

# Serial measurements of serum human epididymal protein 4 in patients at risk for ovarian cancer

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## Summary

**Objective:** Patients with a BRCA1 or BRCA2 gene mutation have an increased lifetime risk of developing epithelial ovarian cancer (EOC). Screening with CA125 and ultrasound is ineffective for detection of EOC at an early stage and does not reduce the mortality rate of EOC. Therefore, women at risk of developing ovarian cancer are recommended to have risk-reducing salpingo-oophorectomy (RRSO). The benefit of the serum marker human epididymis protein 4 (HE4) in screening programmes is still unknown. Therefore, the authors evaluated serial serum HE4 measurements in patients at high risk of developing EOC based on a familiar or genetic predisposition. **Materials and Methods:** Patients with BRCA1 or BRCA2 mutation or familiar predisposition that developed EOC during screening were selected from the hospital based cancer registry. HE4 was measured in consecutive serum samples. **Results:** Cross-linking the hospital-based cancer registry with the serum bank resulted in 182 patients who had developed EOC between 1994 and 2013. More than one serum sample was available of 52 patients but of these only seven patients underwent regular screening. HE4 demonstrated a rapid increase in serum levels just before or at time of diagnosis instead of a longer lead time before diagnosis. This is comparable to the concentrations of serum CA125 in consecutive samples. **Conclusion:** This is the first study showing that serum HE4 values suddenly increase just before diagnosis and do not precede the development of symptoms. This does not support the use of HE4 with a fixed cut-off value for screening in patients at high risk for EOC.

**Key words:** Biomarker; HE4; Screening; BRCA; Epithelial ovarian cancer.

## Introduction

In the Netherlands, the risk of developing epithelial ovarian cancer (EOC) in the general population before the age of 70 is estimated to be 0.7% [1]. Despite this relatively low prevalence, EOC causes the most gynecological cancer-related deaths and mortality rates remain unchanged for the past four decades. Risk factors that increase the lifetime risk of developing EOC are early menarche, nulliparity, late menopause, age, and a familiar predisposition for EOC. It is estimated that 5-10% of cases of EOC are caused by a hereditary predisposition. A germline mutation in the BRCA1 or BRCA2 gene is the most common genetic mutation in women at risk for developing EOC. Women with a germline mutation in the BRCA1 or BRCA2 gene have a 39% and 16% lifetime risk of developing EOC, respectively [2].

Despite various efforts, screening tests for the general population have failed to reduce the mortality rate of EOC. Screening modalities are also ineffective for women with an elevated risk to develop EOC. Studies evaluating screening with transvaginal ultrasound (TVU) and serum CA125 show that these tests lead to false-positive results and un-

necessary surgical interventions [3]. Given the lack of effective screening modalities, women with a BRCA1 or BRCA2 mutation are recommended to have risk-reducing prophylactic bilateral salpingo-oophorectomy (RRSO) at the age of 35-40 years and 40-45 years, respectively. A recent meta-analysis showed that RRSO reduces the ovarian cancer risk by 80% for both BRCA1 and BRCA2 mutation carriers and leads to a reduction in all-cause mortality by 70% [4]. However, the accompanying decline in serum estrogen and androgen levels and other side effects reduces the quality of life of premenopausal women who have received RRSO.

Serum CA125 was the topic of several studies that evaluated the use of biomarkers in the screening of patients for EOC. A large prospective study that included women with an average risk of EOC showed that single (semi) annual CA125 measurements using a fixed cut-off value combined with TVU failed to significantly reduce ovarian cancer mortality rate [3]. Similarly, Hermsen *et al.* [5] did not find a beneficial effect of annual gynecological screening on ovarian cancer mortality. Their multicenter observational follow-up study conducted in the Netherlands included 883

Revised manuscript accepted for publication September 5, 2016

BRCA1 or BRCA2 mutation carriers, of whom only ten women were diagnosed with EOC during follow-up. The observed number of cases did not differ significantly from the expected number based on reference curves [6]. In five of these ten cases, EOC was diagnosed in between screening moments and there was no difference in stage distribution between EOC cases. These results indicate that annual screening with serum CA125 and TVU is unlikely to reduce ovarian cancer related mortality. [5]

