RESEARCH ARTICLE

Diagnostic markers for the detection of ovarian cancer in BRCA1 mutation carriers

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Abstract

Background
Screening for ovarian cancer (OC) in women at high risk consists of a combination of carbohydrate antigen 125 (CA125) and transvaginal ultrasound, despite their low sensitivity and specificity. This could be improved by the combination of several biomarkers, which has been shown in average risk patients but has not been investigated until now in female BRCA mutation carriers.

Methods
Using a multiplex, bead-based, immunoassay system, we analyzed the concentrations of leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor, CA125 and human epididymis antigen 4 in 26 healthy wild type women, 26 healthy BRCA1 mutation carriers, 28 wildtype OC patients and 26 OC patients with BRCA1 mutation.

Results
Using the ROC analysis, we found a high overall sensitivity of 94.3% in differentiating healthy controls from OC patients with comparable results in the wildtype subgroup (sensitivity 92.8%, AUC = 0.988; p = 5.2e-14) as well as in BRCA1 mutation carriers (sensitivity 95.2%, AUC = 0.978; p = 1.7e-15) at an overall specificity of 92.3%.

The used algorithm also allowed to identify healthy BRCA1 mutation carriers when compared to healthy wildtype women (sensitivity 88.4%, specificity 80.7%, AUC = 0.895; p = 6e-08), while this was less pronounced in patients with OC (sensitivity 66.7%, specificity 67.8%, AUC = 0.724; p = 0.00065).

Conclusion
We have developed an algorithm, which can differentiate between healthy women and OC patients and have for the first time shown, that such an algorithm can also be used in BRCA
Introduction

Ovarian cancer is the most lethal cancer among gynaecological malignancies with a 5-year survival rate of patients diagnosed with advanced disease ranging from 20% to 25%. Only 20% of patients are diagnosed at stage I and II because of missing screening strategies [1].

While the lifetime risk of developing OC in the general population is about 1% to 2%, women with deleterious BRCA mutations have a cumulative lifetime risk of developing OC of approximately 45% in BRCA1 carriers and 20% in BRCA2 carriers [2,3]. While the early detection of breast cancer with the combination of mammography and MRI has a high success rate in this high risk population [4], the combination of transvaginal ultrasound (TVU) and CA125 for the early detection of ovarian cancer has suboptimal results. CA125 has a sensitivity of less than 60% in early stage OC with moderate improvement by the addition of TVU [5–9].

Furthermore, the incidence of serous tubal intraepithelial carcinomas (STIC) has been reported in a range from 0.6–7% in BRCA mutation carriers [10,11]. Therefore BRCA1 or BRCA2 mutation carriers are recommended to undergo risk reducing salpingo-oophorectomy (RRSO) by age 40 or after the completion of childbearing [12]. RRSO reduces the risk of OC by 85–90% and the risk of breast cancer by about 50% [13] and may also impact cancer-specific and overall mortality [14].

Studies have reported rates of RRSO in BRCA mutation carriers ranging from 12% to 78% underlining the importance of exact information about benefits and possible side-effects of this intervention in the presence of psychooncologists, because recommendations for OC screening for those who choose to forego or delay RRSO, are conflicting [15].

Therefore a better screening program for the early detection of OC is warranted for a better surveillance during the childbearing period, as a stop gap solution until mutation carriers decide to undergo RRSO and for those carriers deciding to forego RRSO.

Limited data exist regarding the combination of biomarkers to improve the early detection of OC in BRCA mutation carriers, although promising results have been reported using the combination of HE4 with CA125 [16,17]. Using a six biomarker panel consisting of macrophage inhibitory factor (MIF), prolactin, CA125, leptin, osteopontin and insulin like growth factor 2 (IGF2), Visintin et al reported an improved differentiation between disease free and ovarian cancer patients compared to CA125 alone in patients without a family history of OC (sensitivity of 95.3% vs 75%; specificity of 99.4% vs 95%) [18]. In this setting the combination of these six biomarkers with blood based gene expression achieved further improvement showing a sensitivity of 97.8% and a specificity of 99.6% [19].

To our knowledge, there exist no data evaluating the feasibility of the Milliplex 6-plex Ovarian Cancer Panel Kit combined with HE4 for the detection of ovarian cancer in BRCA mutation carriers. We therefore evaluated the serum concentrations of these seven biomarkers in healthy non carriers, healthy BRCA1 mutation carriers, patients with sporadic OC and BRCA1 mutation carriers with OC.

