NOVEL BIOMARKERS IN THE DIAGNOSIS OF OVARIAN CANCER

Doctoral (PhD) Dissertation
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1 INTRODUCTION

Amongst all gynecological cancers, ovarian cancer poses the most difficult in diagnosis and treatment. This type of gynecological malignancy is either diagnosed by chance (e.g. during a diagnostic laparoscopy) owing to the fact that in early stages it is almost symptomless, or when it is already in an advanced stage that gives rise to symptoms. For the above-mentioned reasons ovarian cancer has earned in the United States of America the nickname (The Silent Killer). This phenomenon is due to the fact that our current early diagnostic tools (biomarkers) are extremely limited. Its high mortality rate has made it one of the most investigated fields in gynecological oncology.

As the father of medicine Hippocrates of Kos said, "He who diagnoses best also cures best" that is the main reason that we as physicians should strive to discover novel diagnostic tools, that will help us to better serve humankind. Even though we are in the 21st-century our arsenal of weapons against in diagnosing ovarian cancer is extremely limited. As physicians whose solemn duty under oath is to serve humanity we should strive for excellence and not rest until we have obtained the tools necessary to diagnose and fight this malicious disease.

2 AIMS OF THE STUDY

14-3-3 zeta is an important regulatory protein, which mediates intracellular signaling pathways by interfering with approximately 100 cellular proteins, including oncogenes and protooncogenes. Recently, two independent research groups, Waldemarson et al. and He et al., advocated 14-3-3 zeta as a potential biomarker for EOC. In addition, Kobayashi et al. recently demonstrated that 14-3-3 zeta protein is present in malignant ascites of patients with EOC, and is secreted by ascetic monocytes and macrophages. However, while the role of 14-3-3 zeta protein as an intracellular adaptor protein has been widely investigated, the function of the secreted protein is unclear.

1. The goal of the current pilot study was to assess the potential for 14-3-3 zeta protein to serve as a novel biomarker for monitoring patients with FIGO stage II-III EOC that undergo chemotherapy.

3.1 Materials and methods

3.1.1 Patients and follow-up study design

This prospective study was approved by the University of Pecs Institutional Ethical Review Board (#4076.316-251/KK15/2011), and written informed consent was obtained from all enrolled patients.

Peripheral blood samples were collected preoperatively from 13 patients admitted for six cycles of first line chemotherapy (paclitaxel/carboplatin; Hungarian OEP Chemotherapy protocol # 7167).

Chemotherapy dosage was calculated using (AUC5-7.5) for carboplatin and (175 mg/m^2) for paclitaxel, and treatments were administered in 21 d intervals at the University of Pecs Medical Center Department of Obstetrics and Gynecology/Oncology (Pecs, Hungary) in 2012.

Blood samples were collected 1-2 h before each treatment into citrate tubes. These tubes were then centrifuged (5000 rpm for 10 min), and blood plasma samples were collected and stored at −80 °C. When needed, samples were thawed at room temperature, and then were thoroughly vortexed as indicated by the manufacturer's recommendation.

In all patients, aged 51 to 73 y (mean, 60 y), only sub-optimal gynecological surgery could be performed. Computed tomography (CT) scans were performed prior to and following the completion of chemotherapy treatment. These images were used to assess changes in both target and non-target lesions. Each diagnosis was verified according to histopathology studies of the original tumors. Histopathological grade and stage of disease (according to FIGO criteria) were available for all malignant cases, and included FIGO stage IIa (n = 2), stage IIIb (n = 2), and stage IIIc (n = 9) cases (Table 1).

Patient no.	Histology	FIGO stage	Age, y	Tumor grade*
1.	Serous	III C	69	High
2.	Serous	III C	57	High
3.	Serous	III B	51	High
4.	Serous	II A	50	High
5.	Serous	III C	67	High
6.	Serous	III C	64	High
7.	Serous	III B	73	High
8.	Adenosquamous	II B	51	High
9.	Adenosquamous	III C	69	High
10.	Serous	III C	55	High
11.	Serous	III C	71	High
12.	Serous	III C	60	High
13.	Serous	III C	53	High

*According to International Federation of Obstetrics and Gynecology (FIGO) criteria.

Table 1: *Clinicopathological features of the patients enrolled in this study that underwent six cycles of paclitaxel/carboplatin-based chemotherapy for treatment of EOC.*

3.1.2 Computed tomography (CT) scans

CT scans were performed in the Department of Radiology (University of Pecs, Hungary). Contiguous 5 mm axial slices obtained through the abdomen and pelvis. Prior to examination high-concentration iodinated contrast agent was administered intravenously (Iomeron 400, Bracco Diagnostic Imaging). Field-of-view was adjusted to body habitus (to include the whole body including the skin). Target and non-target lesions were defined based on Responsive Evaluation Criteria In Solid Tumors (RECIST) 1.1 guidelines (www.recist.com). These guidelines are a set of rules published in January 2009 by an international collaboration including the European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States of America and the National Cancer Institute of Canada Clinical Trials Group that define when cancer patients improve ("respond"), stay the same ("stable") or worsen ("progression") during treatments. Today, the majority of clinical trials evaluating cancer treatment for objective response in solid tumors are using RECIST 1.1. A lesion was measurable and defined as a target lesion if the tumor was ≥ 10 mm along its longest diameter (LD) on a CT axial image with ≤ 5 mm reconstruction intervals, or if lymph nodes were ≥ 15 mm along their short axis on CT images. Non-target lesions were considered to be: masses with a diameter < 10 mm, lymph nodes with a diameter of 10-14 mm along the short axis. Ascites, pleural or pericardial effusion, abdominal masses, or organomegaly were identified by physical

exam. Furthermore, these could not be measured by reproducible imaging techniques. CT scans were performed 1-2 weeks after radical surgeries were performed, 1-2 weeks prior to chemotherapy, and 1-2 weeks after the final chemotherapy treatment.

