Published in final edited form as:

Gynecol Oncol. 2015 June ; 137(3): 430–435. doi:10.1016/j.ygyno.2015.03.044.

Detection of the HE4 protein in urine as a biomarker for ovarian neoplasms: clinical correlates

John B. Liao¹, Yuen Yee Yip², Elizabeth M. Swisher¹, Kathy Agnew¹, Karl Erik Hellstrom², and Ingegerd Hellstrom^{2,*}

¹Department of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle, WA 98195

²Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195

Abstract

Objectives—Measure HE4 levels in urine from normal donors, patients with LMP tumors and ovarian cancer patients and correlate levels with clinical factors in ovarian cancer patients.

Methods—Archived samples from controls, patients with LMP tumors and ovarian cancer were tested using commercial assays, including serially collected serum and urine samples from women treated for stage III/IV serous ovarian cancer.

Results—Five of 6 patients with Stage I/II and 26 of 36 Stage III/IV serous ovarian cancer patients had HE4-positive urines, similar to serum samples (4 of 5 Stage I/II and 26 of 34 Stage III/IV) when tested at the same level of specificity (95%). Urine HE4 was more sensitive in LMP tumors: 9 of 32 urines (28%) HE4-positive versus 3 of 68 sera (4.4%, $P=0.002$). Mean levels of serum CA125 and HE4 decreased comparably in patients during initial treatment regardless of their primary platinum response, but mean urine HE4 levels decreased only 7% in primary platinum resistant patients while decreasing 68% in those who were sensitive. By 7 months after diagnosis, urine HE4 levels were higher in primary platinum resistant patients compared to those who proved to be sensitive ($p=0.051$) and persisted 12 months after diagnosis ($p=0.014$). HE4 values in urine also became positive in advance of clinical recurrence in several women while serum HE4 and serum CA-125 remained normal.

Conclusions—Measuring HE4 in urine complements serum assays for the detection of ovarian cancer and may allow identification of patients at high risk for primary platinum resistance.

Keywords

ovarian cancer; urine; ELISA; HE4; LMP

© 2015 Published by Elsevier Inc.

*Corresponding author. ihellstr@u.washington.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Earlier detection of primary ovarian cancer is likely to improve survival, and early prediction of responses to treatment and relapses may be clinically beneficial. While measuring CA125 in serum has high sensitivity for detecting ovarian cancer, there is a need for markers to complement CA125 whose levels are frequently elevated also in women with benign disease [1–3]. A promising marker for this purpose is HE4 which is encoded by the WFDC2 gene [4] and is frequently amplified in ovarian cancer while its expression in normal tissues, including ovary, is low [5–7]. HE4 is a member of a family of stable 4-disulfide core proteins, which include the Wp protein [8], a secretory leukocyte protease inhibitor [9] and elafin [10], all of which are secreted. Applying an ELISA to measure HE4 in serum demonstrated that the sensitivity is similar to that of CA125, while HE4 is less frequently positive in patients with nonmalignant disease [11]. Assaying sera from women with a pelvic mass for both CA125 and HE4 facilitates the detection of those patients who have ovarian cancer [12] and may also complement CA-125 in the diagnosis of early ovarian cancer [13].

Identification of biomarkers in urine would provide a non-invasive way of detecting OvC and facilitate frequent, longitudinal testing of women who belong to high risk groups or have been treated for ovarian cancer and are at risk to relapse. Measurement of analytes in urine may also enhance the performance of the assay. The protein profile in urine may be less complex than that seen in blood and certain proteins may be more stable in urine than in blood. Human chorionic gonadotropin is one such analyte where the enhanced sensitivity and unique properties of the urine assay can be used to identify false positives seen with a blood assay due to heterophilic antibodies [14]. Several markers have been identified in the urine of women with ovarian cancer including eosinophil-derived neurotoxin, a fragment of osteopontin [15], mesothelin [16, 17], and Bcl-2 [18]. Likewise, we used a commercially available kit for serum testing and demonstrated that measuring HE4 in urine can identify patients with ovarian cancer with a sensitivity similar to that for assaying sera [19]. We now report an extended analysis of additional patients, including women who received cytoreductive treatment for advanced ovarian cancer and were followed by serial assays for HE4 and CA125 in serum and HE4 in urine.