Other approaches have been employed to improve the screening capability of CA125 levels. For example, Menon *et al.* showed that the use of changes in serial CA125 measurements instead of a fixed serum marker threshold leads to a doubling in detection rate of EOC [7]. However, whether using dynamic measurements of CA125 instead of fixed thresholds, is a better indicator of disease occurrence or improves cancer-related mortality, remains to be seen. Taken together, these studies underscore the urgent need for other biomarkers that can be used for screening purposes. One possibly candidate is serum biomarker human epididymis protein 4 (HE4). HE4 has proven to have a higher specificity and comparable sensitivity to CA125 for the differentiation between a benign and malignant ovarian mass [8, 9]. In this study, the authors performed a retrospective analysis to evaluate serial serum HE4 measurements in a group of high-risk patients based on a familiar or genetic predisposition that were diagnosed with EOC during screening. They found that both HE4 and CA125 serum concentrations increase just before diagnosis, suggesting that both biomarkers fail to detect EOC in an early stage.

## Materials and Methods

Because the majority of women with BRCA mutations or from HBOC (hereditary breast and ovarian cancer) families undergo RRSO, a prospective study to evaluate the use of HE4 is nearly impossible. Also, to screen more than 800 women like in the previous study by Hermesen *et al.* [5] to find only ten women was considered to be inefficient. Therefore, the authors searched the hospital-based cancer registry for patients with a BRCA1 or BRCA2 gene mutation with a confirmed diagnosis of EOC and cross-linked these data with the serum bank to check whether multiple serum samples were available for analysis. Only patients with more than one serum samples available before diagnosis were included. Patients were excluded when the last serum sample preceding diagnosis was obtained more than one year before diagnosis. Expert review of the pathology had to be available. The authors defined cases as 'screen-detected' when the diagnosis of ovarian cancer was made at regular screening with CA125 and ultrasound made by an expert in ultrasonography, and as 'interval-detected' cases when diagnosis was made after development of physical symptoms, while previous screening had not revealed any abnormalities. Cases detected at RRSO, with no abnormalities found at preoperative screening, were called 'occult' carcinomas. All 'cases' of EOC were matched with three age-matched controls of patients with a BRCA1 or BRCA2 gene mutation who underwent RRSO and histological examination did not reveal (pre) malignant lesions in either ovaries or tubes.

Venous blood samples were collected at different time points

before diagnosis using standard sampling tubes without additives. After allowing blood to clot for at minimum half an hour, the blood was centrifuged for ten minutes at 1,700 g and serum was aliquoted in three cryovials. The serum was stored at -30°C until measurement. Serum CA125 values were already known for most patients, but measurements done before 2003 were repeated with stored serum because of a change in measurement procedure over time.

HE4 and CA125 concentrations were measured using the electrochemiluminescence immunoassay (ECLIA). The upper limit of normal (ULN) for CA-125 was set at 35 kU/L for premenopausal women and at 20 kU/L for postmenopausal women [10]. Cut-off values for HE4 were based on age and in the present hospital established at 60 pmol/L for patients younger than 40 years, 75 pmol/L for patients between 40-60 years, and finally 90 pmol/L for patients >60 years of age [11].

## Results

Cross-linking the hospital-based cancer registry with the serum bank resulted in 182 patients with a BRCA1 or BRCA2 gene mutation or HBOC family and EOC between 1994-2013. The majority of these women presented as expected in Stage III EOC. Often the EOC diagnosis was the reason to offer genetic counseling and thus screening was not performed. These women were not eligible for this study. Serum samples from before time of diagnosis of EOC of 52 patients were available. Of these, in 43 patients serum samples were not obtained for screening but for other purposes e.g preoperative workup, and only nine patients underwent actual screening before they were diagnosed with EOC. Two patients were excluded because the time between last serum sample and diagnosis exceeded one year (1.6 and 3.7 years, respectively). This resulted in seven patients that were included for final analysis of serum CA125 and HE4 level.

One of these seven cases was screened for EOC because of a HBOC family. The other six patients had a BRCA mutation (four BRCA1 and two BRCA2). Three cases were 'screen-detected', two were interval cases, and one was detected at RRSO. The remaining one could not be further specified according to these three groups because EOC was diagnosed after RRSO was performed.

Patients' characteristics are summarized in Table 1 and a short description of each patient is provided in the accompanying legend. Patients are ordered based on the three groups (screen-detected *vs.* interval-detected *vs.* RRSO-detected) that were described previously. One case (number 7) is shown in *italic* because it could not be classified into one of these groups. In this specific case, the patient was diagnosed with EOC one year after RRSO. Diagnosis was made after the onset of clinical symptoms and an elevated serum CA125 concentration was found during annual screening. Screening in this case was performed for the residual risk of extra-ovarian cancer. All patients with screen-detected EOC were diagnosed with advanced disease (FIGO Stage IIIC), while one of the interval-detected cases was diag-

Table 1. — Patients' characteristics.