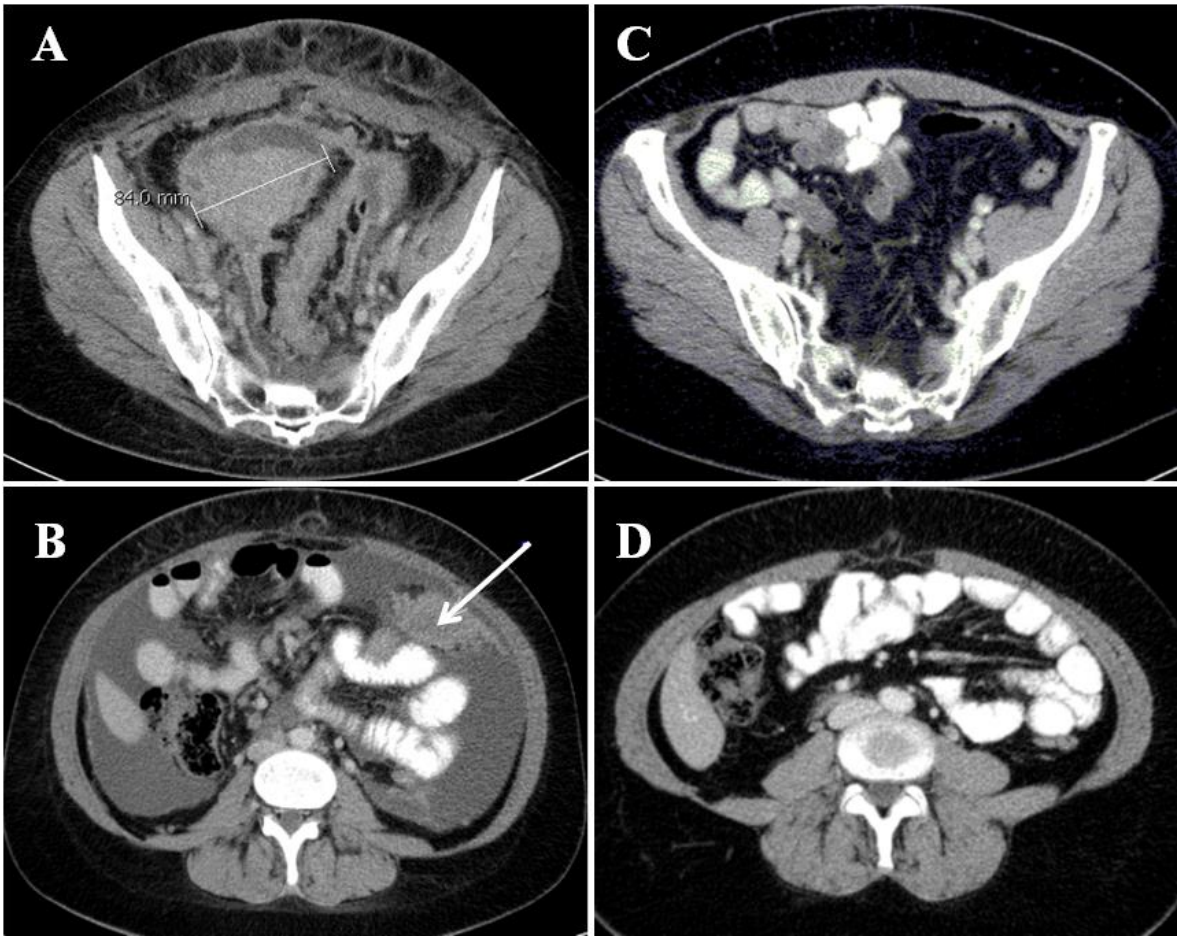


Figure 1: *Axial CT slices of patient # 3 after contrast material was intravenously administered. A target lesion with a SLD of 84 mm is localized near the minor pelvis before* ***(A)*** *and after* ***(C)*** *six cycles of first-line chemotherapy.* ***(B)*** *The arrow represents a mesenteric peritoneal carcinosis (non-target lesion) in the same patient that is level with the lower edge of the liver prior to chemotherapy. A significant amount of ascites associated with the non-target lesion is also observed* ***(B)****.* ***(D)*** *Both non-target lesions are absent after the completion of chemotherapy.*

3.1.3 Laboratory methods

3.1.4 Enzyme-linked immunosorbent assay (ELISA)

Levels of serum CA-125 (Fujirebio Diagnostics, Malvern, PA; Catalog #: 400-10, Lot. # 29191), HE4 (Fujirebio Diagnostics, Malvern, PA; Catalog #: 404-10, Lot# 28373), and 14-3-3 zeta protein (Cusabio Biotech, Wuhan, China; Catalog #: CSB-EL026293HU, Lot. #A26174460) were determined using a quantitative sandwich enzyme immunoassay according to each manufacturer's protocol. Serum concentrations were calculated using Optima 2.10 R2 built-in data calculator software.

3.1.5 Quantitative electrochemiluminescence assay (ECLIA)

Tumor marker levels were measured using a Roche electrochemiluminescent fully automated immunoassay system (ECLIA, Roche Diagnostics, http://www.roche-diagnostics.com). To determine serum levels of CA-125 (Cat. no. 11776223), and HE4 (Cat. no. 05950929), samples were processed using a Roche Cobas e411 analyzer. Master calibration, imprecision, and inaccuracy were checked using bi-level quality controls prior to the analysis of patient serum samples.

