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Original Research

Effectiveness of human epididymis protein 4 (HE4) as predictor of response to first line platinum based chemotherapy

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Objective: To assess the role of HE4 (Human epidydimal protein 4) marker as predictor of response to platinum based chemotherapy. *Methods*: In the current observational prospective study, 35 patients affected by High Grade Serous Ovarian Cancer (HGSOC) were enrolled; among these, 17 patients were platinum sensitive, while 18 were platinum resistant. HE4 levels were measured before surgery, at the III and at the VI cycle of chemotherapy. *Results*: The reduction of 50% or more of HE4 levels at the III cycle of chemotherapy showed a specificity of 100% and a sensitivity of 27%. The negativization (<70 pmol/L) of HE4 at the III cycle of chemotherapy showed a specificity of 100%, with a sensitivity of 39%, in predicting chemotherapy response, while the same parameter at the VI cycle showed a specificity of 82% and a sensitivity of 67%. Moreover the ROC analysis identified the HE4 cut-off value of 62.79 pmol/L as the best cut-off in predicting chemotherapy response, with a sensitivity of 72% and a specificity of 88% at the III cycle. *Discussion*: Our results suggest that HE4 levels during first-line chemotherapy, in particular at the III cycle, could predict chemotherapy response in HGSOC patients.

Keywords

HE4; Ovarian cancer; Marker; Chemotherapy; Platinum

1. Introduction

Nowadays, ovarian cancer is the seventh most frequent tumor in women and remains the most lethal gynecologic malignancy, with five-year survival rates below 45% [1, 2]. Histologically, about 90% of ovarian tumors are epithelial ovarian cancer.

Despite treatment improvements over the last three decades [3–6], a large fraction of advanced-stage disease patients (40–60%) proves to be unresponsive to standard therapy, due to platinum resistance. In order to identify in advance platinum resistant ovarian cancer (OC) patients as well as improving and personalizing the treatment, new predictive and interesting tools are currently studied.

As recommended by the National Comprehensive Cancer Network (NCCN) guideline, follow up is composed of medical examination plus CA-125 (Cancer Antigen 125) and HE4 (Human epidydimal protein 4) dosage every 2 to 4 months for 2 years, then 3 to 6 months for 3 years, then annually after 5 years.

CA-125 sensitivity to detect ovarian cancer recurrence amounts to 83.9% [7], but it is negative in 50% of ovarian cancer early stages and in 10% of advanced stages [8, 9].

Recently FDA has approved the use of HE4 serum marker in clinical practice for diagnosis, monitoring and follow up of ovarian cancer.

From recent studies, HE4 (cut-off 70 pmol/L) seems to be an excellent and useful marker, and in combination with CA-125 for ovarian cancers follow-up showing a 76.47% sensitivity and a 100% specificity in detecting recurrence [10].

Furthermore, HE4 seems to identify successfully patients with poorer prognosis and recurrence in CA-125 negative ovarian cancer patients [11].

In addition, several studies showed a potential role of HE4 in predicting platinum therapy response both *in vivo* [12–18] and *in vitro* clinical studies [19–23]. However, current studies in scientific literature used different parameters to predict chemotherapy response, such as the HE4 reduction of 50% at the III chemotherapy cycle, the negativization at the III cycle or the negativization at the VI cycle of chemotherapy.

Therefore, the aim of the present study is to evaluate the effectiveness of HE4 as predictor of response to first line platinum based chemotherapy and to analyze HE4 performance preoperatively, at III and at VI course of chemotherapy, in order to identify the best parameter, in terms of sensitivity and specificity, in predicting chemotherapy response.

2. Materials and methods

From January 2017 to July 2019 were screened all patients referred to the Division of Gynecologic Oncology of the Uni-

versity Campus Bio-Medico of Rome, affected by suspected epithelial ovarian cancer, and prospectively enrolled.

Inclusion criteria were: age between 18 and 70 years, histopathological diagnosis of High Grade Serous Ovarian Cancer (HGSOC), HE4 test positive at diagnosis, Eastern Cooperative Oncology Group (ECOG) performance status 0–2, according to World Health Organization (WHO) criteria, Residual Tumor after surgical debulking <1 cm (RT <1 cm).