Materials and methods

Ethics, consents

The ethical board of the Medical University of Vienna approved this study. Patients had to sign an informed consent prior to inclusion into the study.
Patient population

**Ovarian cancer group.** The disease group (n = 54) included wildtype women with newly diagnosed OC (n = 28) and *BRCA1* mutation carriers with OC (n = 26). Eleven samples of wildtype OC patients were obtained from the biobank of the Medical University of Vienna and 17 samples of wildtype OC patients as well as 26 samples of *BRCA1* mutation carriers with OC from the tumorbank ovarian cancer (TOC) of the Charité, Medical University of Berlin. Median age in the wildtype group was 57.6 yrs (33.6–82.1) and in the *BRCA1* mutation carrier group 53.0 yrs (41.0–78.0). All samples were collected prior to surgery. Table 1 shows detailed information about histology and stage of the disease.

**Control group.** The healthy control group (n = 52) included age-matched sera from healthy wildtype women (n = 26) and healthy *BRCA1* mutation carriers (n = 26) obtained from the biobank of the Medical University of Vienna. Median age in the healthy wildtype group was 67.1 yrs (60.7–72.4) and 36.4 yrs (26.4–61.7) in healthy *BRCA1* mutation carriers. We included only *BRCA1* mutation carriers who have not undergone risk reducing surgery. (Table 1)

Sample collection

Ten mL of peripheral blood was drawn from subjects using standardized phlebotomy procedures [20]. Samples were processed within two to four hours using guidelines set by the

| Table 1. Patients characteristics of the healthy control group (wildtype and *BRCA1*) and patients tumor characteristics of the group of ovarian cancer patients (wildtype and *BRCA1*). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Wildtype healthy (n = 26) | *BRCA1* healthy (n = 26) | Wildtype OC (n = 28) | *BRCA1* OC (n = 26) |
| **Median age (yrs) (range)** | 67.1 (60.7–72.4) | 36.4 (26.4–61.7) | 57.6 (33.6–82.1) | 53.0 (41.0–78.0) |
| **OC histology** |                   |                   |                   |                   |
| serous         | 23 (82.1%)        | 22 (84.6%)        |                   |                   |
| mucinous       | 0 (0.0%)          | 1 (3.8%)          |                   |                   |
| endometroid    | 2 (7.2%)          | 1 (3.8%)          |                   |                   |
| NA             | 3 (10.7%)         | 2 (7.8%)          |                   |                   |
| **Grading**    |                   |                   |                   |                   |
| G1             | 0 (0.0%)          | 0 (0.0%)          |                   |                   |
| G2             | 6 (21.4%)         | 7 (26.9%)         |                   |                   |
| G3             | 20 (71.4%)        | 19 (73.1%)        |                   |                   |
| NA             | 2 (7.2%)          | 0 (0.0%)          |                   |                   |
| **FIGO**       |                   |                   |                   |                   |
| 1a             | 0 (0.0%)          | 1 (3.8%)          |                   |                   |
| 1b             | 0 (0.0%)          | 0 (0.0%)          |                   |                   |
| 1c             | 0 (0.0%)          | 2 (7.8%)          |                   |                   |
| 2a             | 1 (3.6%)          | 0 (0.0%)          |                   |                   |
| 2b             | 1 (3.6%)          | 0 (0.0%)          |                   |                   |
| 2c             | 1 (3.6%)          | 0 (0.0%)          |                   |                   |
| 3a             | 1 (3.6%)          | 0 (0.0%)          |                   |                   |
| 3b             | 6 (21.4%)         | 0 (0.0%)          |                   |                   |
| 3c             | 14 (50.0%)        | 19 (73.0%)        |                   |                   |
| 4              | 4 (14.2%)         | 4 (15.4%)         |                   |                   |

Abbreviations: OC = ovarian cancer

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National Cancer Institute Inter-Group Specimen Banking Committee and stored at -80˚C in the sera bank.

**Multiplex analysis**

Serum determinations for MIF, leptin, prolactin, OPN, CA125, and IGF II were performed using the beadlyte 6-plex Ovarian Cancer Panel Kit and a kit for the analysis of HE4, both from Millipore, according to the manufacturers instructions [18,19]. The 6-plex Ovarian Cancer Panel Kit included two panels: one for prolactin, leptin, OPN, MIF and CA125 (Beadlyte 5-plex Ovarian Cancer Panel) and a separate panel for IGF-II (Beadlyte Anti-Human IGF-II Bead Set). Because of data suggesting that HE4 plays a role in OC, we added a separate kit for the detection of HE4 (Beadlyte Anti-Human HE4 Bead Set).