3.1.6 Risk for Ovarian Malignancy Algorithm (ROMA) index

The ROMA used serum levels of HE4 and CA-125 measured either by ELISA and ECLIA, and was calculated using an Excel spreadsheet with preset formulas to generate the predictive index (PI) value for EOC as follows:

For postmenopausal women: PI = -8.09 + 1.04*LN [HE4] + 0.732*LN [CA125]

A ROMA value was then calculated as follows: ROMA value (%) = exp(PI) / [1 + exp(PI)]*100. According to the manufacturer's manual, the detection of HE4 by ECLIA and CA-125 by ELISA in post-menopausal women identified an EOC high-risk index value equal to, or higher than, 25.3 %.

3.1.7 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistic 20 (IBM Corporation) at the University of Pecs, Institute of Bioanalysis. The sample size (n) was 13, and comparisons were made between treatments and between methods according to the Wilcoxon signed-rank test. To

evaluate trends between the number of treatments and serum levels of tumor markers, linear regression and correlation analyses were applied. To examine the relationship between tumor marker levels and CT scan results, Spearman's rank correlation coefficient was used. Mean data are reported ± standard error of the mean (SEM). Statistical significance was set at $p < 0.05$, or $p < 0.01$.

3.2 Results

3.2.1 Radiologic assessment following therapeutic procedures

CT scans were obtained one to two weeks after radical gynecological surgeries were performed. After an initial laparotomy, 10 out of 13 (76.92 %) patients were found to have residual tumor present prior to induction of paclitaxel/carboplatin-based chemotherapy. After six consecutive cycles of treatment within 21 d intervals, CT scans were repeated. At this point, residual tumor with a LD value >1 cm was only detected in 4 out of 13 (30.76 %) patients (Table 2). Based on the detection of 26 non-target lesions in pre-chemotherapy CT scans, and only 3 non-target lesions in post-chemotherapy scans, the efficacy of chemotherapy for EOC treatment is demonstrated (Figure 1).

Patient no.	SLD of target lesions 10 (+/- 4) d before chemotherapy (mm)	SLD of target lesions 10 (+/- 4) d after the last cycle of chemotherapy (mm)	Non-target lesion changes*
1	22	0	-1
2	56	0	-1
3	84	0	-1
4	0	0	0
5	137	0	-4
6	0	0	0
7	0	0	0
8	441	202	-1
9	256	18	-3
10	147	0	-3
11	125	0	-1
12	46	18	-1
13	39	14	-3

SLD: sum of length diameter; according to RECIST 1.1 guidelines.
*Negative values represent a decrease in lesion size.

Table 2: *Evaluation of tumor size before and after paclitaxel/carboplatin chemotherapy using CT scans.*

3.2.2 Detection of CA-125, HE4 and 14-3-3 Zeta protein

Levels of CA-125, HE4, and 14-3-3 zeta protein were monitored throughout the treatment period by ELISA and ECLIA.

On the first day of chemotherapy, the mean concentration of CA-125 was 147.87 ± 55.98 U/ml and 648.26 ± 186.52 U/ml, respectively. After completing the sixth cycle of chemotherapy, CA-125 levels were lower, with the mean concentrations detected being 58.54 ± 30.89 U/ml and 119.70 ± 22.75 U/ml, respectively. Similarly, mean serum levels of HE4 detected on the first day of chemotherapy by ELISA were 455.32 ± 106.39 pM, and decreased to 120.52 ± 23.76 pM upon completion of chemotherapy.

Using the ECLIA method, serum levels of HE4 were 1383.49 ± 577.23 pM on the first day of chemotherapy, and decreased to 70.12 ± 26.44 pM upon completion of chemotherapy. ROMA index values were subsequently calculated, and decreased from 58.17 ± 10.05 % to 28.95 ± 7.67 %, and from 69.62 ± 9.91 % to 30.78 ± 7.91 % for ELISA and ECLIA, respectively.

According to Wilcoxon statistical analyses, the differences in the values determined at the start of treatment versus upon completion of treatment were significant ($p < 0.05$), thus further demonstrating the effectiveness of chemotherapy for EOC (Figure 2/A-F, Table 3).

For 14-3-3 zeta protein levels detected in patients prior to chemotherapy by ELISA, the mean concentration was 1.93 ± 0.57 ng/ml. In contrast, the mean concentration of 14-3-3 zeta protein for healthy postmenopausal women (mean age, 58 y), was 0.39 ± 0.11 ng/ml. Subsequently, at the start of chemotherapy, the mean serum level of 14-3-3 zeta protein in EOC patients was 2.38 ± 1.44 pg/ml, and 2.17 ± 1.71 pg/ml after the final treatment. Neither the difference in levels detected for EOC patients and healthy postmenopausal patients, either at the beginning or end of chemotherapy, was found to be significant (Figure 2/G).