Exclusion criteria were: altered hepatic function (transaminases >2.5× the upper normal level—ULN-, bilirubin >1.5× UNL), altered renal function (creatinine clearance <60 mL/min and/or serum creatinine >2.0 mg/100 mL), altered hematological function (absolute neutrophil count $<1.5 \times 10^9$/L or platelets $<100 \times 10^9$/L or hemoglobin <9 g/dL), severe or uncontrolled diseases, other systemic and not compensated diseases or mental illnesses at the diagnosis, no previous surgical, chemotherapeutic or radiotherapeutic treatments for other cancers, pregnancy.

All patients underwent cytoreductive surgery and started six courses of chemotherapy, according to standard Protocols (Carboplatin and Taxol) within 30 days from surgery [24–27].

Other histologic type of ovarian cancers were eventually excluded.

A written informed consensus was obtained. The study was approved by the Institutional Review Board (IRB) of Campus Biomedico (ref. 0512).

Carboplatin doses were calculated according to the Calvert formula, with target AUC (Area Under the Curve) 6 [28]. Taxol dose was 175 mg/mq.

We considered HE4 <70 pmol/L as a normal value. HE4 was dosed routinely at the diagnosis, before surgical debulking and during every chemotherapy course. HE4 was measured using ELISA test, in particular the kit HE4 EIA, produced by Fujirebio Diagnostic Inc. (Malvem, PA, USA). It is a direct, not competitive, immunoblot assay, in solid phase, based on the "sandwich" technique: it consists in the use of two murine monoclonal antibodies (2H5 and 3D8) directed against two HE4 epitopes in C-WFDC (whey acidic protein four-disulfide core domain protein 2) domain [29, 30].

As suggested by Gynecologic Cancer Intergroup (GCIG), the response was evaluated using RECIST 1.1 criteria [31]. For each patient we evaluated the HE4 value before surgery and at each chemotherapy course, particularly at the III and VI cycle, in correlation with the disease recurrence.

Considering the period between the end of the six courses of chemotherapy and the disease recurrence, patients were classified in:

(I) Platinum resistant: disease progression during chemotherapy treatment or within 6 months after the end of chemotherapy

(II) Platinum sensitive: recurrence after 6 months or more, or without recurrence [32, 33].

Recurrence was diagnosed by clinical examination, CA-125 serum levels and chest-abdomen-pelvis computed tomography (CT) scan (or whole body positon emission tomography-CT, PET-CT), performed according to follow up or in case of CA-125 high serum levels [34].

Table 1. Patients' characteristics.

Patients' characteristics	
Median Age, n (range)	61 (31–80)
Median BMI, n (range)	24 (20–31)
Performance status (ECOG 0–5), n (%)	
0	29 (82.85%)
1	6 (17.14%)
FIGO stage, n (%)	
IIB	2 (5.71%)
IIIA	1 (2.85%)
IIIB	4 (11.42%)
IIIC	28 (80%)
Residual tumor n (%)	
0	15 (42.85%)
<1	20 (57.14%)
Hystological subtype n (%)	
High Grade Serous Ovarian Cancer	35 (100%)

FIGO, The International Federation of Gynecology and Obstetrics.

All data were recorded and analyzed using a Microsoft Excel spreadsheet.

The receiver operating characteristic curve (ROC) has been performed using HE4 levels before surgery, at the III and at the VI cycle of chemotherapy, which are the most used values in literature, in order to find a cut-off value at each moment.

Sensitivity, specificity, area under the curve (AUC), likelihood ratio+, likelihood ratio– and post-test probability have been assessed. The HE4 variations over the time for each patient have been shown using Excel software. Statistical analysis has been performed using MedCalc Statistical Software version 19.1.3 (MedCalc Software bv, Ostend, Belgium; https://www.medcalc.org; 2019). The p value < 0.05 has been considered statistically significant.

3. Results

During the chosen timeframe, 60 patients affected by HGSOC were selected for the present study: 10 patients underwent a non-optimal cytoreductive surgery (RT >1), 9 patients had previous or concomitant tumors, 4 patients received a palliative treatment and 2 patients developed hepatopathies during the treatment. Therefore, 35 patients fulfilled our inclusion criteria and were enrolled in the study.

Patients' median age was 61 years (range 31–80). More characteristics are reported in Table 1.