Case number	Age at diagnosis of EOC (years)	Mutation	Screening yes/no, interval (months)	RRSO	Screen-detected vs interval cases vs RRSO detected	Histological diagnosis	FIGO Stage
1	52	BRCA 2	Yes, 6	No	Screen detected	Serous adenocarcinoma	IIIC
2	47	BRCA 1	Yes, 6	No	Screen detected	Serous adenocarcinoma	IIIC
3	50	BRCA 1	Yes, 6-12	No	Screen detected	Adenocarcinoma nos	IIIC
4	32	BRCA 1	Yes, 6-12	No	Interval detected	Mucinous adenocarcinoma	IV
5	49	NA <sup>b</sup>	Yes, 12	No	Interval detected	Endometrioid adenocarcinoma	IC
6	54	BRCA 2	Yes, 6	Yes	RRSO	detected adenocarcinoma nos	IAI
7	60	BRCA 1	No	Yes	NA <sup>a</sup>	Serous adenocarcinoma	IIIC

EOC= epithelial ovarian cancer; RRSO= risk reducing salpingo-oophorectomy; NA= not applicable

<sup>a</sup> Case was diagnosed with extra-ovarian adenocarcinoma a few years after RRSO.

<sup>b</sup> Patient was screened because of a familiarly high risk for breast and ovarian cancer, but a mutation was never found.

Case description:

#1: detected by screening based on an elevated serum CA125 value.

#2: diagnosed with ovarian cancer before RRSO based on a preoperative elevated serum CA125 value.

#3: detected by screening based on an elevated serum CA125 value

#4: detected between screening moments by the development of abdominal complaints.

#5: detected between screening moments by the development of abdominal complaints.

#6: detected at RRSO, with normal preoperative serum CA125 value

#7: developed EOC one year after RRSO.

Table 2. — Overview of screening moments and corresponding serum CA125 and HE4 values

Case number	Age at diagnosis of EOC (years)		Time					Diagnosis
			t = -5	t = -4	t = -3	t = -2	t = -1	
1	52	CA125 (kU/L)	12	16	12	21	142 <sup>a</sup>	248 <sup>a</sup>
		HE4 (pmol/L)	37	56	38	45	236 <sup>a</sup>	-
2	47	CA125 (kU/L)	-	-	-	10	1352 <sup>a</sup>	1463 <sup>a</sup>
		HE4 (pmol/L)	-	-	-	58	98 <sup>a</sup>	-
3	50	CA125 (kU/L)	-	18	20	14	10	105 <sup>a</sup>
		HE4 (pmol/L)	-	-	-	40	56	-
4	32	CA125 (kU/L)	-	-	37	27	49 <sup>a</sup>	82 <sup>a</sup>
		HE4 (pmol/L)	-	-	40	41	63 <sup>a</sup>	-
5	49	CA125 (kU/L)	-	-	17	13	6	562 <sup>a</sup>
		HE4 (pmol/L)	-	-	57	48	9	-
6	54	CA125 (kU/L)	-	-	18	24	19	29
		HE4 (pmol/L)	-	-	-7	8 <sup>a</sup>	72	-
7 <sup>b</sup>	60	CA125 (kU/L)	15	16	17	105 <sup>a</sup>	370 <sup>a</sup>	893 <sup>a</sup>
		HE4 (pmol/L)	75	74	87 <sup>a</sup>	324 <sup>a</sup>	930 <sup>a</sup>	-

t = -5, -4, -3 etc. reflects screening moments, where t = -1 is closest to diagnosis and respectively 1, 11, 3, 12, 3 and 2 months prior to diagnosis for case 1 to 7. Interval between screening moments is different for each case (see Table 1).

<sup>a</sup> Indicates that serum values are above threshold (threshold CA125: 35 kU/L for premenopausal and 20 kU/L for postmenopausal women; threshold HE4 60 pmol/L for <40 years, 75 pmol/L for 40-60 years and 90 pmol/L for > 60 years)

<sup>b</sup> case was screened after RRSO for the residual risk of extra-ovarian cancer.

Suspicion of EOC was raised at t = -2.

nosed with early-stage EOC (FIGO IC). The one 'occult' carcinoma detected by RRSO comprised an early stage adenocarcinoma confined to one of the ovaries.