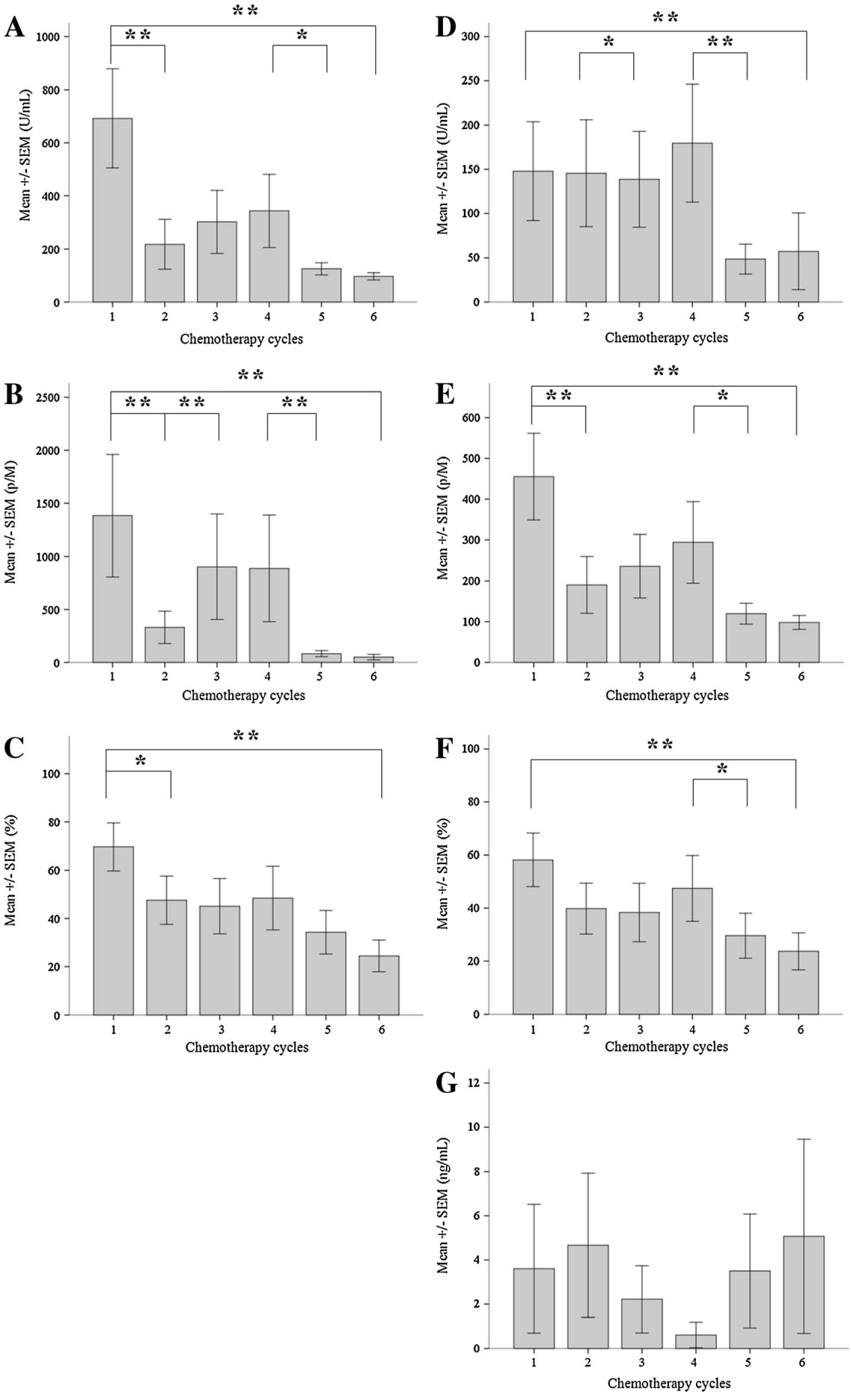

A
Mean +/- SEM (U/mL)
Chemotherapy cycles
B
Mean +/- SEM (p/M)
Chemotherapy cycles
C
Mean +/- SEM (%)
Chemotherapy cycles
D
Mean +/- SEM (U/mL)
Chemotherapy cycles
E
Mean +/- SEM (p/M)
Chemotherapy cycles
F
Mean +/- SEM (%)
Chemotherapy cycles
G
Mean +/- SEM (ng/mL)
Chemotherapy cycles

Figure 2: *Mean levels of serum biomarkers (CA-125, HE4 and 14-3-3 zeta protein) measured with ELISA and ECLIA methods, and the ROMA index values. Average values of CA-125 (U/mL) +/− SEM determined by ECLIA* ***(A)*** *and by ELISA* ***(D)*** *are shown according to the chemotherapy cycles (consistently on each x-axis from 1 to 6). Mean concentrations of HE4 (p/M), measured by ECLIA* ***(B)*** *and by ELISA* ***(E)*** *are also shown. Furthermore average values determined by ELISA* ***(G)*** *of 14-3-3 zeta protein (ng/ml) are depicted. Based on the above-mentioned data, we calculated the postmenopausal ROMA index values (%) for ECLIA* ***(C)*** *and for ELISA* ***(F)*** *techniques. We present the mean levels of 14-3-3 zeta protein (ng/mL) for each of the treatment days. Wilcoxon signed-rank test analysis was performed for each diagram, and statistical significance represented with an asterisk (*) was set at $p < 0.05$, and (**) when $p < 0.01$.*

