Abstract

To understand Cancer Antigen 125 (CA125) testing in primary care in relation to a national guideline we conducted a retrospective observational study including CA125 data from a well-defined region in the UK, from 2003-2014. 51,033 CA125 tests from 30,737 women, stratified by month and year of testing, location of test request, and patient age. Absolute numbers and rates of testing, rates and proportions of positive and negative tests, and frequencies of single and repeat tests were calculated. Negative binomial and logistic regression were used to test the effect of the guideline's introduction. Primary care testing spiked in the three months following the release of the guideline. However, there was no difference in the increase in testing observed across age groups. The proportion of positive tests decreased over time despite both the rates of positive and negative tests increasing. Retesting and repeat testing were associated with the initial CA125 value with no significant difference between women whose first test was 30-35IU/L and >35IU/L. Large studies using linked data are required to investigate the impact of increasing CA125 testing on onward intervention and patient outcomes. CA125 guidelines should be refined to avoid over-investigation in low risk age-groups.

KEY WORDS: Primary Care, Ovarian Cancer, CA125, Guidelines, Diagnosis.

Introduction

Ovarian cancer has been classified as "harder to suspect" as symptoms are typically vague and non-specific (Lyratzopoulos et al., 2014). Consequently it is diagnosed at a late-stage in 80% of cases in the UK, a third presenting as an emergency, and overall 5-year survival is 46% reducing to 40% in late-stage disease (Sundar et al., 2015). The effectiveness of ovarian cancer screening and primary care initiated symptom-triggered diagnostic testing remains unclear (Buys et al., 2011, Jacobs et al., 2016, NICE, 2015). Currently a symptom-triggered approach is recommended in the UK to investigate bloating, unexplained weight or appetite loss, early satiety, fatigue, a change in bowel habit, increased urinary urgency or frequency, and abdominal pain . These symptoms have low positive predictive values (PPV) in primary care as they are also present in many benign conditions (Hamilton et al., 2009) and their use in isolation may lead to unnecessary follow up of false positives. However a window of opportunity exists for targeted investigation as patients with ovarian cancer of all stages may present with these symptoms up to 36 months before diagnosis (Bankhead et al., 2005, Goff et al., 2004, Smith et al., 2005, Hamilton et al., 2009).

Cancer Antigen 125 (CA125) is a cell-surface antigen which is potentially elevated in the serum of patients with ovarian cancer and its measurement has been recommended by NICE in symptomatic women in primary care (NICE 2011) with values above 35 IU/L being used to trigger referral for ultrasound. The CA125 threshold of 35 IU/L was derived as the 99th percentile of a distribution of results taken from a healthy population (Diamandis et al., 2002) and is therefore raised in 1-2% of healthy individuals, dependent on the relative bias of the CA125 method. CA125 is also raised in 5% of benign gynecological conditions and in 28% of non-gynaecological cancers (The Association for Clinical Biochemistry and Laboratory Medicine, 2012). There is currently no other biomarker or biomarker panel that has shown greater accuracy than CA125 for Ovarian cancer in primary care although other biomarkers such as human epididymis protein 4 (HE4) and the risk of ovarian malignancy algorithm (ROMA) index continue to be evaluated (Zhu et al., 2011, Soletormos et al., 2016, Sundar et al., 2015, Wei et al., 2016).

To reduce the impact of false positive CA125 measurements, NICE recommend that in UK primary care: (1) only women with persistent or frequent symptoms (particularly over 12 times a month) should first be offered a CA125 test; (2) CA125 should be used "especially" in women over 50 years (the postmenopausal); (3) a single raised value >35IU/L should first trigger an Ultrasound Scan (USS) of the abdomen and pelvis and only if both tests are abnormal then an urgent referral is indicated (although NICE provide no guidance on what constitutes an abnormal USS) (NICE, 2015). Following referral to secondary care, the Royal College of Obstetrics and Gynaecology (RCOG) recommends that the Risk of Malignancy Index (RMI) score is used to determine ongoing management, combining CA125, menopausal status, and USS findings (RCOG, 2016, RCOG and BSGE, 2011). There is no established pathway for the management of cases referred with a raised CA125 alone.