The average preoperative HE4 value was 608,5 (75.8–2631) pmol/L. The average values of HE4 at the III and VI chemotherapy cycle were respectively 68.9 (12.4–160.3) pmol/L and 85.6 (16.3–488.9) pmol/L.

After six courses of chemotherapy (CT), patients were followed for at least 12 months, and were divided in platinum resistant or platinum sensitive.

Table 2. Reporting the results of the statistical analysis performed using as cut-off the values most used in scientific literature.

	Area under the curve	Cut-off value	Sensitivity	Specificity	*p* value	Likelihood ratio+	Likelihood ratio–	Post-test probability
Pre-surg.	0.575	>212.60	67%	65%	$p > 0.05$	1.91	0.51	67%
During 3° cycle	0.824	>62.79	72%	88%	$p < 0.001$	6.00	0.32	87%
During 6° cycle	0.709	>56.21	75%	69%	$p < 0.05$	2.42	0.36	72%

Table 3. Area under the curve, cut-off value, sensitivity, specificity, *p* value, likelihood ratio+, likelihood ratio- and post-test probability resulting from ROC analysis of level of HE4 measured before surgery, during the 3rd and the 6th cycle of chemotherapy to predict chemotherapy response.

	Sensitivity	Specificity	Likelihood ratio+	Likelihood ratio–	Post-test odd	Post-test probability
HE4 <50% at III cycle	27	99.90	270.00	0.73	180.00	0.99
HE4 <70 pmol/L at III cycle	39	99.90	390.00	0.61	260.00	1.00
HE4 <70 pmol/L at VI cycle	67	82.00	3.72	0.40	2.48	0.71

In particular, 17 patients of 35 (48.6%) were platinum sensitive (recurrence after 6 months), while 18 patients (51.4%) were platinum resistant (recurrence within 6 months).

HE4 levels were assessed preoperatively and at each cycle of chemotherapy.

In the following results we analyzed the significance (A) of HE4 reduction of 50% at the III cycle of chemotherapy, (B) of HE4 negativization at the III and (C) at the VI cycle, and the preoperative HE4 value, since they are all criteria studied before in literature, as markers of chemotherapy response.

(A) The reduction of less or more than 50% of HE4 during the 3rd cycle of chemotherapy has been evaluated as potential index of platinum response, and it has shown a sensitivity of 27%, a specificity of 100%, a likelihood ratio of 270 and a post-test probability of 100%.

(B) The negativization of HE4 at III cycle of chemotherapy, using the consensus cut-off value of 70 pmol/L has been evaluated, and it has shown a sensitivity of 39%, a specificity of 100%, a likelihood ratio of 390 and a post-test probability of 100% in predicting chemotherapy response.

(C) Then, the negativization of HE4 at VI cycle of chemotherapy, using the consensus cut-off value of 70 pmol/L has been evaluated, and it has shown a sensitivity of 67%, a specificity of 82%, a likelihood ratio of 3.7 and a post-test probability of 82% in predicting chemotherapy response (Table 2).

The ROC analysis has shown that the presurgical level of HE4 was not a good prognostic marker to predict chemotherapy response ($p > 0.05$), while the level of HE4 measured at the 3rd and the 6th cycle of chemotherapy has given statistically significant results (Fig. 1).

In addition, the ROC analys identified the HE4 cut-off value of 62.79 pmol/L as the best cut-off in predicting chemotherapy response, with a sensitivity of 72% and a specificity of 88% at the III cycle, while at the VI cycle the assessed value of 56.21 pmol/L has 75% of sensitivity and 69% of specificity, being both statistically significant.

Sensitivity, specificity, area under the curve (AUC), likelihood ratio+, likelihood ratio– and post-test probability has been reported (Table 3).

4. Discussion and conclusions

In the personalized medicine era, in which therapy is tailored to genotipical and phenotipical patients' peculiarities, the first line therapy of epithelial ovarian cancer is still based on standard protocols, composed of platinum and carbotaxol chemotherapy.

Approximately 40–60% of patients submitted to first-line therapy become resistant to platinum and will experience a fail of response to the treatment, or a condition of disease progression while chemotherapy or an early relapse within 6 months from the end of chemotherapy.