Patient no.	Chemo Cyle no.	ELISA			ECLIA			ELISA
		CA-125 (U/mL)	HE4 (p/M)	ROMA %	CA-125 (U/mL)	HE4 (p/M)	ROMA %	14-3-3 Z (ng/mL)
1	1	23.38	95.87	26.19	105.70	79.63	46.90	38.28
	2	18.09	65.94	16.60	76.82	30.69	20.57	37.95
	3	7.78	64.47	9.49	73.55	15.77	11.20	18.78
	4	NA	NA	NA	NA	NA	NA	NA
	5	10.71	67.85	12.26	77.76	8.58	6.5	32.01
	6	7.90	62.81	9.35	75.21	9	6.60	39.82
2	1	26.93	334.49	58.99	353.50	176.10	83	0
	2	24.28	102.01	28	116.10	106.20	56	0
	3	19.31	66.42	17.37	79.16	29.24	20.10	0
	4	10.16	87.74	14.97	88.92	18.22	14.30	0
	5	3.33	81.38	6.7	88.28	15.06	12	0
	6	0.08	85.94	0.49	87.33	13.11	10.50	0
3	1	1.45	43.73	2	83.1	52.97	32.6	0
	2	0	49.24	0	67.49	28.04	17.70	0
	3	3.47	52.41	4.48	69.24	23.49	15.4	0
	4	NA	NA	NA	NA	NA	NA	NA
	5	0	44.09	0	52.38	17.25	9.7	0
	6	9.12	52.37	8.68	62.76	24.63	15.10	0
4	1	0	48.51	0	55.06	6.78	4	1.34
	2	0	55.9	0	58.43	6.91	4.30	1.42
	3	3.63	47.48	4.18	54.72	6.71	4	0.39
	4	2.55	45.86	3.15	53.12	6.08	3.50	0.32
	5	4.35	51.67	5.16	58.04	6.59	4.10	0.26
	6	NA	NA	NA	NA	NA	NA	NA
5	1	9.23	101.40	15.93	133.4	14.60	15.20	0
	2	12.40	93.07	17.77	107.40	8.69	8.20	0
	3	2.78	85.49	6.21	103.30	7.08	6.50	0
	4	14.24	69.76	15.03	75.26	6.66	4.90	0

	5	9.10	75.68	12.20	80.91	7.95	6.20	0
	6	9.73	74.47	12.55	80.21	7.89	6.10	0
6	1	153.04	904	93.53	1500	701.5	98.30	0
	2	445.65	904	96.94	1218	644.20	97.90	0
	3	368.55	714.78	95.57	843.10	394.10	95.50	0
	4	249.51	541.89	92.41	540.10	243.90	90.30	0
	5	95.43	259.90	73.68	227.30	84.13	65.40	0
	6	39.94	167.57	48.43	142	46.16	38.30	0
7	1	77.86	118.41	51.49	111.90	68.66	44.10	4.36
	2	17.01	122.23	26.50	124.90	22.64	22.64	15.02
	3	9.25	120.03	18.50	127	18.20	17.80	8.28
	4	8	107.76	15.47	111.10	16.34	15	6.38
	5	1.07	53.14	1.98	132.50	15.39	15.80	13.13
	6	9.03	202.84	27.82	186.80	26.99	30.30	5.74
8	1	475.23	259.74	90.07	248.50	4810	99.2	0.04
	2	500	90.83	75.92	80.07	1265	92.7	0
	3	189.18	67.99	53.34	70.60	642.10	85.20	0
	4	500	904	97.18	1500	5000	99.8	0
	5	172.63	57.11	47.22	57.21	254.10	65.30	0
	6	402.03	62.74	64.64	57.66	256.20	65.60	0
9	1	21.16	904	77.35	1500	185.40	93.70	2.46
	2	29.53	170.69	43.41	176.10	61.61	49.50	1.61
	3	4.97	97.08	10.38	103.70	21.29	18.10	1.32
	4	8.13	96.13	14.09	102.70	15.37	13.50	0
	5	5.90	105.57	12.56	108.90	13.93	12.80	0.13
	6	8.57	84.25	12.96	87.24	15.70	12.40	0.06
10	1	500	904	97.18	1500	5000	99.8	0
	2	189.91	62.04	51.08	60.85	243.10	65.30	0
	3	500	441.44	94.22	514.20	4789	99.5	0
	4	500	149.23	84.07	155.70	833.50	93.10	0
	5	84	127.48	54.97	121.70	197.90	71.60	0

	6	29.43	90.79	28.37	92.78	54.27	35	0
11	1	7.85	904	62.12	1500	117.70	90.20	0
	2	9.58	201.95	28.59	200.90	36.28	38.40	0
	3	0.93	131.04	4.42	125.90	18.88	18.30	0
	4	3.75	105.53	9.34	104.40	13.74	12.30	0
	5	2.65	107.64	7.54	107.80	13.41	12.30	0
	6	NA	NA	NA	NA	NA	NA	NA
12	1	126.02	397.04	84.2	404.30	1772	98.30	0.38
	2	500	365.72	93.07	323.20	1517	97.70	0
	3	192.79	273.67	83.2	258.90	761.40	94.70	0.09
	4	178.79	223.37	79.09	192.20	360.10	86.80	0
	5	121.86	168.58	68.17	169	265.70	81.30	0
	6	NA	NA	NA	NA	NA	NA	NA
13	1	500	904	97.18	1500	5000	99.8	0
	2	NA	NA	NA	NA	NA	NA	NA
	3	500	904	97.18	1500	5000	99.8	0
	4	500	904	97.18	853.90	3241	99.50	0
	5	120.65	355.03	82.16	349.30	171.90	82.5	0
	6	NA	NA	NA	NA	NA	NA	NA