The aim of this study was to describe patterns of CA125 testing in Oxfordshire, UK, by age-group over an 11-year period from 2003-2014 to include the release of release of the NICE guidance in terms of: (1) trends in CA125 use; (2) the proportion of positive tests in primary care; and (3) the frequency of testing in primary care patients with multiple CA125 samples.

Methods

Study Setting

The Oxford University Hospitals Trust (OUHT) Clinical Biochemistry laboratory serves the county of Oxfordshire with a population of approximately 660,000. As consultant head of biochemistry one of the researchers (BS) had full access to the data held on the laboratory information system which holds all results produced since the mid 1980's. For analysis all data was fully anonymized. We estimated the number of women residing in the catchment area for the laboratory by deriving weights from population pyramid data for mid-2012 and multiplying these by the total population size. We assumed that the population was 660,000 in 2012 and increased at a rate of 0.96% per year since 2002 based on regional population data (Oxford City Council, 2013). All CA125 analyses were undertaken using a single method, the Siemens Centaur XP analyser (Siemens Healthcare, Frimley, UK) by chemiluminescence immunoassay. Method reproducibility, expressed as % coefficient of variation was 5.3% at 28.9 IU/L, 6.3% at 69.7 IU/L, 6.3% at 188.0 IU/L.

Study Sample

We searched the laboratory database for all CA-125 tests from 2002 to 2014, recording the sex and date of birth of the patient, specimen date, requesting doctor, requestor location, and CA125 value. Samples from males were excluded. Requestor location was categorized into Primary Care, Secondary Care, Research, Out of Oxfordshire, Hospice, Unknown. Only Primary Care and Secondary Care samples were included in the analysis. The NICE guidance CG 122 (NICE 2011) uses age 50 years to guide patient investigation and therefore age was divided into four categories, using cut points of 25, 50 and 75 years in order to obtain broad age quartiles. Because the UK NICE Ovarian Cancer guidelines were released on April 27th 2011, we organized data into three-month periods starting in May, August, November and February (NICE, 2011). In order to have data for complete years, we examined data from May 2003 to April 2014.

Statistical Analysis

Testing over time

We calculated the number of women having their first CA125 test from either primary or secondary care by age-category per three-month interval and the rates of new tests (per 10,000 women) by dividing the total number of tests by the estimated population. We calculated the total number of CA125 samples in each year over the study period. We used negative binomial regression to assess the effect of the introduction of the national guideline on Ca125 testing controlling for age specific trends in testing. The model was specified such that we could test both the immediate effect of the guideline on testing, the effect on the trend over time, and interaction of the trend for each age quartile.

Positive tests over time

Using a cut-off value of 35 IU/L, we examined the proportions and rates of positive CA125 results (>35 UI/L) in women undergoing their first CA125 test, and assessed the impact of the guideline. We stratified tests into yearly intervals from May 1st to April 30th because of the low number of positive tests in women less than 50 years of age. We used

logistic regression to test the effect of the guideline release on the probability of the first CA125 value being positive controlling for age and trend over time.

Retesting and serial testing

We also looked at the number of tests performed in primary care in the 2 years following the first CA125 test in each patient and how this changed over time. As we did not have access to clinical information about the reason for testing, we assumed that all tests requested from primary care were for diagnosis and not for monitoring treatment, as the vast majority of monitoring following diagnosis is led by hospital specialists in Oxfordshire. We classified women either having one or two CA125 tests in a 2-year period, or having three or more tests in a 2-year period. In order for each test to have a full two years following the first test we included only tests that were undertaken between May 1st 2003 and 30st April 2012 in this analysis. We tested the impact of the guidelines on intensity of CA125 testing following the release of the guideline using logistic regression models adjusted for age and year and the value of the first test result. We also examined the proportion and the odds of GPs second test (retesting) and of performing serial testing in primary care in relation to the first Ca125 value. All analyses were performed using R version 3.3.0

Results

51,033 tests were processed and 30,737 women tested throughout the study period. The number of tests increased 6-fold from 1659 in 2003 to 10,107 in 2013.

