Predicting value of HE4 and CA125 markers for optimal cytoreductive surgery in ovarian cancer patients

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Abstract

We conducted a cross-sectional study to evaluate the role of serum levels of CA125 and HE4 in predicting optimal cytoreductive surgery. Eligible women who had been diagnosed with ovarian cancer based on both clinical and imaging criteria were enrolled in this study. Serum levels of CA 125 and HE4 were checked before surgery and all patients underwent complete surgical staging. After completion of the pathological evaluation, data were entered in SPSS version 23. One hundred and ten individuals were enrolled in our study. We divided cases between two groups: stage I to IIIb and stage IIIc to IV. Serum level of HE4 >170 pmol/L can predict optimal cytoreductive surgery before operation. (sensitivity:80% and specificity 70 %) and serum level of CA 125 > 320 U/mL can predict optimal cytoreductive surgery before operation. (sensitivity:80% and specificity 70%). Our data demonstrated a negative predictive value of about 80% for both HE4 and CA125. Based on these cut-off, unnecessary surgery can be avoided in many cases, however, it is unwise to ignore clinical performance and radiological findings. Nevertheless, we can say the evaluation of tumor markers is feasible and helpful in predicting optimal surgery.

Key Words: HE4; CA 125 Ovarian neoplasm; optimal cytoreduction.
residual tissue < 2 cm changed into no macroscopic residual tumor.\textsuperscript{10} Advanced ovarian cancer has raised a multitude of questions about contemplating the best procedure and taking some drastic measures. There is no consensus among experts. Several studies shed some light on the matter of performing laparoscopy in advanced ovarian cancer, but laparoscopy is an invasive procedure with its own risks.\textsuperscript{11} Abdominal pelvic Computed Tomography (CT) has been used profusely to reveal omental cake, liver metastasis, retroperitoneal lymphadenopathy but there is no consensus that CT scan may predict whether the tumor is operable or not.\textsuperscript{15} Because all those previous studies suggest to find a cut-off level for pre-surgical diagnosis, we designed a cross-sectional study to evaluate the role of serum levels of CA125 and HE4 in predicting cytoreductive surgery.

**Materials and Methods**

**Study Design**

We conduct a cross-sectional study in a tertiary center hospital in Tehran, Iran, between 2015 – 2020. In this study, codes of ethics were obtained and general guidelines of ethics in medical science research were followed. This proposal has been approved by the ethical committee in our research center with the code of IR.IUMS.FMD.REC.1399. 843. The project was approved by the research department of the Faculty of Medicine. Informed consent was obtained from patients. One hundred and ten patients with ovarian neoplasm were enrolled in this study. All of them were referred from other centers with the diagnosis of ovarian neoplasm made by transvaginal sonography (TVS) or CT scan. Just for the matter of giving transparency in the diagnosis process to build up trust, we performed TVS again in our center as conceivable imaging to confirm pre-surgical diagnosis of ovarian neoplasm.

**Inclusion and exclusion criteria**

The inclusion criteria were as follows: individuals between 20-80 years old of age who were diagnosed with ovarian neoplasm based on clinical and pathological characteristics, eligible for complete staging surgery or cytoreductive surgery composed of unilateral cystectomy/oophorectomy for benign neoplasm and hysterectomy, bilateral salpingo-oophorectomy, omentectomy and retroperitoneal lymphadenectomy in malignant ovarian neoplasm. Exclusion criteria included smokers, history of hepatic, cardiac, or renal insufficiency, history of prior malignancy at other parts of the body, patients who did not have acceptable performance before surgery and took neo-adjuvant chemotherapy, and patients with thromboembolism.

**Method description and data collection**

For all cases, serum levels of CA 125 and HE4 were checked one week before surgery just in one laboratory. The blood sample was analyzed through chemiluminescent micro-particle immunoassay specific mean for CA125 (ARCHITECT CA125II assay; Abbott GmbH, Wiesbaden, Germany) or HE4 (ARCHITECT HE4 assay; Abbott GmbH Wiesbaden, Germany). After completion of the surgery, all ovarian neoplasms were evaluated by one pathologist who was an expert in Gynecology Oncology. Several variables such as benign or malignant morphology, stage of malignancy, histological type of tumor, grade of tumor, and lymph node involvement were collected.