MATERIALS AND METHODS

Study population

Archived urine samples were obtained, one sample/subject from 187 controls (100 premenopausal +87 postmenopausal), including healthy women (n=60), women with benign ovarian disease (n=79), benign gynecological disease excluding ovaries (n=3) and women with cancer not involving the ovary (n=63) (Table 1). Urine and serum samples were concomitantly obtained also from patients with Stage I/II (n=6) or Stage III/IV invasive serous ovarian cancer (n=36) which consists of 1 low-grade serous carcinoma, 40 high-grade serous carcinomas and 1 serous carcinoma with unknown grade, from women with non-serous ovarian cancers, which include clear cell, endometrioid, granulosa cell, adenocarcinoma, poorly differentiated, non-mucinous carcinoma, pseudomyxoma peritonei, and malignant mixed mullerian tumor/carcinosarcoma histologies (n=18) and from 68

women with ovarian neoplasms of low malignant potential (LMP). Borderline tumors included all histologies (serous, mucinous, endometrioid) Nineteen patients with Stage III/IV serous ovarian cancer who showed primary platinum response, had urine and serum samples obtained at time of surgery as well as serial serum and urine samples collected approximately once every four months. All cancer cases were surgically staged according to guidelines by the International Federation of Gynecologic Oncology (FIGO 1998). After collection, urine samples were centrifuged and the non-cellular supernatant stored at -80° . Samples were assayed for HE4 as well as for creatinine to relate the findings to the varying concentration of protein in urine samples.

Sample collection

Urine and serum samples and clinical information were obtained from the University of Washington Gynecologic Oncology Tissue Bank as approved by the Human Subjects Division of the Institutional Review Board.

Enzyme-linked immunosorbent assay

HE4 levels were measured by a quantitative enzyme-linked immunosorbent assay (ELISA) provided as a kit by Fujirebio Diagnostics Inc (FDI) according to manufacturer's instructions. Streptavidin coated microstrips were washed once with washing buffer (Tris-HCl buffer with Tween 20). Calibrators, biotinylated anti-HE4 monoclonal antibody (Mab) 2H5 and samples from patients or controls were added into streptavidin-coated microstrips and incubated for 1 hr with shaking. Subsequently, the strips were washed and incubated with horseradish peroxidase (HRP) labeled anti-HE4 Mab 3D8 for 1 hr with shaking. After washing, buffered substrate/chromogen reagent (hydrogen peroxide and 3,3',5' tetra-methyl-benzidine) was added to each well. After 30 min, the reaction was terminated by the addition of stop solution (0.12 M hydrochloric acid). Plates were read at 405 nm wavelength using a microplate reader (Fusion universal microplate analyzer, Fusion instrument company, Meriden, CT) within 15 min. Urine samples were diluted 1:40 prior to testing with tracer diluent (phosphate buffered saline with BSA, blocking agents, detergents, an inert blue dye and a non-azide antimicrobial preservative) as provided with the kit.

Urinary creatinine levels were measured using a kit and (Cayman Chemical Company, Ann Arbor, MI) following the manufacturer's protocol. ELISA data from urine samples were normalized by calculating the ratio HE4 (pM)/creatinine (mg/dl). As in the initial study, data for HE4 in urine were normalized by establishing a ratio between HE4 and creatinine. [19]

Statistical analysis

Graphs and statistical comparisons were completed using GraphPad Prism 6 software. Unpaired t-tests and Fisher's exact test were used. Significance was defined as $p < 0.05$ for all statistical tests.

RESULTS

HE4/creatinine ratios were significantly higher in urine samples from women with early or advanced ovarian cancer or LMP as compared to controls ($p < 0.001$) and they were higher in

urines from women with serous ovarian cancer than in those with non-serous ovarian cancer (Figure 1). No significant differences in HE4/creatinine ratios were observed between pre and post menopausal women or those with or without other cancers. At three different cut-offs for the ratio between HE4 versus creatinine, 2.5, 3.5 and 5.0, respectively, 9, 5 and 2 % of the control samples were positive, representing a specificity level of 91, 95 and 98% (Table 2). Frequencies of positive samples in urines from patients with early or late serous ovarian cancer, non-serous ovarian cancer or LMP, were statistically different from control urines at all three cutoffs. Urine HE4 from women with serous ovarian cancer were positive more frequently than urine HE4 from women with other ovarian cancer and urine HE4 from a subgroup of women with LMP were positive with 13% of samples being positive even at 99% specificity.

