four to five patients at an early stage of this fatal disease can be diagnosed, and recommendation of a single screening test for a specific population at risk, is not yet available [10, 11]. Early detection of the disease, specifically in Stage I, may help increase the rates of patients' survival up to 90% [12].

The present findings supported a valuable diagnostic role

Table 2. — *The diagnostic performance for the studied markers.*

	AUC	*p*	95% CI (LL - UL)	Cut off	Sensitivity	Specificity	PPV	NPV
CA125	0.850*	<0.001*	0.8 – 0.9	>35	50.0	92.1	73.7	80.7
CA72.4	0.540	0.389	0.5 – 0.6	>4	7.0	100.0	100.0	70.6
HE4	0.849*	<0.001*	0.8 – 0.9	≥140	68.42	93.7	83.0	86.9
M.CSF	0.818*	<0.001*	0.7 – 0.9	≥1044	32.14	100.0	100.0	77.0

*CA 125 U/ml, CA 72.4 ng/ml, HE4 pmol/l, and M.CSF pg/ml. *Statistically significant at p ≤ 0.05.*

Table 3. — *Diagnostic performance for all markers separately, and in combination.*

	AUC	*p*	95% CI		Sensitivity	
			LL	UL	Specificity 90	Specificity 95
CA125	0.850	<0.001*	0.795	0.905	53.57	50.0
CA72.4	0.540	0.389	0.451	0.629	15.79	12.28
HE4	0.849	<0.001*	0.782	0.915	71.93	70.18
M.CSF	0.818	<0.001*	0.744	0.891	58.93	51.79
CA125,CA72.4	0 .851	<0.001*	0.796	0.906	53.57	48.21
CA125,HE4	0.949	<0.001*	0.921	0.977	83.93	62.50
CA125,M.CSF	0.922	<0.001*	0.885	0.959	62.50	60.71
CA72.4,HE4	0.851	<0.001*	0.784	0.918	70.18	68.42
CA72.4,M.CSF	0.820	<0.001*	0.746	0.894	58.93	51.79
HE4,M.CSF	0.948	<0.001*	0.915	0.982	80.36	80.36
CA125, CA72.4, HE4	0.949	<0.001*	0.922	0.977	83.93	62.50
CA125, CA72.4, M.CSF	0.923	<0.001*	0.886	0.960	62.50	60.71
CA125, HE4, M.CSF	0.978	<0.001*	0.959	0.996	91.07	87.50
CA72.4, HE4, M.CSF	0.949	<0.001*	0.915	0.982	80.36	80.36
CA125, CA72.4, HE4, M.CSF	0.978	<0.001*	0.960	0.996	91.07	87.50

Table 4. — *Sensitivity and predictive values for each marker at a fixed specificity (90 and 95).*

	Sensitivity	NPV	PPV	Sensitivity	NPV	PPV
CA125	53.57	68.18	81.29	50.0	73.68	80.69
CA72.4	15.79	39.13	70.19	12.28	50.0	70.0
HE4	71.93	68.33	87.10	70.18	83.33	87.50
M.CSF	58.93	68.75	82.96	51.79	78.38	81.51

for each of the four studied markers; namely: CA-125, CA72-4, HE-4, and M-CSF, with significantly higher serum levels in patients in Stage I disease, than controls. Sölétormos *et al.* described a low sensitivity (50–62% for early stage epithelial ovarian cancer) and limited specificity (94–98.5%) for CA125 to be a cause for not using it as a screening test in asymptomatic women[5]. When adding other markers, such as CA72-4, HE-4, and M-CSF, to CA-125, the current study confirmed a significant increased diagnostic potential for Stage I disease, in terms of higher sensitivities at a fixed specificity of 90, and 95%. Skates *et al.* verified an increase in sensitivity for early-stage ovarian cancer from 45% to 70% at a fixed specificity of 98%, by addition of CA 72-4 and M-CSF to CA-125 in screening preoperatively [13]. At present, CA125 remains the most important biomarker for epithelial ovarian cancer, excluding tumors of mucinous origin [5]. Wu *et al.* evaluated HE-4 in patients with pelvic masses, and they reported a pooled sensitivity and specificity to diagnose ovarian cancer to be 83 up to 88% and 90 up to 92%, respectively [14], which supports the present work (sensitivity 70-72% at a specificity of 90-95%). The European Group on Tumor Markers (EGTM)' stated that HE-4 measurements, either alone or in combinations with CA125, may be considered for differential diagnosis of pelvic masses especially in premenopausal patients [5]

Lenhard *et al.* and Anastasi *et al.* confirmed that CA72-4 serum levels do rise with ovarian cancer but not with endometriosis and thus, CA72-4 evaluation may have a role in the differentiation between malignant and benign ovarian conditions [8, 15]. The present findings match the previous recommendations by Będkowska *et al.*, who

suggested a significant benefit of M-CSF in diagnosing epithelial ovarian cancer, particularly in combination with CA 125 and HE4 for the detection in the early stages [16]. Bian *et al.* suggested that serum CA72-4, CA15-3, and CA125 could be a good combination in the diagnosis of ovarian cancer [17]. The present work confirmed and added a value to their suggestion, by adding two other markers, presumably boosting early detection potential.

Shadfan *et al.* postulated that the most promising markers' panel tested after evaluating sensitivity, specificity, and biological variability, were a combination of CA125, HE4, MMP-7, and CA72-4 with the highest sensitivity and specificity [10].

No other previous study to the present authors' knowledge, combined those four markers to evaluate their diagnostic performance in early-stage ovarian cancer in a prospective analysis on a relatively large cohort. The present study's limitations may include: relatively-non-homogenous sample size, lack of long term follow up, and not combining imaging reports to the diagnostic performance assessment.

In conclusion, early detection of ovarian cancer is one of the most important targets that will improve the survival rates as well as the quality of life for those patients. Using non-invasive and simple markers' panel for early detection of ovarian cancer is the ultimate goal for the best clinical practice. No single marker can be used for this purpose due to either decreased sensitivity or specificity, although using multiple markers can boost the diagnostic performance panel used, to be reliable enough for the diagnosis. Based on the present results, the authors suggest using the four studied markers in combination for satisfactory and reliable diagnostic performance.

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