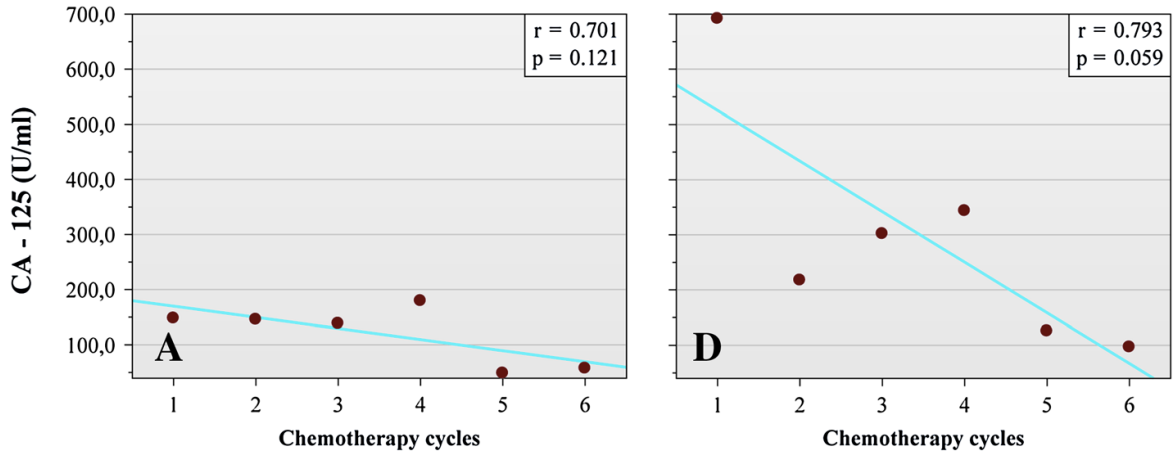
Table 3: *Levels of CA-125, HE4, and 14-3-3 Z protein determined by ELISA and ECLIA methods.*

3.2.3 Correlation between radiological findings and serum parameters

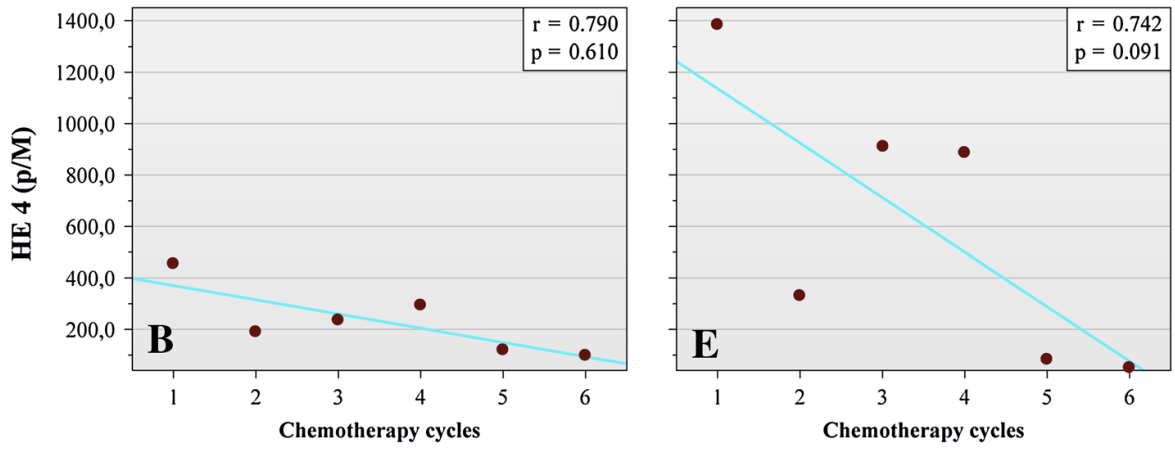
Neither ELISA nor ECLIA measurements of CA-125 and HE4 serum biomarkers provided significant linear regression correlations. However, the ROMA index values that were calculated based on these values did provide a strong significant regression correlation ($r = 0.840$, $p = 0.036$ and $r = 0.920$, $p = 0.009$, respectively) (Figure 3/C and F). Moreover, with a p-value margin of 0.01, a significant linear correlation was found for all ECLIA measurements.

Linear regression analysis of 14-3-3 zeta protein levels at each treatment day, showed no significant correlation between the mean serum values and the chemotherapy cycles ($r = 0.073$; $p = 0.089$). (Figure 3/G)

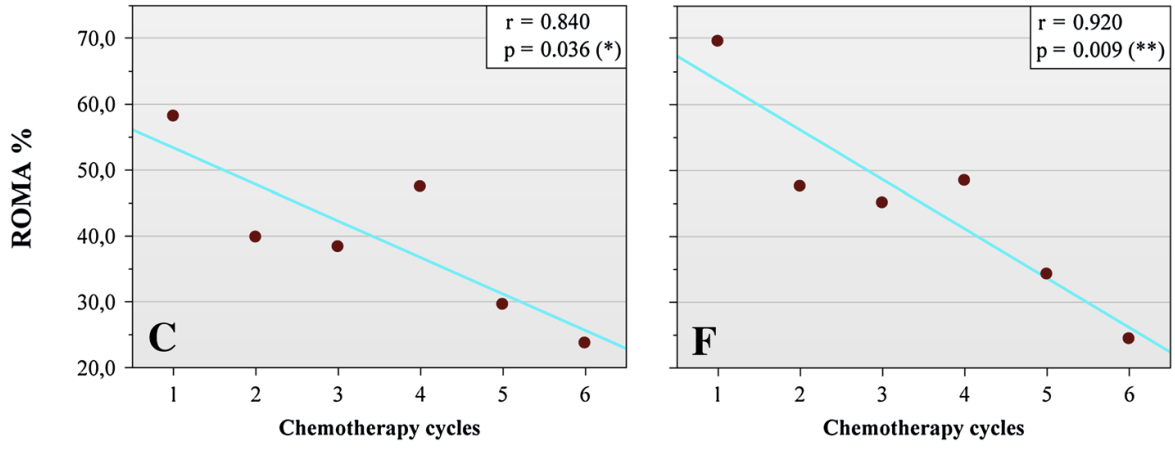
Spearman's correlation analysis further identified a significant correlation between CA-125 serum levels determined by ELISA and the largest tumor diameter measured by CT scans obtained following chemotherapy ($p = 0.011$). Levels of HE4 detected by ECLIA were also found to significantly correlate with tumor diameter ($p = 0.04$), while levels of 14-3-3 zeta protein did not significantly correlate with any of the examined parameters.