Testing over time

The largest change in CA125 testing was in primary care, where the number of patients being tested for the first time increased 10-fold from 528 to 5082 (Figure 1a upper panel). The rates of CA125 tests per 10,000 women broadly reflected the trend for increasing numbers of women being tested. However, rates of CA125 tests in primary care increased sharply by 76% (95% C.I. 55% to 100%) in the three months following the introduction of the national guideline (Figure 1b upper panel). The rates of testing increased at an average of 24% (95% C.I. 22% to 26%) per year before the guideline and reduced to 5% (95% C.I. 1% to 12%) per year thereafter (Figure 1b upper panel and Table 1). In contrast, the testing remained relatively stable in secondary care, with no change in the absolute number of tests requested nor the rate of testing over time (Figure 1a and 1b lower panels).

Most women tested in primary care were aged 25-50 or 50-75 years but due to the smaller numbers of older women in the population the greatest increase in rates were observed in the 50-75 and 75+ year age-groups (Figure 1). Despite this, the sharp increase following the introduction of the national guidelines does not differ significantly between age-quartiles (Appendix Table 1).

Positive tests

Among all the primary care patients being tested for the first time, 5% of test results were positive (>35IU/L), varying by age quartile: 3%, 5%, 5% and 10% respectively. Rates of positive tests increased by an average of 11% (95% C.I. 8% to 14%) per year before the guideline was introduced, sharply increasing by 65% (95% C.I. 50% to 81%) in the year following the guideline, then decreasing at an average of 6% (95% C.I. 1% to 11%) per year following this (Figure 2a and Appendix Table 2).

Although it appears there are variations in the trend of rates of positive and negative tests between age-groups, we found no statistical evidence of this as three of four age-groups clustered on each plot (Figure 2a upper and lower). In contrast to testing rates over time, the introduction of the national guideline had no significant effect on the proportion of positives tests (OR = 0.89, 95% C.I. 0.72 to 1.10). Furthermore, the odds of a positive result decreased over time at an average of 10% per year (95% C.I. 7% to 13%) as the number of women with CA125 test results <35IU/L increased to a greater extent than the number of women with test results >35IU/L. There was a significant difference between age-groups in the proportion of women with positive tests, with ≥75yr olds twice as likely to test positive than the 25 to 50 year olds and 25-50yr olds (OR=1.96, 95% C.I. 1.66 to 2.31) (Figure 2b and Appendix Table 3).

Repeat vs serial testing over time

84% of women tested in primary care had a single CA125 test on record, 12% had two tests and 4% had three or more CA125 tests. These proportions changed over time from 80%, 13%, and 7% in 2003-5, to 87%, 11%, and 2% in 2012-14 (Figure 3). Tests in women aged 50 – 75 (OR = 1.57, 95% C.I. 1.28 to 1.93) and in women aged ≥75yrs (OR = 1.65, 95% C.I. 1.27 to 2.13) were more likely to be tested more than twice in 2 years rather than once or twice over 2 years compared to women aged 25-50 year. The introduction of the guideline had no effect on the probability that tests were used more than twice (OR = 0.81, 95% C.I. 0.62 to 1.06) rather than once or twice. The trend in the odds of tests being used more than twice decreased by 5% (95% C.I 2% to 8%) per year (Figure 3a and Appendix Table 4).

The value of the first test was associated with an increase in the odds of further testing being done (Figure 3b). If the first test result was 30-35 IU/L, both the odds of retesting (OR = 0.88, 95% C.I. 0.63 to 1.24) and the odds of serial testing (OR=0.68, 95% C.I. 0.42 to 1.06) were not significantly different from women with first tests result > 35 IU/L. However, if the first test was 25-30 IU/L, then the odds of retesting and the odds of serial testing were much less compared to first tests result > 35 IU/L (OR = 0.39, 95% C.I. 0.28 to 0.53) and OR = 0.28 (95% C.I. 0.24 to 0.33) respectively (Figure 3b and Appendix Table 5).