**Statistical analysis**

Missing Luminex values, i.e. values below analyte-specific detection limits, were imputed with analyte-corresponding PBS-values divided by square root of two. The geometric mean of two calibrator samples were used as reference to level plate specific differences and all values were log-2 transformed to get (near) parametric distributions.

Statistical differences over all four/five groups (CTRL WT, CTRL BRCA, Ca WT, and Ca BRCA) were calculated by one-way analysis of variance (ANOVA) with subsequent–if significant–post-hoc tests according Tukey’s ‘Honest Significant Difference’ method. Multivariable discriminative models were built by logistic regressions and a cut-off defined by maximizing specificity and sensitivity simultaneously (R-package OptimalCutpoints). Receiver Operating Characteristic (ROC) curves, corresponding area under these ROC curves (AUC), and p-values are presented. (Two-sided) p-values below 0.05 were considered as statistically significant. All analyses were performed with R version 3.3.3 [21] and R-packages: ROCR v1.0–7 [22] and OptimalCutpoints v1.1–3 [23].

**Results**

**Differentiation between healthy women and OC patients**

We performed multiplex analysis and evaluated the serum determination of the seven biomarkers (CA125, MIF, Leptin, OPN, Prolactin, IGF2 and HE4) in healthy wildtype women, healthy women with *BRCA1* germline mutation, wildtype OC patients and *BRCA1* mutation carriers with OC. Since 17 samples of patients with wildtype OC and 26 samples of *BRCA1* mutation carriers with OC came from one institution (tumorbank ovarian cancer (TOC) of the Charité, Medical University of Berlin), we performed a test for interaction in order to identify possible laboratory or sampling bias. We found no significant difference for CA125, leptin, OPN, MIF, IGF2 and HE4. However, the prolactin levels in the respective subgroups differed significantly between the two centers and we therefore excluded prolactin from our algorithm (see S1 Fig).

The individual serum levels of the evaluated biomarkers are shown in Fig 1A–1F. CA125 (Fig 1A) and MIF (Fig 1B) were shown to be the best single markers to differentiate between healthy women and OC patients in wildtype as well as *BRCA1* mutation carriers (MIF: p = 4.64e-16; CA-125: p = 9.19e-23, respectively) followed by Leptin (p = 0.00000108; Fig 1C). While different levels of HE4 (Fig 1D) lead to a significant difference between healthy wildtype women and wildtype OC patients (p = 0.000898), we found no significant difference in the HE4 levels of healthy *BRCA1* mutation carriers and those with OC. We found no significant differences between the four groups comparing their levels of IGF2 (P = 0.138; Fig 1E) or OPN (p = 0.528; Fig 1F).
Serum biomarkers in BRCA1 mutation carriers

A. CA123

B. MIF

C. Leptin

D. HE4

E. IGFB2

F. CFSP
We then adjusted our results for age and looked at correlations between the level of the investigated biomarkers and age (S2–S5 Figs, Table 2). We found a positive correlation of MIF with age (R = 0.42, p < 0.05) and a negative correlation of IGF2 with age (R = -0.35, p < 0.1) in the wildtype control group. We found no such correlations in the BRCA1 groups where the difference in age between healthy mutation carriers and those with OC were significant.

Differentiation between wildtype women and BRCA1 mutation carriers

We then investigated if there are differences in the levels of these six biomarkers between wildtype women and BRCA1 mutation carriers. In the healthy cohort, we found significant higher levels of CA125 (Fig 1A) and lower levels of Leptin (Fig 1E) in BRCA1 mutation carriers. In the OC cohort, Leptin was the only biomarker with significantly different (lower) levels between wildtype OC patients and BRCA1 mutation carriers (Fig 1E).

ROC Analysis

Since IGF2 levels were not available from all patients, we restricted our algorithm to five biomarkers (MIF, Leptin, CA125, OPN and HE4) using area under the receiver operating characteristic (ROC) curves (AUCs). When we evaluated the value of the five biomarkers in differentiating between healthy women and OC patients regardless of their mutation status, we found a sensitivity of 94.3% (AUC = 0.981; p = 6.4e-20; Fig 2A). We then calculated the ROC AUC separately for the wildtype subgroup showing a sensitivity of 92.8% (AUC = 0.988; p = 5.2e-14; Fig 2B) and the subgroup of BRCA1 mutation carriers resulting in a sensitivity of 95.2% (AUC = 0.978; p = 1.7e-15; Fig 2C) at an overall sensitivity of 92.3%.