**Statistical analysis**

Data were gathered and then entered SPSS version 23. Numerical variables were evaluated by Mean ± Standard Deviation (SD) and categorical variables by count & percentage. T-test and Chi-2 (or Exact Fisher) test were used for quantitative and qualitative variables, respectively. ANOVA and T-tests were used for evaluating the association between serum levels of tumor markers and clinic-pathological factors after surgery. To assess the predictive value (sensitivity, specificity, positive, and negative predictive values) of tumor markers, a cut-off of serum level of each one was extracted for prediction of optimal surgery, using the ROC curve (Receiver Operating Characteristic curve) considering its AUC (area under the curve), and $p < 0.05$ was considered significant.

**Results**

A total of 110 patients were enrolled in this study. They were between 20-80 years old. The mean and standard deviation for age was 49.1 ± 13.8. The mean for CA125 serum level was 732.82 U/mL ± 1293.29, and the mean for HE4 serum level was 597.78 pmol/L ± 1049.25.

Table 1 shows clinicopathological factors of patients' and their association with serum levels of HE4 and CA125. The serum level of HE4 was significantly higher in malignancies with lymph node involvement ($p = 0.003$), omental involvement ($p = 0.001$), and in papillary serous tumors ($p = 0.001$) and in higher stage ($p = 0.005$), while there was no significant association between HE4 level with histopathological grade ($p = 0.015$). There was no significant association between CA125 level with lymph node involvement, omental involvement, stages, grade and type of tumor. Among all 110 women enrolled in our study, 9 patients (8.2%) had benign ovarian neoplasm, 7 patients (6.4%) had a borderline ovarian cancer and 94 patients (85.5%) had malignant ovarian neoplasm. Patients with benign ovarian neoplasms were excluded and we carried out the analysis on 101 cases. Based on the tumor type, in 94 cases with malignant neoplasm, 54 cases had papillary serous subtype, 9 cases had endometrioid adenocarcinoma, 8 cases had clear cell carcinoma and the rest of the cases had other types of ovarian cancer.

Given the abundant evidence, unlike women with stage I to IIb, in patients with stage IIIc or stage IV, optimal cytoreductive surgery may be out of reach. Therefore, it
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is assumed that in advanced ovarian cancer, neoadjuvant chemotherapy followed by cytoreductive surgery is more acceptable.

Thus, in Table 2 we divided cases between two groups: stage I to IIIb and stage IIIc to IV. This table revealed that serum level of HE4 >170 pmol/l can predict optimal cytoreductive surgery before operation (sensitivity: 80% and specificity 70%) and serum level of CA 125 >320 UI/mL can predict optimal cytoreductive surgery before operation (sensitivity: 80% and specificity 70%).

Table 1. Clinic-pathologic factors and their association with serum level of HE4 and CA 125