Archived samples of serum and urine obtained on the same day were tested for HE4 at similar specificity levels (95% for urine and 94% for serum according to manufacturer's instructions) (Table 3). The frequency of positive data for urine and serum samples was similar except that urines from the LMP group were positive significantly more frequently than controls while no difference was observed between sera from LMP patients and controls.

We also examined a subset of Stage III and IV ovarian cancer patients with serial measurements of urine HE4, serum HE4, and serum CA125 from the date of initial surgery and at subsequent time points (Table 4). Nineteen patients with known primary platinum response status had samples available at the time of surgery/initial diagnosis along with follow-up samples. Serial measurements were plotted for initial and follow-up samples obtained 4–7 months and 8–12 months after surgery with patients segregated by primary platinum sensitivity or resistance defined as having a recurrence within 6 months of completing primary platinum chemotherapy (Figure 2, all subfigures). Measurements for serum CA125 decreased from baseline to the 4–7 month time point regardless of whether patients ultimately proved to be platinum sensitive (97% decrease) or resistant (97% decrease). This was also seen for mean serum HE4 levels, which decreased 70% in platinum sensitive patients and 57% in platinum resistant patients (Figure 2B). However, mean urine HE4 levels remained stable (7% decrease) in patients that proved to be platinum resistant while they decreased 68% in those that proved to be platinum sensitive. While serum CA125 levels were statistically higher in primary platinum resistant patients at the time of surgery, this significance was lost by the first time point at 4–7 months with chemotherapy in both resistant and sensitive patients. (Figure 2C.) Urine HE4 levels approached statistical significance by the 4–7 months time point ($p=0.051$) and was significantly higher ($p=0.014$) in platinum resistant patients compared to platinum sensitive patients by 12 months (Figure 2A.) [by changing remained to was I feel we don't need to make further changes here to satisfy reviewer]

Six of nineteen patients achieved clinical remissions with primary treatment, had normalized serum CA-125 levels at 6–12 months after diagnosis and also had at least 5 serial urine HE4 measurements available for analysis. All six had stage 3C (A2, A3, A4, A5, A6) or 4 (A1) disease. Four patients had serous histology (A1, A3, A4, A5). There was 1 carcinosarcoma (A2) and one poorly differentiated carcinoma (A6). These patients were followed

longitudinally with serial measurements for 22 to 53 months (mean 38 months) after diagnosis. In three patients (A1, A2, and A3) who were in clinical remission following therapy but later relapsed, the urine samples were repeatedly positive for HE4 while the serum samples were negative for both HE4 and CA125 (Figure 3). When these patients subsequently developed clinically detectable recurrences, the serum values for both HE4 and CA125 were positive as were the urine values for HE4. One patient (A4) had no clinical evidence of disease during the observation period and had negative urine and serum assays. Two patients (A5 and A6) recurred without elevation in any of the markers.

DISCUSSION

The WDFC2 gene which encodes the HE4 protein was cloned in 1991 [4] and was shown by microarray analysis to be overexpressed in ovarian cancer [5, 6]. A double determinant “sandwich” ELISA for HE4 was constructed and showed that sera from women with ovarian cancer were positive more frequently than sera from women who had benign ovarian disease or were healthy [11]. The findings suggested that HE4 is a useful biomarker for ovarian cancer and appears to be better than CA125 in distinguishing patients with ovarian cancer from those with benign disease. They were confirmed and extended in a large prospective study of women with pelvic masses, where measurement of both HE4 and CA125 in serum better identified ovarian cancer than measurement of CA125 alone [20]. A kit measuring HE4 is now commercially available as is a clinical assay, ROMA, which combines assays of HE4 and CA125 in serum [21].

HE4, like several other tumor biomarkers, can be detected in urine as a potentially useful diagnostic tool [19]; however, the number of patient and control samples previously studied was small and there was no direct comparison of studies on serum and urine samples obtained from patients on the same occasion. In this study, we analyzed the ratios between HE4 and creatinine levels in urine from controls and from patients with ovarian neoplasms, including early and late ovarian cancer and LMP. Studies on archived samples demonstrated that assaying urine and serum has similar sensitivity, except that urine samples from women with LMP are more frequently positive for HE4 than concomitantly obtained serum samples which do not differ from controls. Further studies are needed to learn whether the women who have positive urines are at greater risk to have recurrence of their LMP or develop ovarian cancer.