Discussion

Summary of findings

These data confirm that testing for CA125 in primary care increased dramatically between 2003 and 2014, most notably and most rapidly in the three months following the release of the 2011 NICE guideline, but perhaps unexpectedly on a background trend of already increasing CA125 use in primary care. Higher rates of testing were observed in postmenopausal females (>50years) although increases in testing were observed in all age groups with no statistically significant difference between them. Both the rates of positive and negative tests increased as testing increased over time, but the proportion of positive tests decreased as the increase in the number of negative tests was far greater. The proportion of females receiving two or more tests in a 2 year period reduced over time suggesting an increase in testing to investigate symptoms in primary care. Retesting and serial testing in primary care were both associated with the initial CA125 value in all age-groups with no significant difference between women whose first test was 30-35IU/L and >35IU/L.

Strengths and Limitations

We present a consecutive sample of 51,033 CA125 tests analysed using the same method over a 10 year period, by the only laboratory serving the population of a well-defined region allowing comprehensive analysis of trends in CA125 use over time in relation to population level cancer incidence data. Whilst the population studied is typical of the wider UK population in terms of age, Oxfordshire has lower than average deprivation scores and so help seeking behavior leading to CA125 testing in primary care may not be representative of more deprived regions of the UK. We could not access data on the patient demographics to confirm or refute this. Nor could we access past medical history, the symptoms leading to primary care testing, nor other investigations performed before or after CA125 (imaging and histology). Consequently, we are unable to confirm whether CA125 results were true or false positive (or negative). Our findings without these data still have direct relevance to referral volume to secondary care where all positive CA125s must be investigated. We have also made the assumption that all CA125 requests were made to investigate ovarian cancer, although CA125 may have been requested against primary care guidance or following specialist advice as a marker for another malignancy, in particular before the introduction of the 2011 guideline. To reduce the risk of this we restricted the first two analyses to the first primary care CA125 sample in females.

Interpretation

To our knowledge this is the largest published analysis of primary care CA125 data to date. Barrett et al described the route to diagnosis from primary care of 212 primary ovarian cancer cases diagnosed in the Exeter region of South-West England between 2000 and 2007: only 44 had CA125 values in their record, all of which were raised and most likely taken in secondary care 30 days after other investigative action was taken (Barrett et al., 2010). A conference abstract compared the number of primary care CA125 requests received by Airedale District General Hospital's laboratory in rural Yorkshire in the 6 month period leading up to (486 requests) and following (1314 requests) the 2011 NICE guideline release (Ford et al., 2013). Our data support this finding but show that some of the increase was likely a continuation of an upwards trend in primary care testing observed throughout the preceding decade. Despite most raised CA125 values being followed up in the hospital record, there was no change

in the number of diagnoses made in Airedale, and the most common cause of an increased CA125 was benign pathology (Ford et al., 2013). A more recent audit from two NHS hospitals showed that only 10% of urgent referrals for suspected ovarian cancer were preceded by a CA125 then USS test, that a third (37.78%) of patients referred were younger than 50 years old, and a similar proportion were referred without any initial primary care testing (Rai et al., 2015): our analysis confirms that CA125 continues to be used in pre-menopausal women for whom false positive results are much more likely due to benign conditions such as the follicular phase of the menstrual cycle, pregnancy, ovarian cysts, and pelvic inflammatory disease.

The prospective Diagnosing Ovarian Cancer Early (DOvE) pilot study reported that in women over 50 years, a symptom triggered strategy led to a reduction in tumour volume at diagnosis (without improvement in stage at diagnosis) allowing a higher rate of complete surgical resection (Gilbert et al., 2012). In DOvE, all patients received CA125 testing at an initial assessment after referral for abdominal and non-specific symptoms lasting for >2 weeks, and an ultrasound was performed, regardless of the CA125 result, 2 weeks later (Gilbert et al., 2012). DoVE (in symptomatic patients) and UKCTOCS (in a screened population) have found that a multi-modal strategy involving both CA125 and ultrasound is most effective at detecting ovarian cancer, as CA125 can detect low-volume disease before ultrasound can (Gilbert et al., 2012, Jacobs et al., 2016).