We then investigated if these 5 biomarkers are able to differentiate between wildtype and BRCA1 mutation carriers and found a sensitivity of 88.4% at a specificity of 80.7% (AUC = 0.895; p = 6e-08) in the healthy subgroup (Fig 2D) and a sensitivity of 66.7% at a specificity of 67.8% at a specificity of 67.8% (AUC = 0.724; p = 0.00065) in the subgroup of OC patients (Fig 2E).

Discussion

To date, screening for OC in women at high risk consists of the combination of CA125 and TVU, although evidence is insufficient to demonstrate that these tests provide a survival benefit [8,9]. Improvement of OC screening strategies in high risk women is urgently warranted not only for BRCA1 mutation carriers who decide to postpone or forego RRSO, but also for women with a family history who should follow early detection programs for breast and ovarian cancer.

Table 2. Correlations of the levels of the investigated biomarkers with age in the four study groups.

|        | age | CA125 | MIF  | Leptin | HE4  | IGF2 | OPN | Prolactin |
|--------|-----|-------|------|--------|------|------|-----|----------|
| Co WT  | 67,1| 0,42  | -0,35|        |      |      |     |          |
| Co BRCA1| 36,4|       |      |        |      |      |     |          |
| CA WT  | 57,6| 0,49  | -0,50| 0,47   |      |      |     |          |
| CA BRCA1| 53,0|       |      |        |      |      |     |          |

**bold**: p<0.05, **bold, italic**: p<0.100, black: not sign.
Fig 2. Sensitivity and specificity. Sensitivity and specificity of the combination of five biomarkers (CA125, MIF, Leptin, HE4, OPN) in differentiating between healthy women and OC patients (A), healthy wildtype women and wildtype OC patients (B), healthy BRCA1 mutation carriers and BRCA1 mutation carriers with OC (C), healthy wildtype women and healthy BRCA1 mutation carriers (D) and wildtype OC patients and BRCA1 mutation carriers with OC (E).

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We here present the first study comparing the serum levels of a combination of five biomarkers (MIF, leptin, OPN, CA125 and HE4) in healthy wildtype women, healthy BRCA1 mutation carriers, wildtype OC patients and BRCA1 mutation carriers with OC.

Our data demonstrate that an algorithm based on these five proteins is able to significantly differentiate between healthy and OC patients in wildtype patients as well as BRCA1 mutation carriers.

In average risk women, a wide range of diagnostic approaches like panels of biomarkers, algorithms, ultrasound and other imaging methods have been investigated to improve the early detection of OC [24–28].

Concentrating on biomarkers, HE4 has been reported to be superior to CA125 in separating benign, borderline ovarian tumors, cancers of the fallopian tubes, as well as early stage epithelial OC [29–39]. Together with CA125 and menopausal status it has been incorporated into the Risk of Ovarian Malignancy Algorithm (ROMA) [40] and in combination with CA125-II, apolipoprotein A-1, follicle stimulating hormone, and transferrin into the Overa-Test in order to discern malignant from benign pelvic masses [41].

Osteopontin is another interesting biomarker, which has been shown to ameliorate the discriminating ability between benign and malign pelvic masses when combined with HE4 and CA125 [42]. Furthermore, El-Tanani MK et al demonstrated that BRCA1 mutation lead to OPN overexpression resulting in proliferation of breast cancer cells in a rat mammary model system [43], thus OPN could also be an important biomarker in the development of OC in BRCA1 mutation carriers.

There is some evidence that lower levels of leptin [44] and higher levels of prolactin [45] might be associated with increased risk of ovarian cancer.

The combination of the above mentioned six biomarkers (MIF, OPN, CA125, IGF II, leptin and prolactin) has been shown to improve differentiation between disease free and ovarian cancer patients compared to CA125 alone in patients without a family history of OC [18,19]. Other studies reported similar benefits when combining these six biomarkers with p53 [46] or interleukin 18 (IL-18) and fibroblast growth factor 2 (FGF-2) [47].

Although these developments seem to be promising, recent data suggest that CA125 is still the best single marker for the early detection of OC [48–50] in average risk women and limited data exists regarding the use of these biomarkers as OC screening in high risk women.