HE4 may have additional roles in guiding treatment of ovarian cancer beyond early detection. Blood HE4 measured in patients being treated for ovarian cancer has been shown to correlate with surgical outcome and survival [22, 23] and may predict platinum response with first line chemotherapy. Angioli and colleagues reported that the reduction of HE4 levels by the third cycle of chemotherapy predicted primary platinum response with a positive predictive value of 0.68 and a negative predictive value of 0.85 [24]. In our study, mean levels of blood tests for both CA125 and HE4 both decreased in response to primary surgery and chemotherapy when measured 4–7 months later, irrespective of primary platinum response. Patients who proved to be platinum resistant, however, had stable urine HE4 levels over the period covering initial surgery and chemotherapy, while those who proved to be platinum sensitive had decrease urine HE4 levels. Our findings thus indicate

that urine HE4 levels may be effective for early identification of primary platinum resistant patients and should be studied further to evaluate the use of HE4 urine levels to predict chemotherapy response.

Because of the established role of HE4 in the early detection of ovarian cancers [20] and the increased sensitivity of other urine markers [16], we examined whether urine HE4 levels could signal recurrences prior to serum CA125 or serum HE4 levels. We examined patients for whom we had at least 5 longitudinal measurements of urine HE4 and also achieved a clinical remission after primary treatment (6 of 19). Three women (A1, A2, and A3) who had no evidence of disease following cytoreductive surgery and adjuvant chemotherapy but later relapsed, had urine that was positive for HE4 before sera became positive for HE4 or CA125 prior to clinical diagnosis of relapse. One patient (A4) who remained clinically tumor free was negative for HE4 in urine and for HE4 and CA125 in serum. Two patients (A5 and A6) recurred with normal values in both serum markers and urine HE4.

In addition to confirming that the sensitivity of assaying HE4 in serum and urine is similar [19], our present study reports a number of differences in measuring HE4 in urine rather than serum that are important for further study. Patients with tumors of low malignant potential were more often positive for HE4 in urine compared to serum, suggesting that this may be a better route for detecting or monitoring this disease. The levels of HE4 in urine also suggest a relationship to primary platinum resistance that is not evident in serum levels. If these findings can be confirmed on a larger cohort they are highly significant and could aid the identification of patients whose ovarian cancers are primary platinum resistant who may be candidates for additional biologic agents or maintenance strategies. Our longitudinal studies on treated patients indicate that HE4 is detectable in urine earlier than in serum before the patients relapse; however, these data need to be confirmed on a larger cohort. Further studies are also needed to evaluate whether the more frequent detection of HE4 in urine samples from women with LMP implies that those women differ with respect to risk for recurrence and whether serial monitoring of urines from women belonging to high-risk groups improves the chance for earlier detection of ovarian cancer.

ACKNOWLEDGEMENTS

This work was supported by NIH grants 1R01CA1344867-01 and by a grant from Fujirebio Diagnostics, Inc., to Dr. I. Hellstrom, and by the Wendy Feuer Research Fund for the Prevention and Treatment of Ovarian Cancer. Dr. J. Liao is a scholar supported by the National Institutes of Health's Women's Reproductive Health Research Program at the University of Washington (5K12HD001264-12). We thank Dr. N. Kiviat for support and Drs. C. Fermer and O. Nilsson, Fujirebio Diagnostics, Inc. for discussions. We also thank patients and the group of women who donated urine samples for use as controls.

REFERENCES

1. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. *Hum Reprod.* 1989; 4:1–12. [PubMed: 2651469]
2. Berek JS, Bast RC Jr. Ovarian cancer screening. The use of serial complementary tumor markers to improve sensitivity and specificity for early detection. *Cancer.* 1995; 76:2092–2096. [PubMed: 8635006]
3. Maggino T, Gadducci A, D'Addario V, Pecorelli S, Lissoni A, Stella M, Romagnolo C, Federghini M, Zucca S, Trio D, et al. Prospective multicenter study on CA 125 in postmenopausal pelvic masses. *Gynecol Oncol.* 1994; 54:117–123. [PubMed: 8063233]