Table 2 gives an overview of serum CA125 and HE4 concentrations at different screening moments. Time between screening moments and time between last screening moment (t = -1) and diagnosis (t = 0) is different for each case and provided in the accompanying legend. Serum concentrations at time of diagnosis were obtained from serum collected a few days before surgery. In most cases "t = -1"

is the moment of the clinical suspicion of EOC, based on either screening or clinical symptoms. One exception to this is case 7, in which "t = -2" is the moment clinical suspicion of EOC is raised.

Figures 1 and 2 show serum CA125 and HE4 concentrations, respectively, during screening for all cases. Both biomarkers show a rapid increase of serum levels instead of a gradual rise. One patient (case 7) developed abdominal pain one year after RRSO and an elevated serum CA125 was found (Table 2, t = -2) during annual screening. TVU did

Figure 1. Course of serum CA125 concentrations in time, all cases

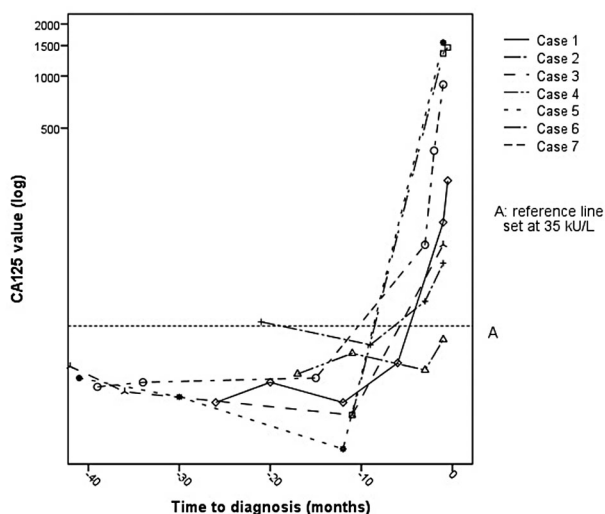


Figure 1. — Course of serum CA125 concentrations in time of all cases

Figure 2 Course of serum HE4 concentrations in time, all cases

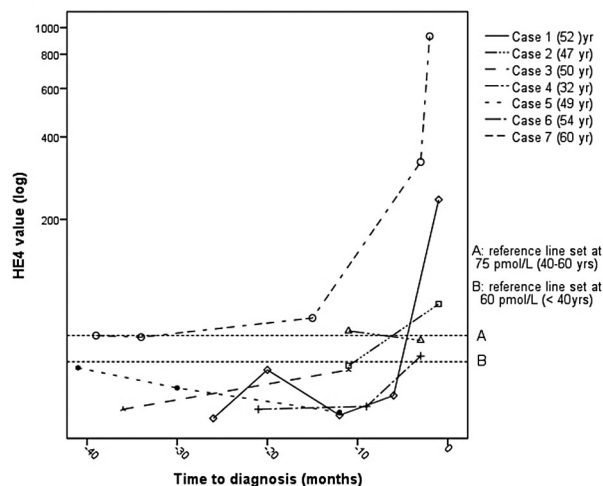


Figure 2. — Course of serum HE4 concentrations in time of all cases

not show any abnormalities at this moment. Serum CA125 measurement was repeated and showed a rapid increase three weeks later ( $t = -1$ ) and patient was planned for surgery. In this case serum HE4 showed an earlier increase in serum concentration (87 pmol/L at  $t = -3$ ) compared to serum CA125 (Figures 1 and 2, Table 2). Time between  $t = -2$  and  $t = -3$  was 12 months.

In case number 6 detected at RRSO, serum HE4 value showed an elevated value one year before diagnosis while serum CA125 was normal. In all age-matched control patients, both serum CA125 and HE4 show normal concentrations during preoperative screening (results not shown). These data suggest that HE4 is just as effective as CA125 in its usefulness as a screening biomarker for EOC.

**Discussion**

In this study, the authors evaluated the possible use of serial serum HE4 measurements as screening tool to detect EOC in an early stage in patients at risk. Unfortunately, the present results showed a rapid increase of serum HE4 levels just before time of diagnosis instead of a longer lead-time before diagnosis. The increase in HE4 levels mirror the course of serum CA125 presented here and results from a previous study [5]. Only in one case that underwent screening after RRSO, an earlier increased serum HE4 concentration compared to serum CA125 was found. Based on these results, serum HE4 is not useful as biomarker in screening for EOC in a high-risk population. It is therefore even more unlikely to be useful in a general population where the incidence of EOC is lower.