We have identified no research demonstrating that any biomarker, such as HE4, or diagnostic strategy, for example repeat single CA125 measurements or CA125 trend, (Jacobs et al., 2016, Zhu et al., 2011) that has superior diagnostic accuracy. HE4 has undergone significant evaluation in secondary care and meta-analysis indicate that it is more specific for ovarian cancer than CA125 (Bandiera et al., 2011, Escudero et al., 2011, Yu et al., 2012). However, most of these studies have been conducted in patients with a pre-identified pelvic mass or pre-identified cancer, with some studies including healthy controls. While HE4 has potential utility in primary care, it has never been evaluated in this setting (NICE, 2011, Sundar et al., 2015).

Our finding that the likelihood of repeat and serial testing is no different between initial CA125 values of 30-35IU/L and >35IU/L is novel, and reflects the clinical reality that GPs will not be reassured by a CA125 result under but close to the threshold in a symptomatic patient.

Myers et al showed that CA125 testing has greater accuracy in postmenopausal women (Sensitivity 69-87%; Specificity 81-100%) where the prevalence of disease is higher than in pre-menopausal women (Sensitivity 50-74%; Specificity 26-95%) (Myers et al., 2006). Using our data from 2013, we can estimate that there were 5 patients with positive CA125 results for every 1 patient with ovarian cancer in the <50yrs age-group compared to 2 to 1 in those >50yrs. NICE decided against using age as a threshold for testing so that the 20% of pre-menopausal Ovarian cancer patients would not be disadvantaged. NICE recommended that CA125 testing is carried out in symptomatic women "(especially if 50 or over)", however an alternative strategy could be to recommend a higher CA125 threshold in the pre-menopausal given the many causes of false positives in this age-group (NICE, 2011). At present, these data remain unreported in primary care populations.

Conclusions

Collaborative primary and secondary care research is needed to develop more detailed pathways for the investigation and management of patients presenting with symptoms of Ovarian cancer to further quantify the potential physical and economic costs of further investigation of symptomatic women investigated with CA125 in primary care. These data are available through electronic health records: our team and others are working on data linkage to answer these questions.

Contributors: BDN, TJ, BS, and JLO contributed to the conception and design of the study. BDN, MML, DW, TJ, BS, and JLO were responsible for the analysis and interpretation of the data. BDN, MML, DW, TJ, BS, and JLO drafted the manuscript. All authors were involved in the final revision of the article. All of the authors approved the final article.

Funding: No specific funding.

Disclaimer: The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Disclosure of interests: None declared.

Acknowledgements: Nil

Ethical Approval: Ethical approval was not required for this observational study.

Provenance and peer review: Not commissioned; externally peer reviewed.

Data sharing statement: No additional data are available.

References

BANDIERA, E., ROMANI, C., SPECCHIA, C., ZANOTTI, L., GALLI, C., RUGGERI, G., TOGNON, G., BIGNOTTI, E., TASSI, R. A., ODICINO, F., CAIMI, L., SARTORI, E., SANTIN, A. D., PECORELLI, S. & RAVAGGI, A. 2011. Serum human epididymis protein 4 and risk for ovarian malignancy algorithm as new diagnostic and prognostic tools for epithelial ovarian cancer management. *Cancer Epidemiol Biomarkers Prev,* 20**,** 2496-506.

BANKHEAD, C. R., KEHOE, S. T. & AUSTOKER, J. 2005. Symptoms associated with diagnosis of ovarian cancer: a systematic review. *Bjog,* 112**,** 857-65.

BARRETT, J., SHARP, D. J., STAPLEY, S., STABB, C. & HAMILTON, W. 2010. Pathways to the diagnosis of ovarian cancer in the UK: a cohort study in primary care. *Bjog,* 117**,** 610-4.

BUYS, S. S., PARTRIDGE, E., BLACK, A., JOHNSON, C. C., LAMERATO, L., ISAACS, C., REDING, D. J., GREENLEE, R. T., YOKOCHI, L. A., KESSEL, B., CRAWFORD, E. D., CHURCH, T. R., ANDRIOLE, G. L., WEISSFELD, J. L., FOUAD, M. N., CHIA, D., O'BRIEN, B., RAGARD, L. R., CLAPP, J. D., RATHMELL, J. M., RILEY, T. L., HARTGE, P., PINSKY, P. F., ZHU, C. S., IZMIRLIAN, G., KRAMER, B. S., MILLER, A. B., XU, J. L., PROROK, P. C., GOHAGAN, J. K. & BERG, C. D. 2011. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. *Jama,* 305**,** 2295-303.