4. Kirchoff C, Habben I, Ivell R, Krull N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitor. *Biol. Reprod.* 1991; 45:350–357. [PubMed: 1686187]
5. Schummer M, Ng W, Bumgarner R, Nelson P, Schummer B, Bednarski D, Hassell L, Baldwin R, Karlan B, Hood L. Comparative hybridization of an array of 21 500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. *Gene.* 1999; 238:375–385. [PubMed: 10570965]
6. Wang K, Gan L, Jeffery E, Gayle M, Gown AM, Skelly M, Nelson PS, Ng WV, Schummer M, Hood L, Mulligan J. Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarray. *Gene.* 1999; 229:101–108. [PubMed: 10095109]
7. Ono K, Tanaka T, Tsunoda T, Kitahara O, Kihara C, Okamoto A, Ochiai K, Takagi T, Nakamura Y. Identification by cDNA microarray of genes involved in ovarian carcinogenesis. *Cancer Res.* 2000; 60:5007–5011. [PubMed: 11016619]
8. Dandekar AM, Robinson EA, Appella E, Quasba PK. Complete sequence analysis of cDNA clones encoding rat whey phosphoprotein: homology to a protease inhibitor. *Proc. Natl Acad. Sci.* 1982; 79:3987–3991. [PubMed: 6955785]
9. Heinzel R, Appelhans H, Gassen G, Seemuler U, Machleidt W, Fritz H, Steffens G. Molecular cloning and expression of cDNA for human antileukoprotease from cervix uterus. *Eur. J. Biochem.* 1986; 160:61–67. [PubMed: 3533531]
10. Wiedow O, Schroder JM, Gregory H, Young JA, Christophers E. Elafin: an elastase-specific inhibitor of human skin. Purification, characterization, and complete amino acid sequence. *J. Biol. Chem.* 1990; 265:14791–14795. [PubMed: 2394696]
11. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, Drescher C, Urban N, Hellstrom KE. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer Res.* 2003; 63:3695–3700. [PubMed: 12839961]
12. Moore RG, McMeekin DS, A K, DiSilvestro P, Miller MC, Allard WJ, Gajewski W, Kurman R, Bast RCJ, Skates SJ. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. *Gynecol Oncol.* 2009; 112:40–46. [PubMed: 18851871]
13. Hellstrom I, Hellstrom KE. Two new biomarkers, mesothelin and HE4, for diagnosis of ovarian carcinoma. *Expert Opinion Med Diagnosis.* 2011; 5:227–240.
14. ACOG Committee Opinion: number 678. Avoiding inappropriate clinical decisions based on false-positive human chorionic gonadotropin test results. Committee on Gynecologic Practice, ACOG. *Obstet Gynecol.* 2002; 100(5 Pt 1):1057–1059. [PubMed: 12434782]
15. Ye B, Skates S, Mok SC, Horick N, Rosenberg HF, Vitonis A, et al. Proteomic-based discovery and characterization of glycosylated eosinophil-derived neurotoxin and COOH-terminal osteopontin fragments for ovarian cancer in urine. *Clin Cancer Res.* 2006; 12:432–441. [PubMed: 16428483]
16. Badgwell D, Lu Z, Cole L, Fritsche H, Atkinson EN, Somers E, Allard J, Moore RG, Lu KH, Bast JRC. Urinary mesothelin provides greater sensitivity for early stage ovarian cancer than serum mesothelin, urinary hCG free beta subunit and urinary hCG beta core fragment. *Gynecol Oncol.* 2008; 106:490–497. [PubMed: 17532030]
17. Hellstrom I, Hellstrom KE. SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. *Adv Exp Med Biol.* 2008; 622:15–21. [PubMed: 18546615]
18. Anderson NS, Bermudez Y, Badgwell D, Chen R, Nicosia SV, Bast JRC, Kruk PA. Urinary levels of Bcl-2 are elevated in ovarian cancer patients. *Gynecol Oncol.* 2009; 112:60–67. [PubMed: 19007973]
19. Hellstrom I, Heagerty PJ, Swisher EM, Liu P, Jaffar J, Agnew K, Hellstrom KE. Detection of the HE4 protein in urine as a biomarker for ovarian neoplasms. *Cancer Letters.* 2010; 296:43–48. [PubMed: 20381233]
20. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Vercy T, Steinhoff M, Messerlian G, DiSilvestro P, Granai CO, Bast RCJ. The use of multiple novel tumor biomarkers for the detection