| Variables          | Number | HE4          | CA 125        |
|--------------------|--------|--------------|---------------|
|                    |        | Mean± SD     |               |
| Age:               |        |              |               |
| < 50               | 48     | 338.96 ± 518.80 | 739.96 ± 1681.06 |
| ≥ 50               | 53     | 832.17 ± 1325.17 | 726.35 ± 813.76  |
| P-value            |        | 0.018        | 0.958         |
| Tumor Type:        |        |              |               |
| Papillary          | 54     | 917.04 ± 1317.94 | 906.64 ± 1087.37 |
| serous             |        | 230.97 ± 367.94 | 533.12 ± 1482.41 |
| Other types        | 47     |              |               |
| P-value            |        | 0.001        | 0.149         |
| Grade:             |        |              |               |
| 1                  | 22     | 231.78 ± 280.68 | 410.09 ± 616.02  |
| 2                  | 7      | 503.00 ± 497.55 | 962.23 ± 1196.37 |
| 3                  | 72     | 718.82 ± 1203.80 | 809.13 ± 1441.94 |
| P-value            |        | 0.158        | 0.402         |
| Lymph node         |        |              |               |
| involvement:       |        |              |               |
| Positive           | 41     | 970.65 ± 1465.17 | 943.66 ± 1134.26 |
| Negative           | 60     | 342.98 ± 497.94 | 588.74 ± 1382.27 |
| P-value            |        | 0.003        | 0.177         |
| Omental involvement: |      |              |               |
| Positive           | 47     | 978.27 ± 1394.84 | 957.59 ± 1134.23 |
| Negative           | 54     | 266.60 ± 385.34 | 537.19 ± 1308.38 |
| P-value            |        | 0.001        | 0.103         |
| Stage:             |        |              |               |
| I                  | 40     | 203.44 ± 341.65 | 477.44 ± 1599.59 |
| II                 | 11     | 365.82 ± 448.01 | 561.43 ± 564.20  |
| III                | 45     | 955.09 ± 1412.33 | 969.92 ± 1101.87 |
| IV                 | 5      | 1047.00 ± 742.01 | 1019.00 ± 1117.37 |
| P-value            |        | 0.005        | 0.323         |

Considering the ROC curve analysis, AUC (area under the curve) and 95% CI (confidence interval) of the lower stage (I-IIIB) in comparison with the higher stage (IIIC-IV) HE4 and CA125 were 82.3% (74.1-90.5) and 79.3% (70.3-88.2), respectively (Figure 1).

Discussion
It is intuitively obvious and incontrovertibly true that cytoreductive surgery followed by chemotherapy is

Table 2. Predictive value of HE4 and CA 125 considering cut-off values for optimal cytoreductive surgery before the operation (Stage I –IIIb versus IIIc-IV).

| Description | HE4 cut-off | CA125 cut-off |
|-------------|-------------|---------------|
| Sen         | 70          | 320           |
| Spec        | 70          | 320           |
| PPV         | 70          | 320           |
| NPV         | 70          | 320           |
| Sen         | 140         | 80            |
| Spec        | 140         | 70            |
| PPV         | 140         | 66            |
| NPV         | 140         | 0.68          |
| Sen         | 170         | 80            |
| Spec        | 170         | 70            |
| PPV         | 170         | 0.68          |
| NPV         | 170         | 0.81          |

| Value       | 98          | 320           |
|-------------|-------------|---------------|
| PPV         | 0.58        | 0.71          |
| NPV         | 0.96        | 0.82          |

Sen: Sensitivity; Spec: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value.
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considered the standard of care in ovarian cancer. However, no defined criteria have been determined so far to prove exactly who is the best candidate for optimal surgery. Finding the criteria will represent a breakthrough in the management of ovarian cancer especially in advanced cases. Optimal debulking surgery will have achieved in 80% of cases by oncologist gynecologists who have expertise in ultra-radical surgery. Therefore, in the remaining 20% of patients, debulking surgery was not appropriate and these patient groups should initially receive chemotherapy and this intervention is a waste of time for chemotherapy. Paramount importance of timing justifies surgeons’ predilection for initial chemotherapy. So the ideal timing of cytoreductive surgery has considerable significance. We sift through the evidence to find articles that addressed the prediction value of tumor markers and radiologic assessment in ovarian cancer. Some studies have suggested CT scans before surgery to evaluate operability. For instance, Bristow et al. (2000) have claimed radiologic criteria with a sensitivity of 67.6% and specificity of 74% for predicting optimal surgery. These criteria were as follows: peritoneal thickening and peritoneal implants >2 cm, bowel mesentery tumor > 2cm, suprarenal lymph node >1 cm, omental extension to the spleen, pelvic sidewall involvement, and hydro ureter. But this method could raise concerns about clinical complexity. Some experts put diagnostic laparoscopy forward as a predicting measure. The accuracy rate was variable from 77.3% to 100%. But in many oncologic centers in developing countries, facilities are inadequate for performing laparoscopy, moreover, it is an invasive procedure. On one hand, serum level of CA 125 has helped predict optimal cytoreductive surgery, on the other hand, a poor negative predictive value of serum level of CA 125 was reported. Finding the most eligible criteria for predicting successful surgery in ovarian cancer has been an insurmountable problem in gynecology oncology so far, therefore, we assessed the preoperational serum level of CA 125 and HE4 to find a reliable cut-off. Firstly, we considered three cut-offs for serum level of HE4: 70, 140 and 170 Pmol/L, and our result showed that the HE4 value of 170 Pmol is the best cut-off for our main goal.