CA - 125 (U/ml)
700,0
600,0
500,0
400,0
300,0
200,0
100,0
r = 0.701
p = 0.121
A
1
2
3
4
5
6
Chemotherapy cycles

r = 0.793
p = 0.059
D
1
2
3
4
5
6
Chemotherapy cycles


HE 4 (p/M)
1400,0
1200,0
1000,0
800,0
600,0
400,0
200,0
r = 0.790
p = 0.610
B
1
2
3
4
5
6
Chemotherapy cycles

r = 0.742
p = 0.091
E
1
2
3
4
5
6
Chemotherapy cycles


ROMA %
70,0
60,0
50,0
40,0
30,0
20,0
r = 0.840
p = 0.036 (*)
C
1
2
3
4
5
6
Chemotherapy cycles

r = 0.920
p = 0.009 (**)
F
1
2
3
4
5
6
Chemotherapy cycles

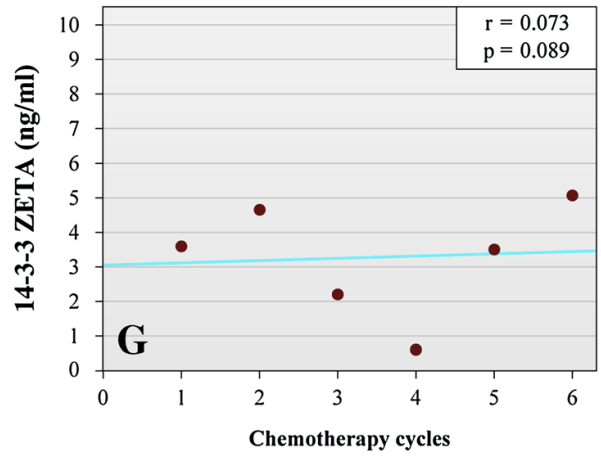

14-3-3 ZETA (ng/ml)
10
9
8
7
6
5
4
3
2
1
0
r = 0.073
p = 0.089
G
0
1
2
3
4
5
6
Chemotherapy cycles

Figure 3: *Changes in CA-125, HE4, and 14-3-3 zeta protein serum levels, and ROMA index values during the six cycles of paclitaxel/carboplatin-based chemotherapy that were performed. Mean concentrations of CA-125 determined by ELISA* ***(A)*** *and by ECLIA* ***(D)*** *are shown for each of the treatment days. Mean levels of HE4 determined by ELISA* ***(B)*** *and ECLIA* ***(E)*** *are also shown for each of the treatment days. Mean concentrations of 14-3-3 zeta protein were determined by ELISA* ***(G)*** *are represented at each chemotherapy day. Postmenopausal ROMA index values were calculated based on ELISA* ***(C)*** *and ECLIA* ***(F)*** *data. Linear regression analysis was performed for each diagram (see r value), and statistical significance was set at* $p < 0.05$.

3.3 Discussion

Several studies have demonstrated the limitations associated with depending on any single tumor marker for the detection of EOC. Initially, CA-125 was widely used. However, other malignant and benign diseases also express CA-125, thereby limiting its reliability as a tumor marker. In particular, CA-125 has a high false-positive rate among women with benign gynecological conditions such as endometriosis, and a low sensitivity in identifying patients with early-stage ovarian cancer.

Accordingly, when EOC is diagnosed, 80 % of cases are in an advanced stage of disease (e.g., FIGO III-IV). To improve the specificity and sensitivity of an ovarian cancer diagnosis, additional tumor markers have been investigated. One novel tumor marker is HE4, which contains two whey acid protein (WAP) domains and eight cysteine residues that constitute a four-disulphide bond core. HE4 localizes to human chromosome 20q12-13.1 and its expression significantly increases during malignant transformation. However, HE4 is expressed in normal tissues as well, and therefore, is not tumor specific. Correspondingly, it has been hypothesized that the function of HE4 is related to both spermiotelcosis (a protease inhibitor involved in sperm maturation) and natural immunity, although the details of HE4 function remain to be clarified.

As a tumor marker for the early detection of ovarian cancer, Moore et al. reported a sensitivity of 72.9 % and a specificity of 95 % for HE4. Moreover, when both HE4 and CA-125 were detected, the sensitivity increased to 76.4 %. Therefore, the detection of more than one biomarker resulted in a 33.1 % increase in the sensitivity of CA-125, and a 3.5 % increase in HE4 sensitivity.

In the present study, ROMA values provided a PI based on the postmenopausal status of a patient, and the presence and levels of biomarkers CA-125 and HE4. As such, this PI relies on an accurate determination of serum levels of HE4 and CA-125.

Moreover, in a recent study, the ROMA was found to be more effective in predicting ovarian cancer than the widely used risk of malignancy index (RMI), which employs ultrasound findings, CA-125 concentrations, and menopausal status.