Platinum-sensitive ovarian cancer shows a median survival of 2 years, ranging from 3 months to over 10 years. Conversely, platinum-resistant ovarian cancer, expresses a median survival of 9–12 months and less than 15% of patients respond to subsequent chemotherapy [35, 36].

Thus it is crucial to underline and correctly classify patients as platinum sensitive and platinum resistant, in order to differentiate the therapeutic approach.

HE4 seems to have a role in detecting and predicting ovarian cancer recurrence, alone or in association with CA-125 [37, 38].

Many authors analyzed the role of HE4 as prognostic factor, assessed at the time of diagnosis, after surgery, throughout terapies and follow up, and used together with FIGO stage, residual tumor and other mostly used prognostic factors.

In this perspective, as we showed in a recent review [39], seven clinical studies [12–18] showed the efficacy of HE4 as marker in predicting chemosensitivity, but the results are still controversial, since too many different criteria were used.

Furthermore, five *in vitro* studies [19–23] showed how HE4 overexpression could be related to platinum resistance, because HE4 overexpressing clones inhibit platinum induced apoptosis.

To date, among the seven clinical studies, four authors measured the preoperative value of HE4 and demonstrated how an high preoperative HE4 value was a strong predictor of chemoresistance, with a high sensitivity (median value 91.5%) [15–18].

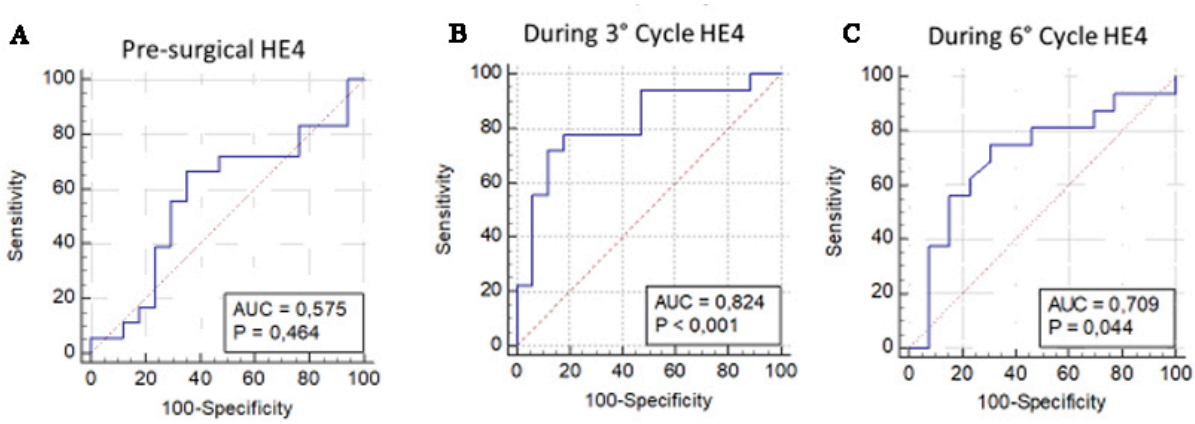

Fig. 1. ROC analysis of level of HE4 measured before surgery, during the 1st, the 3rd and the 6th cycle of chemotherapy to predict ovarian cancer relapse. (A) ROC analysis of level of HE4 measured before surgery to predict ovarian cancer relapse. (B) ROC analysis of level of HE4 measured at the third cycle of chemotherapy to predict ovarian cancer relapse. (C) ROC analysis of level of HE4 measured at the VI cycle of chemotherapy to predict ovarian cancer relapse.

In order to compare our results with those currently in literature, in the present study HE4 was registered also before surgery, and its value proved to be significant as predictor of chemotherapy response. Anyway, the results from ROC analysis showed the higher sensitivity and specificity of HE4 value at III and VI cycle of chemotherapy, compared with the preoperative value. Besides we believe that the preoperative HE4 value is an unreliable indicator, since it's strongly related to tumour load.

Two authors focused on HE4 blood levels measured at the third course of chemotherapy, with the purpose of assessing the role of HE4 as marker of chemotherapy response [13, 14]. In particular, Steffensen *et al.* [14] analyzed the dynamics of serum HE4 levels, showing that HE4 value at the set cut-off at the upper third percentile has a Positive Predictive Value (PPV) of 57.4% alone, and a PPV of 64.3% in combination with CA-125 in detecting chemotherapy response.