The rationale for screening lies in the fact that detection

of ovarian carcinoma at an early stage results in a reduced overall mortality rate. As mentioned earlier, screening for EOC using TVU and serum CA125 lack sensitivity and lead to unnecessary surgery without reducing mortality [3, 12] In the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial, women from the general population were randomized to either an intervention group who were offered annual screening with CA125 and TVU or a control group receiving standard care. This study demonstrated a ratio of surgery to invasive cancer of 19.5 to 1, and the majority of EOC cases in both groups were diagnosed at an advanced stage (FIGO III-IV). [12] Long-term follow up of the PLCO screening trial did not show a beneficial effect of screening on EOC mortality. Moreover, a 15% complication rate was associated with surgeries performed following a false-positive screening test result. [3]

A change in serum CA125 concentrations within the normal range instead of a single-threshold rule was used in the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Although the impact on the EOC mortality rate is not published yet, the number of screen-detected EOC cases, using serial CA125 measurement, was doubled. [7] Another study by Drescher *et al.* evaluating a longitudinal screening algorithm using serial CA125 values has also shown to detect EOC earlier than when using a single-threshold screening strategy [13]. It would be very interesting to measure serum HE4 concentrations and observe subtle changes of HE4 in these patients as well. In the present limited number of patients changes in HE4 concentrations within the normal range did not lead to extra detection rate.

Data of the PLCO trial was used to evaluate the potential

use of six candidate serum markers (HE4, mesothelin, matrix metalloproteinase 7 (MMP7), SLPI, Spondin2, and insulin-like growth factor binding protein 2 (IGFBP2)), as second step measurement in patients with increased CA125 concentrations [14]. These markers were selected based on the results of a previous study that systematically evaluated the performance of candidate serum makers for early detection of EOC [15]. The results showed that HE4 performed better than TVU as a second step screen, confirming 32% of cancers with increasing CA125 concentrations compared to 20% of cancers confirmed with TVU. [14] However, these results do not make screening itself effective. In line with the study of Hermesen *et al.* [5] with CA125, the authors found that serum HE4 suddenly increases before diagnosis and does not precede the development of clinical symptoms. Based on the results of this study, in spite of the limited number of patients, it is not likely that serial serum HE4 has a benefit over CA125 measurement in terms of screening in a high risk population.

This study is limited by its retrospective design. Unfortunately, not all serum samples of every patient were available for the retrospective determination of serum HE4. A difficulty in the analysis is caused by the differences in screening interval between cases and the duration of screening. However, this is the reality of screening in clinical practice. The use of HE4 in screening for each individual patient was evaluated and shown not to be effective.

As mentioned before, screening for EOC is not efficient in terms of mortality, and after the results of the study of Olivier *et al.* [16], screening with CA125 and TVU in high risk women was no longer advised in the national protocol. This resulted in an increase in the percentage of woman undergoing RRSO. As a consequence, the retrospective inclusion of women who had undergone screening for EOC was difficult. Despite this the authors were able to identify seven patients that could be further analyzed. To double this number, more than 1,000 women with a high risk of developing EOC should be screened at regular intervals. However, with the change in national protocol advising RRSO in these women, a prospective study is not feasible.

In this study, the authors used the same predefined age dependent single-threshold cut-off values for serum HE4 for all patients. However, an earlier study showed that serial CA125 measurements by using Risk of Ovarian Cancer Algorithm (ROCA), which takes into account an individual serial profile in comparison with cases and controls, could lead to improvement in screening performance [7, 17]. This could also be the case for serum HE4 and might lead to different results. Besides age, there might be more factors that influence serum HE4 levels, e.g. smoking and kidney function, and that could be of value to consider when evaluating HE4 for screening purposes [14, 18].

Despite these limitations, this is the first study showing that serum HE4 values suddenly increase just before diag-

nosis and do not precede the development of symptoms. These data indicate that HE4 as screening strategy is equivalent to CA125, but both markers fail to detect EOC at an early stage.

## Acknowledgements

The authors would like to thank the Starz foundation and Fujirebio Diagnostics Inc. for partially funding this study and Roche Diagnostics for providing the study reagents.