Furthermore, when the specificity was set to 75 %, the RMI had a sensitivity of 84.6 %. For the same specificity, the sensitivity of the ROMA was significantly higher (94.3 %). Although biomarker concentrations can be assayed by various methods (e.g., ELISA, chemiluminescent microparticle immunoassay), a recent study conducted by Ruggeri et al. demonstrated that chemiluminescent immunoassays are more adequate and more reproducible than commercially available ELISA kits that are characterized by interassay imprecision percentages (CV %) ranging from 6.8-10.3 %, compared to < 4 % for ECLIA. The results of the present study are consistent with these findings, and they further support the use of the ECLIA method for routine determinations of CA-125 and HE4 levels. Furthermore, the deviation in accuracy for ELISA versus ECLIA can be attributed to the fully automated format of ECLIA, while ELISAs are manual assays that also require testing samples in duplicate.

14-3-3 zeta protein plays an important role in several different biological mechanisms. For example, it has been reported to be an adaptor protein for intracellular signaling since it contains tandem repeats of phosphoserine motifs that have the capacity to bind upstream and downstream signaling molecules (27-30). 14-3-3 zeta protein also facilitates cell migration by forming a ternary complex with integrin alpha-4 and paxillin.

However, 14-3-3 zeta also has potential roles in cancerogenesis, based on its ability to bind NF-kappa B, beta-catenin, and Bcl-2, and to augment cancer cell proliferation. Furthermore, 14-3-3 zeta protein has been shown to block activation of p38 mitogen-activated protein kinase (MAPK), thereby mediating an anti-apoptotic mechanism. Numerous investigations have also suggested that 14-3-3 zeta protein is a key molecule in the malignant pathological processes of several malignancies, including oral, esophageal, lung, and breast cancers, as well as B cell lymphoma.

Recently, He et al. reported that 14-3-3 zeta protein represents a candidate biomarker and a metastasis-promoting factor in ovarian cancer based on a serum proteomic analysis of a nude mouse xenograft model containing SKOV-3 cells and a mass spectrometry [liquid chromatography-tandem mass spectrometry (LC-MS/MS)] analysis to identify metastasis-related serum proteins. Significantly higher expression of 14-3-3 zeta was detected in EOC patients than in patients with benign gynecological diseases. Furthermore, compared to CA-125, serum levels of 14-3-3 zeta protein were significantly upregulated when microscopic peritoneal metastasis was present, or when both ovaries were involved. Accordingly, the authors suggested that 14-3-3 zeta protein may be a useful tool in differentiating FIGO stage Ib and Ic ovarian cancers from stage Ia ovarian cancers in the clinic.

However, the results of the present study are not consistent with these findings. For example, significant differences in the serum levels of 14-3-3 zeta protein were not detected in healthy menopausal women versus patients with advanced stage EOC. Furthermore, significant changes in serum levels of 14-3-3 zeta protein was not detected during the six consecutive cycles of chemotherapy treatment that were administered (Figure 3/G), although CT scans and CA-125 and HE4 levels unambiguously indicated the efficacy of the treatment.

A possible explanation for these results is the insufficient number of patients enrolled in the current study. Thus, future studies should include a larger cohort in order to identify statistically significant changes. It is also possible that serum proteins may undergo degradation, even when stored at -80 °C. In particular, it may be that 14-3-3 zeta is an unstable protein that needs to be assayed shortly after collection.

Furthermore, an intriguing possibility is that 14-3-3 zeta may bind proteins activated by chemotherapeutic agents, or present as a result of chemotherapy, thereby obscuring detection of 14-3-3 zeta protein in serum. In the future a large-scale clinical investigation is necessary to evaluate the efficacy of 14-3-3 zeta protein, and to determine the sensitivity and the specificity of this biomarker comparing it to CA-125 and to HE4.

3.4 Conclusion

In conclusion, determination of CA-125 and HE4 serum levels for the ROMA represents a useful tool for the prediction of chemotherapy efficacy for EOC patients. However, based on our current findings, levels of 14-3-3 zeta protein were not found to reliably correlate with the clinical behavior of EOC, and therefore we question if it would be a useful biomarker for this disease.

4 PUBLICATIONS OF THE AUTHOR

Original Research Articles upon which the Dissertation is based on:

Hatzipetros I, Gocze P, Koszegi T, Jaray A, Szereday L, Polgar B, Farkas B
Investigating the clinical potential for 14-3-3 zeta protein to serve as a biomarker for epithelial ovarian cancer.
JOURNAL OF OVARIAN RESEARCH 2013 Nov. 15;6(1):79 PMID: 24238270 **IF: 2.429**

Other Original Research Articles:

Hatzipetros I, Gocze PM, Cziraky K, Kovacs K, Kalman E, Farkas B.
Assessment of cells in the ascitic fluid of women with ovarian hyperstimulation syndrome: the clinical implications for subsequent ovarian malignancy.
REPRODUCTIVE BIOLOGY AND ENDOCRINOLOGY 2013 Sep 12;11(1):91. doi: 10.1186/1477-7827-11-91. PMID**:** 24028152 **IF: 2.144**

Letters to the Editor:

Hatzipetros I, Gocze PM, Farkas B.
Oral Contraceptive Pills as Primary Prevention for Ovarian Cancer: A Systematic Review and Meta-analysis.
OBSTETRICS AND GYNECOLOGY 2013 Nov;122(5) p. 1114. doi: 10.1097/01.AOG.0000435076.57585.21. PMID: 24150022 **IF: 4.798**

Hatzipetros I, Drozgyik I, Bodis J.
What is new in gynecologic oncology? Thought-provoking articles from the past year.
OBSTETRICS AND GYNECOLOGY 2013 Aug;122(2 Pt 1):393-4. doi: 10.1097/AOG.0b013e31829bd8a2. PMID: 23969813 **IF: 4.798**

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