In high risk women, it has been suggested that higher cut-off levels and frequent CA125 testing could improve the low sensitivity and specificity of the current early detection program [51]. Our data, which show significantly higher levels of CA125 in healthy BRCA1 mutation carriers and lower levels of leptin when compared to healthy wildtype women, confirm the suggested necessity of individual adjustment of CA125 cut-off levels.

Our study has several limitations like the small sample size and the possible laboratory or sampling bias, because samples have been obtained from two different biobanks. Although all OC samples have been collected prior to surgery following a standardized protocol in the two centers, we performed a test for interaction and found no significant difference for CA125, leptin, OPN, MIF, IGF2 and HE4. However, the prolactin levels differed significantly between the two centers and we therefore excluded prolactin from our algorithm.

Furthermore our study is limited by the difference in age between healthy BRCA1 mutation carriers and those with OC as age could impact the expression of biomarkers. While we found a positive correlation of the level of MIF in the healthy wildtype group, we found no such correlation in healthy BRCA1 mutation carriers or those with OC. We found slightly higher levels of MIF in the healthy wildtype group (oldest cohort) compared to the healthy BRCA1 mutation carriers (youngest cohort), but in both OC groups (WT and BRCA1) the levels of MIF were significantly higher than in the healthy control groups. Furthermore, we found a negative
correlation between the levels of IGF2 and age and therefore suggest that the higher levels of IGF2 (p = n.s.) in healthy BRCA1 mutation carriers result from the younger age in this group.

Taken together we have developed an algorithm, which can differentiate between healthy women and OC patients with a sensitivity of 94.3% and a specificity of 92.3% and have for the first time shown, that such an algorithm can also be used in BRCA mutation carriers with a sensitivity of 95.2% and a specificity of 92.3%.

However, to verify if this algorithm could improve the early detection of OC in high risk women, larger prospective trials with mainly early stage OC cases are warranted.

Supporting information

S1 Fig. Serum levels of prolactin. Different serum levels of Prolactin with significant differences between samples from the two biobanks (Vienna and Berlin). We therefore excluded Prolactin from our calculations.

S2 Fig. Correlations of the investigated biomarkers with age in the Co WT group. Correlation coefficient (R); *p<0.1 (not sign.); * p<0.05; ** p<0.01; *** p<0.001(Spearman).

S3 Fig. Correlations of the investigated biomarkers with age in the CA WT group. Correlation coefficient (R); *p<0.1 (not sign.); * p<0.05; ** p<0.01; *** p<0.001(Spearman).

S4 Fig. Correlations of the investigated biomarkers with age in the Co BRCA1 group. Correlation coefficient (R); *p<0.1 (not sign.); * p<0.05; ** p<0.01; *** p<0.001(Spearman).

S5 Fig. Correlations of the investigated biomarkers with age in the CA BRCA1 group. Correlation coefficient (R); *p<0.1 (not sign.); * p<0.05; ** p<0.01; *** p<0.001(Spearman).

Author Contributions

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References
1. Smith RA, Andrews KS, Brooks D, Fedewa SA, Manassaram-Baptiste D, Saslow D, et al. Cancer Screening in the United States, 2017: A review of current american cancer society guidelines and current issues in cancer screening. Ca Cancer J Clin. 2017; 67:100–121. https://doi.org/10.3322/caac.21392 PMID: 2810806

2. King MC, Marks JH, Mandell JB. New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science. 2003; 302:643–646. https://doi.org/10.1126/science.1088795 PMID: 14576434

3. Singer CF, Tea MK, Pristauz G, Hubalek M, Rappaport C, Riedl CC, et al. Clinical Practice Guideline for the prevention and early detection of breast and ovarian cancer in women from HBOC (hereditary breast and ovarian cancer) families. Wien Klin Wochenschr. 2015; 127:981–986. https://doi.org/10.1007/s00508-015-0880-x PMID: 26525377

4. Riedl CC, Luft N, Bernhart C, Weber M, Bernathova M, Tea MK, et al. Triple-modality screening trial for familial breast cancer underlines the importance of magnetic resonance imaging and questions the role of mammography and ultrasound regardless of patient mutation status, age, and breast density. J Clin Oncol. 2015; 33:1128–1135. https://doi.org/10.1200/JCO.2014.56.8626 PMID: 25713430

5. Jacobs IJ, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics 2004; 3:355–66. https://doi.org/10.1074/mcp.R400006-MCP200 PMID: 1476655

6. Wilder JL, Pavlik E, Straughn JM, et al. Clinical implications of a rising serum CA-125 within the normal range in patients with epithelial ovarian cancer: a preliminary investigation. Gynaecol Oncol 2003; 89:233–5.