Our group demonstrated in a previous study that HE4 level reduction of almost 47% at the III cycle of chemotherapy was significantly related to platinum response (83% sensitivity, 87% specificity) [38].

In the present study, we considered both HE4 negativization and HE4 reduction of 50% at the III cycle of chemotherapy, reaching a specificity of 100% either way in predicting response to platinum, which may help us to identify platinum resistant and high risk recurrence patients, and to customize diagnostic and therapeutic strategies, building a personalized cancer follow-up care pathway.

Patients with high HE4 levels at the III should be included in a high-risk set of patients, which could be eventually selected and directed to a closer follow-up.

One study, conducted by Hamed *et al.* [12], underlined the role of HE4, assessed at the end of 6 courses of chemotherapy treatment, and his negativization showed a higher sensitivity and a higher specificity than CA-125 in detecting patients' sensitivity to platinum chemotherapy.

In the current study, even HE4 negativization at the VI cycle could be used as criterion of response to chemotherapy, but it showed a lower specificity (82% vs 100%) than the same parameter registered at the III cycle.

Therefore, we can assess that in our study all three criteria previously analyzed, that are HE4 reduction of 50% at the III cycle of chemotherapy, HE4 negativization at the III and at the VI cycle of chemotherapy, seemed to have a role as potential markers of response to platinum chemotherapy.

Moreover we performed a ROC analysis with the aims to choose the most accurate parameter in predicting chemotherapy response and to find the best cut-off for HE4 value with the highest sensitivity and specificity, which could allow us to select the group of patients with high risk of chemoresistance and recurrence. The ROC analysis suggests us that the best indicator of chemotherapy response is the value of HE4 measured at the III cycle and at that moment the HE4 cut-off value of 86.69 pmol/L reaches a specificity of 100%. In other terms, all the patients at the III cycle of chemotherapy with an HE4 value higher than 86.69 pmol/L will be chemoresistant.

The other reason for choosing the value of HE4 at the third cycle of chemotherapy, instead of the value at the VI cycle, as the best predictor of response to platinum based chemotherapy, apart from the higher specificity, is related to the hypothetic possibility, in the future, of eventually switching to second line drugs in advance, avoiding unnecessary toxicity of platinum, that often results in neutropenia and nephrotoxicity.

More clinical trials are requested to find new therapeutic strategies reserved to platinum resistant ovarian cancer patients, which could be enrolled and experience new regimens composed of traditional chemotherapic agents, immunotherapic drugs, anti-angiogenetics, PARP-inhibitors (poly adenosine diphosphate-ribose polymerase) and other biomolecular therapies.

About that, such as the monoclonal anti-body Oregovomab has shown to bind the glycoprotein CA-125, similarly new molecules should be investigated to be able to target the HE4 [40].

These conclusions lead us to last, but certainly not least, consideration, about healthcare costs of ovarian cancer. In 2015 Lazzaro *et al.* [41] performed a retrospective study about the costs of ovarian cancer in a single center in Italy, reporting the sum of €44999 for one year of first line treatment. The use of HE4 in predicting in advance platinum response at the III course of chemotherapy and the resultant individuation of selected chemoresistant patients, could have special implications, such as the development of new therapeutic strategies and target therapies, avoiding long maintenance therapies, with subsequent healthcare costs reduction.

In conclusion, the role of HE4 as marker of response to platinum based chemotherapy is really promising and the applications related are very interesting. Recently our group published a pilot study about the association between BRCA (Breast CAncer genes) status and HE4 serum levels, which could improve the predictive power of therapy response [42].

The main weakness of the present study is certainly represented by the small sample size, also due to the careful selection done. Therefore multicentric studies with larger samples are required to better evaluate the effectiveness of HE4.

Author contributions

PF Conceived and designed the analysis; BM Collected the data; TC, GF, MR, DCNC, MM Contributed data or analysis tools; CM and BD performed the analysis; DDV, BPP, AR, BM Wrote the paper. CM, BD, DDV, BPP and AR contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study. The study was approved by the Institutional Review Board (IRB) of Campus Biomedico (ref. 0512).

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Conflict of interest

The authors declare no conflict of interest. FP is our Guest Board, given his role as the guest board, had no involvement in the peer-review of this article and has no access to information regarding its peer-review.

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