Fig 1. Association between HE4 / CA125 sensitivity and specificity based on stages demonstrated by ROC curve

![ROC Curve](image)
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The negative predictive value is 81% which can be an acceptable cut-off. This means that in cases with ovarian cancer if the serum level of HE4 is less than 140 Pmol/L, optimal surgery can be achieved approximately 80%. and if the serum level of HE4 is more than 140 Pmol/L, postponing the surgery is logical and patients should refer to neo-adjuvant chemotherapy. In our research, we achieved the CA125 value of 320 IU/mL as the best cut-off for predicting optimal debulking. The negative predictive value is 82% which can be a reasonable cut-off. This means that in cases with ovarian cancer if the serum level of CA125 is less than 320 Pmol/L, optimal surgery can be achieved approximately 80% and if the serum level of CA125 is more than 320 Pmol/L, postponing the surgery is logical and the patient should refer to neo-adjuvant chemotherapy. In our study, we divided cases into two groups: stage I to IIIb and stage IIIc-IV. because in advanced ovarian cancer (stage IIIc and IV), due to diffuse involvement in the peritoneal cavity, optimal cytoreductive surgery is not possible in an overwhelming majority of cases. So we designed our evaluation based on this classification. The HE4 value of 262 Pmol was achieved as the best cut-off for predicting optimal debulking in the Angioli et al. study (2013). This data is the same as our study and reported that HE 4 is more reliable than CA 125 in predicting of poor prognosis of ovarian cancer. Moreover, the cut-off of CA 125 IU/mL was 303 and HE4 was 777 Pmol/L. Although our study had some similarities we excluded patients with the underlying disease who were not excluded in their study and it can explain the difference between the two studies. Another study of the determination of reference intervals of serum levels of HE4 in the Chinese Journal of ovarian research reported a level of 472 Pmol/L as a cut-off for lymph node involvement in ovarian cancer. They reported that this cut-off was not associated with grade or type of tumor. In our study higher HE4 was associated with a higher probability of lymph node involvement too. Despite our research, underlying diseases were not excluded in their study. Other studies determined a cut-off of 345 IU/mL for CA 125 and 218 Pmol/L for HE4, and a cut-off of 282 IU/mL for CA 125 and 277 Pmol/L for HE4, our study is similar to them. The most considerable limitation of our study was the low sample size. But we were able to demonstrate two cut-offs for two tumor markers with acceptable negative predictive value for predicting optimal ovarian cancer surgery. Based on our study, it seems that selecting patients for successful optimal surgery or in contrast referring them to neoadjuvant chemotherapy and delaying surgery, can predict the two tumor markers at the pre-operating phase. Further studies with a larger sample size and combination of tumor markers with other clinical variables, should be carried out to achieve the best predictor criteria. To sum up, our data claimed that preoperative serum levels of both HE4 and CA125 are a predictor for optimal debulking to avoid unnecessary surgery.

List of acronyms
CA 125 - Cancer Antigen 125
HE4 - Human Epididymis Protein 4
NPV - Negative Predictive Value
PPV - Positive Predictive Value
ROMA - Risk of Ovarian Malignancy Algorithm
SD - Standard Deviation
Sen - Sensitivity
Spec - Specificity
TVS - Transvaginal sonography

Contributions of Authors
ES and SN have contributed to development of the article and are responsible for conception and design of the study. ES was responsible for acquisition of data. Manuscript drafting was performed by SN. Critical revision was done by NA. Interpretation of data was done by MM. SN was responsible for revision of the manuscript. All authors have read and approved the final version of the article.

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Conflict of Interest
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Ethical Publication Statement
We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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