7. DePriest PD, DeSimone CP. Ultrasound screening for the early detection of ovarian cancer. J Clin Oncol 2003; 21:194–9.

8. Menon U, Gentry-Maharaj A, Hallett R, Ryan A, Burnell M, Sharma A, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK–Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol. 2009; 10:327–340. https://doi.org/10.1016/S1470-2045(09)70026-9 PMID: 19282241

9. Bauss SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening randomized controlled trial. JAMA Oncol. 2011; 305:2295–2303. https://doi.org/10.1001/jama.2011.766 PMID: 21642681

10. Patrone MG, Iniesta MD, Malpica A, Lu KH, Fernandez RO, Salvo G, et al. Clinical outcomes in patients with isolated serous tubal intraepithelial carcinoma (STIC): a comprehensive review. Gynecol Oncol. 2015; 139:568–572. https://doi.org/10.1016/j.ygyno.2015.09.018 PMID: 26407480

11. Domchek SM, Friebele TM, Garber JE, Isaacs C, Mallof E, Eeles R, et al. Occult ovarian cancers identified at risk-reducing salpingo-oophorectomy in a prospective cohort study of BRCA1/2 mutation carriers. Breast Cancer Res Treat. 2010; 124:203–209. https://doi.org/10.1007/s10549-010-0799-x PMID: 20180014

12. Eisen A, Lubinski J, Klijn J, Moller P, Lynch HT, Offit K, et al. Breast cancer risk following bilateral oophorocentomy in BRCA1 and BRCA2 mutation carriers: an international case-control study. J Clin Oncol. 2005; 23:7491–7496. https://doi.org/10.1200/JCO.2004.00.7138 PMID: 16234515

13. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst. 2009; 101:90–97. https://doi.org/10.1093/jnci/djn442 PMID: 19141781

14. Domchek SM, Friebele TM, Neuhausen SL, Wagner T, Evans G, Isaacs C, et al. Mortality after bilateral salpingo-oophorectomy in BRCA1 and BRCA2 mutation carriers: a prospective cohort study. Lancet Oncol. 2006; 7:223–229. https://doi.org/10.1016/S1470-2045(0670595-X PMID: 16510331

15. Mannis GN, Fehninger JE, Creasman JS, Jacoby VL, Beattle MS. Risk-reducing salpingo-oophorectomy and ovarian cancer screening in 1077 women after BRCA testing. JAMA Intern Med. 2013; 173:96–103. https://doi.org/10.1001/2013.jamainternmed.962 PMID: 23247828
16. Chudecka-Glaz A, Cymbaluk-Ploska A, Strojna A, Menkiszak J. HE4 serum levels in patients with BRCA1 gene mutation undergoing prophylactic surgery as well as in other benign and malignant gynecological diseases. Dis Markers. 2017; 2017:9792756. Epub 2017 Jan 15.

17. Karlan BY, Thorpe J, Watabayashi K, Drescher CW, Palomares M, Daly MB, et al. Use of CA125 and HE4 serum markers to predict ovarian cancer in elevated-risk women. Cancer Epidemiol Biomarkers Prev. 2014; 23:1383–1393. https://doi.org/10.1158/1055-9966.EPI-13-1361 PMID: 24789859

18. Visintin I, Feng Z, Longton G, et al. Diagnostic Markers for Early Detection of Ovarian Cancer. Clin Cancer Res 2008; 14(10):1065–72. https://doi.org/10.1158/1078-0432.CCR-07-1569 PMID: 18258665

19. Pils D, Tong D, Hager G, Obermayr G, Aust S, Heinze G, et al. A combined blood based gene expression and plasma protein abundance signature for diagnosis of epithelial ovarian cancer- a study of the OVCA consortium. BMC Cancer 2013; 13:178. https://doi.org/10.1186/1471-2407-13-178 PMID: 23551967

20. Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, et al. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci USA 2005; 102:7667–7682.

21. R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2008:ISBN 3-900051-07-0, URL http://www.R-project.org.

22. Sing T, Sander O, Beerenwinkel N, Lengauer T. ROCr: visualizing classifier performance in R. Bioinformatics. 2005; 21:3940–3941. https://doi.org/10.1093/bioinformatics/bti623 PMID: 16096348

23. Monica Lopez-Raton Maria Xose Rodriguez-Alvarez, Carmen Cadarso Suarez Francisco Gude Samperdo (2014). OptimalCutpoints: An R Package for Selecting Optimal Cutpoints in Diagnostic Tests. Journal of Statistical Software 2014; 61:1–36.