- of ovarian carcinoma in patients with a pelvic mass. *Gynecol Oncol.* 2008; 108:402–408. [PubMed: 18061248]
21. Nolen BM, Lokshin AE. Biomarker testing for ovarian cancer: clinical utility of multiplex assays. *Mol Diagn Ther.* 2013; 17:139–146. [PubMed: 23552992]
22. Braicu EL, Chekerov R, Richter R, Pop C, Nassir M, Lofgren H, Stamatian F, Muallem MZ, Hall, Fotopoulou C, Sehouli J, Pietzner K. HE4 expression in plasma correlates with surgical outcome and overall survival in patients with first ovarian cancer relapse. *Ann Surg Oncol.* 2014; 21(3): 955–962. [PubMed: 24217786]
23. Braicu EL, Fotopoulou C, Van Gorp T, Richter R, Chekerov R, Hall C, Butz H, Castillo-Tong DC, Mahner S, Zeillinger R, Concin N, Vergote I, Sehouli J. Preoperative HE4 expression in plasma predicts surgical outcome in primary ovarian cancer patients: results from the OVCAD study. *Gynecol Oncol.* 2013; 128(2):245–251. [PubMed: 23178313]
24. Angioli R, Capriglione S, Aloisi A, Guzzo F, Luvero D, Miranda A, Damiani P, Montera R, Terravona C, Plotti F. Can HE4 predict platinum response during first-line chemotherapy in ovarian cancer? *Tumor Biol.* 2014; 35(7):7009–7015.

Highlights

Patients with LMP tumors were more often positive for HE4 in urine compared to serum.

Urine HE4 was stable in primary platinum resistance but decreased in sensitive ovarian cancers.

HE4 is detectable in urine earlier than in serum prior to a recurrence.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HE4 in Control Urines vs LMP and OvCa Urines

Fig 1.
Ratios for HE4 in archived urines from controls and from patients with ovarian neoplasms.

A

B.

C.

Figure 2.

Serial measurements of HE4 in urine (Panel A), HE4 in sera (Panel B), and CA-125 in sera (Panel C) plotted for initial and follow-up samples obtained 4–7 months and 8–12 months in primary platinum sensitive and primary platinum resistant ovarian cancer patients.

Figure 3.

Serial data from 6 patients who achieved normalization of CA 125 and had at least 5 serial measurements for serum CA-125, serum HE4, and urine HE4, obtained at time points after primary surgery for advanced stage serous OvC.

Table 1

Donors of control urine samples.

Total Number of subjects (1 sample from each)	187
Pre-menopause	100
Post-menopause	87
Age years (M+/-SD)	48.7 +/- 11.2
Healthy	60
Benign Ovarian disease	79
Benign Gynecological disease (not ovarian)	3
Cancer not involving ovary or Fallopian tube	63

Table 2

Summary of data on HE4 in urine.

Cut-off	Controls	Serous OvC		Non-Serous Stage I–IV	LMP
		Stage I/II	Stage III/IV		
2.5	17/187 (9%)	6/6 (100%)	30/36 (83%)	10/18 (55%)	17/32 (53%)
3.5	10/187 (5%)	5/6 (83%)	26/36 (72%)	7/18 (39%)	9/32 (28%)
5.0	2/187 (1%)	2/6 (33%)	23/36 (64%)	5/18 (28%)	6/32 (13%)

Table 3

Comparison of data from testing sera and urine from controls and women with LMP or ovarian cancer.

Groups	Fraction (%) positive serum	Fraction (%) positive urine at cut-off 3.5
Control	(6%) according to kit	10/187 (5%)
LMP	3/68 (4%)	9/32 (28%) ***#
Serous I/II	4/5 (80%) ***	5/6 (83%) ***
Serous III/IV	26/34 (77%) ***	26/36 (72%) ***
Non-serous (I–IV)	5/18 (28%) ***	8/22 (36%) ***

*** Differences relative to control are significant (p<0.001)

The difference between LMP in urine versus serum is significant (p<0.01)

Table 4

Clinical characteristics of advanced ovarian cancer patients with samples available from time of surgery/
diagnosis.

Patients	19
Mean Age	57.7 years
Stage	
III	13/19 (68.4%)
IV	6/19 (31.6%)
Primary platinum response	
Resistant	7/19 (36.8%)
Sensitive	12/19 (63.2%)
Histology	
Serous	14/19 (73.7%)
Non-serous	5/19 (26.3%)