## References

- [1] "Dutch Cancer Registration [Internet]". Available at: [www.iknl.nl](http://www.iknl.nl)
- [2] Chen S.: "Meta-analysis of BRCA1 and BRCA2 penetrance". *J. Clin. Oncol.*, 2007, 25, 1329.
- [3] Buys S.S., Partridge E., Black A., Johnson C.C., Lamerato L., Isaacs C., *et al.*: "Effect of screening on ovarian cancer mortality". *JAMA*, 2011, 305, 2295.
- [4] Marchetti C., De Felice F., Palaia I., Perniola G., Musella A., Musio D., *et al.*: "Risk-reducing salpingo-oophorectomy: a meta-analysis on impact on ovarian cancer risk and all cause mortality in BRCA 1 and BRCA 2 mutation carriers". *BMC Womens Health*, 2014, 14, 1.
- [5] Hermesen B.B.J., Olivier R.I., Verheijen R.H.M., van Beurden M., de Hullu J.A., Massuger L.F., *et al.*: "No efficacy of annual gynaecological screening in BRCA1/2 mutation carriers; an observational follow-up study". *Br. J. Cancer*, 2007, 96, 1335.
- [6] Antoniou A., Pharoah P.D.P., Narod S., Risch H.A., Eyfjord J.E., Hopper J.L., *et al.*: "Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies". *Am. J. Hum. Genet.*, 2003, 72, 1117.
- [7] Menon U., Ryan A., Kalsi J., Gentry-Maharaj A., Dawnay A., Habib M., *et al.*: "Risk Algorithm Using Serial Biomarker Measurements Doubles the Number of Screen-Detected Cancers Compared With a Single-Threshold Rule in the United Kingdom Collaborative Trial of Ovarian Cancer Screening". *J. Clin. Oncol.*, 2015, 33, 2062.
- [8] Macedo A.C.L., da Rosa M.I., Lumertz S., Medeiros L.R.: "Accuracy of serum human epididymis protein 4 in ovarian cancer diagnosis: a systematic review and meta-analysis". *Int. J. Gynecol. Cancer*, 2014, 24, 1222.
- [9] Ferraro S., Braga F., Lanzoni M., Boracchi P., Biganzoli E.M., Panteghini M.: "Serum human epididymis protein 4 vs. carbohydrate antigen 125 for ovarian cancer diagnosis: a systematic review". *Biochim. Clin.*, 2013, 37, 179.
- [10] Bonfrer J.M.G., Korse C.M., Verstraeten R.A., van Kamp G.J., Hart G.A.M., Kenemans P.: "Clinical evaluation of the Byk LIA-mat CA125 II assay: Discussion of a reference value". *Clin. Chem.*, 1997, 43, 491.
- [11] Moore R.G., Miller M.C., Eklund E.E., Lu K.H., Bast R.C., Lambert-Messerlian G.: "Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age". *Am. J. Obstet. Gynecol.*, 2012, 206, 1.
- [12] Partridge E., Kreimer A.R., Greenlee R.T., Williams C., Xu J.L., Church T.R., *et al.*: "Results from four rounds of ovarian cancer screening in a randomized trial". *Obstet. Gynecol.*, 2009, 113, 775.
- [13] Drescher C.W., Shah C., Thorpe J., O'Briant K., Anderson G.L., Berg C.D., *et al.*: "Longitudinal screening algorithm that incorporates change over time in CA125 levels identifies ovarian cancer earlier than a single-threshold rule". *J. Clin. Oncol.*, 2013, 31, 387.
- [14] Urban N., Thorpe J.D., Bergan L.A., Forrest R.M., Kampani A.V., Scholler N., *et al.*: "Potential role of HE4 in multimodal screening for epithelial ovarian cancer". *J. Natl. Cancer Inst.*, 2011, 103, 1630.
- [15] Palmer C., Duan X., Hawley S., Scholler N., Thorpe J.D., Sahota R.A., *et al.*: "Systematic evaluation of candidate blood markers for

- detecting ovarian cancer". *PLoS One*, 2008, 3, 1.
- [16] Olivier R.I., Lubsen-Brandsma M.A.C., Verhoef S., van Beurden M.: "CA125 and transvaginal ultrasound monitoring in high-risk women cannot prevent the diagnosis of advanced ovarian cancer". *Gynecol. Oncol.*, 2006, 100, 20.
- [17] Skates S.J., Xu F.J., Yu Y.H., Sjøvall K., Einhorn N., Chang Y.C., *et al.*: "Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers". *Cancer*, 1995, 76, 2004.
- [18] Ferraro S., Schiumarini D., Panteghini M.: "Human epididymis protein 4: Factors of variation". *Clin. Chim. Acta*, 2015, 438, 171.

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