24. Leung F, Bernardini MQ, Brown MD, Zheng Y, Molina R, Bast RC Jr, et al. Validation of novel biomarker panel for the detection of ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2016; 25:1333–1340. https://doi.org/10.1158/1055-9965.EPI-15-1299 PMID: 27448593

25. Fortner RT, Vitonis AF, Schock H, Hustin A, Johnson T, Fichorova RN, et al. Correlates of circulating ovarian cancer early detection markers and their contribution to discrimination of early detection models: results from the EPIC cohort. J Ovarian Res. 2017; 10:20. https://doi.org/10.1186/s13048-017-0315-6 PMID: 28320479

26. Dayyani F, Uhlig S, Colson B, Simon K, Rolny V, Morgenstern D, et al. Diagnostic Performance of Risk of Ovarian Cancer Algorithm against CA125 and HE4 in connection with ovarian cancer: a meta-analysis. Int J Gynecol Cancer. 2016; 26:1586–1593. https://doi.org/10.1097/IGC.0000000000000804 PMID: 27540691

27. Ueland FR. A perspective on ovarian cancer biomarkers: past, present and yet-to-come. Diagnostics. 2017; 7:14.

28. El Bairi K, Kandhro AH, Gouri A, Mahfoud W, Louanji N, Saadani B, et al. Emerging diagnostic, prognostic and therapeutic biomarkers for ovarian cancer. Cell Oncol. 2017; 40:105–118.

29. Granato T, Porpora MG, Longo F, Angeloni A, Manganaro L, Anastasi E. HE4 in the differential diagnosis of ovarian masses. Clin Chim Acta. 2015; 446:147–155. https://doi.org/10.1016/j.cca.2015.03.047 PMID: 25892674

30. Macedo AC, da Rosa MI, Lumertz S, Meeiros LR. Accuracy of serum human epididymis protein 4 in ovarian cancer diagnosis; a systematic review and meta-analysis. Int J Gynecol Cancer. 2014; 24:1222–1231. https://doi.org/10.1097/IGC.0000000000000192 PMID: 25078339

31. Lin JY, Qin JB, Li XY, Dong P, Yin BD. Diagnostic value of human epididymis protein 4 compared with mesothelin for ovarian cancer: a systematic review and meta-analysis. Asian Pac J Cancer Prev. 2012; 13:5427–5432. PMID: 23317195

32. Wu L, Dai ZY, Qian YH, Shi Y, Liu FJ, Yang C. Diagnostic value of serum human epididymis protein 4 (HE4) in ovarian carcinoma: a systematic review and meta-analysis. Int J Gynecol Cancer. 2012; 22:1106–1112. https://doi.org/10.1097/IGC.0b013e318263eaf2 PMID: 22854652

33. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2008; 108:402–408. https://doi.org/10.1016/j.ygyno.2007.10.017 PMID: 18061248

34. Moore RG, Miller MC, Disilvestro P, et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstet Gynecol. 2011; 118:280–288. https://doi.org/10.1097/AOG.0b013e318224fecd2 PMID: 21775843

35. Ferraro S, Braga F, Lanzoni M, Boracchi P, Biganzoli EM, Panteghini M. Serum human epididymis protein 4 vs carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review. J Clin Pathol. 2013; 66:273–281. https://doi.org/10.1136/jclinpath-2012-201031 PMID: 23426716

36. Scaletta G, Plotti F, Luvero D, Capriglione S, Monteria R, Miranda A, et al. The role of novel biomarker HE4 in the diagnosis, prognosis and follow-up of ovarian cancer: a systematic review. Expert Rev Anti-Cancer Ther. 2017; 17:827–839. https://doi.org/10.1080/14772562.2017.1360138 PMID: 28756722
37. Angioli R, Plotti F, Capigrilone S, Aloisi A, Montera R, Luvero D, et al. Can the preoperative HE4 level predict optimal cytoreduction in patients with advanced ovarian carcinoma? Gynecol Oncol. 2013; 128:579–583. https://doi.org/10.1016/j.ygyno.2012.11.040 PMID: 23220563

38. Plotti F, Capigrilone S, Terranova C, Montera R, Aloisi A, Damiani P, et al. Does HE4 have a role as biomarker in the recurrence of ovarian cancer? Tumour Biol. 2012; 33:2117–2123. https://doi.org/10.1007/s13277-012-0471-7 PMID: 22875782

39. Capigrilone S, Luvero D, Plotti F, Terranova C, Montera R, Scaletta G, et al. Ovarian cancer recurrence and early detection: may HE4 play a key role in this open challenge? A systematic review of literature. Med Oncol. 2017; 34:164. https://doi.org/10.1007/s12032-017-1026-y PMID: 28825178

40. Terlikowska KM, Dobrzycka B, Wilkowska AM, Mackowiak-Matejczyk B, Sledziewski TK, Kinalsie M, et al. Preoperative HE4, CA125 and ROMA in the differential diagnosis of benign and malignant adnexal masses. J Ovarian Res. 2016; 9:43. https://doi.org/10.1186/s13277-016-0254-7 PMID: 27436085

41. Coleman R, Herzog T, Chan D, Munroe D, Pappas T, Smith A, et al. Validation of a second-generation multivariate index assay for malignancy risk of adnexal masses. J Obstet Gynecol. 2016; 215:82.e1–82.e11.

42. Horala A, Swiatly A, Matysiak J, Banach P, Nowak-Markwitz E., Kokot ZJ. Diagnostic value of serum angiogenesis markers in ovarian cancer using Multiplex Immunoassay. Int J Mol Sci. 2017; 18:123.

43. El-Tanani MK, Campbell FC, Crowe P, Erwin P, Harlin DP, Pharoah P, et al. BRCA1 suppresses osteopontin-mediated breast cancer. J Biol Chem. 2006; 281:26587–26601. https://doi.org/10.1074/jbc.M604032020 PMID: 16807234

44. Jin JH, Kim HJ, Kim CY, Kim YH, Ju W, Kim SC. Association of plasma adiponectin and leptin levels with the development and progression of ovarian cancer. Obstet Gynecol Sci. 2016; 59:279–285. https://doi.org/10.5468/ogs.2016.59.4.279 PMID: 27462594

45. Clendenen TV, Arslan AA, Lokshin AE, Liu M, Lundin E, Koenig KL, et al. Circulating prolactin levels and risk of epithelial ovarian cancer. Cancer Causes Control. 2013; 24:741–748. https://doi.org/10.1007/s10552-013-0156-6 PMID: 23378139

46. Lu D, Kuhn E, Bristow RE, Giuntoli II RL, Krüger Kjaer S, Shih I-M, et al. Comparison of candidate serologic markers for type I and type II ovarian cancer. Gynecol Oncol. 2011; 122:560–566. https://doi.org/10.1016/j.ygyno.2011.05.039 PMID: 21704359

47. He G, Holcroft CA, Beauchamp MC, Yasmeen A, Ferenczy A, Kendall-Dupont J, et al. Combination of serum biomarkers to differentiate malignant from benign ovarian tumors. J Obstet Gynaecol Can. 2012; 34:567–574. https://doi.org/10.1016/S1701-2163(16)35273-2 PMID: 22673173

48. Skates SJ. EPIC early detection of ovarian cancer. Clin Cancer Res. 2016; 22:4542–4544. https://doi.org/10.1158/1078-0432.CCR-16-1391 PMID: 27418634

49. Terry KL, Schock H, Fortner RT, Hüsing A, Fichorova RN, Yamamoto HS, et al. A prospective evaluation of early detection biomarkers for ovarian cancer in the European EPIC cohort. Clin Cancer Res. 2016; 22:4664–4675. https://doi.org/10.1158/1078-0432.CCR-16-0316 PMID: 27060155

50. Sólétormos G, Duffy MJ, Othman Abu Hassan S, Verheijen RH, Tholander B, Bast RC Jr, et al. Clinical Use of Cancer Biomarkers in Epithelial Ovarian Cancers: Updated Guidelines From the European Group on Tumor Markers. Int J Gynecol Cancer. 2016; 26:43–51. https://doi.org/10.1097/IGC.0000000000000586 PMID: 26588231

51. Skates SJ, Greene MH, Buys SS, Mai PL, Brown P, Piedmonte M, et al. Early detection of ovarian cancer using the Risk of Ovarian Cancer Algorithm with frequent CA125 testing in women at increased familial risk- combined results from two screening trials. Clin Cancer Res. 2017; Epub 2017 Jan 31.