DIAMANDIS, E., FRITSCHE, H., LIJA, H., CHAN, D. & SCHWARTZ, M. 2002. *Tumor Markers: Physiology, Pathobiology, Technology, and Clinical Applications*, AACC Press.

ESCUDERO, J. M., AUGE, J. M., FILELLA, X., TORNE, A., PAHISA, J. & MOLINA, R. 2011. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. *Clin Chem,* 57**,** 1534-44.

FORD, A., MARSHALL, O. & CRAWFORD, S. 2013. UK guidance on diagnosis of ovarian cancer: Its impact on services in an acute general hospital (European Cancer Congress conference abstract). *European Journal of Cancer,* 49.

GILBERT, L., BASSO, O., SAMPALIS, J., KARP, I., MARTINS, C., FENG, J., PIEDIMONTE, S., QUINTAL, L., RAMANAKUMAR, A. V., TAKEFMAN, J., GRIGORIE, M. S., ARTHO, G. & KRISHNAMURTHY, S. 2012. Assessment of symptomatic women for early diagnosis of ovarian cancer: results from the prospective DOvE pilot project. *Lancet Oncol,* 13**,** 285-91.

GOFF, B. A., MANDEL, L. S., MELANCON, C. H. & MUNTZ, H. G. 2004. Frequency of symptoms of ovarian cancer in women presenting to primary care clinics. *Jama,* 291**,** 2705-12.

HAMILTON, W., PETERS, T. J., BANKHEAD, C. & SHARP, D. 2009. Risk of ovarian cancer in women with symptoms in primary care: population based case-control study. *Bmj,* 339**,** b2998.

JACOBS, I. J., MENON, U., RYAN, A., GENTRY-MAHARAJ, A., BURNELL, M., KALSI, J. K., AMSO, N. N., APOSTOLIDOU, S., BENJAMIN, E., CRUICKSHANK, D., CRUMP, D. N., DAVIES, S. K., DAWNAY, A., DOBBS, S., FLETCHER, G., FORD, J., GODFREY, K., GUNU, R., HABIB, M., HALLETT, R., HEROD, J., JENKINS, H., KARPINSKYJ, C., LEESON, S., LEWIS, S. J., LISTON, W. R., LOPES, A., MOULD, T., MURDOCH, J., ORAM, D., RABIDEAU, D. J., REYNOLDS, K., SCOTT, I., SEIF, M. W., SHARMA, A., SINGH, N., TAYLOR, J., WARBURTON, F., WIDSCHWENDTER, M., WILLIAMSON, K., WOOLAS, R., FALLOWFIELD, L., MCGUIRE, A. J., CAMPBELL, S., PARMAR, M. & SKATES, S. J. 2016. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. *Lancet,* 387**,** 945-56.

MYERS, E. R., BASTIAN, L. A., HAVRILESKY, L. J., KULASINGAM, S. L., TERPLAN, M. S., CLINE, K. E., GRAY, R. N. & MCCRORY, D. C. 2006. Management of adnexal mass. *Evid Rep Technol Assess (Full Rep)***,** 1-145.

NICE 2011. The recognition and initial management of ovarian cancer (122). National Institute for Health and Care Excellence

NICE 2015. Suspected cancer: recognition and referral (NG12). National Institute for Health and Care Excellence

OXFORD CITY COUNCIL. 2013. *Population estimates and forecasts, 2001 to 2026.* [Online]. Oxford: Oxford City Council. Available: http://www.oxford.gov.uk/PageRender/decC/Population_statistics_occw.htm [Accessed 17 September 2015].

RAI, N., NEVIN, J., DOWNEY, G., ABEDIN, P., BALOGUN, M., KEHOE, S. & SUNDAR, S. 2015. Outcomes following implementation of symptom triggered diagnostic testing for ovarian cancer. *Eur J Obstet Gynecol Reprod Biol,* 187**,** 64-69.

RCOG 2016. The Management of Ovarian Cysts in Postmenopausal Women. *Green-top Guideline.* Royal College of Obstetrics and Gynaecology.

RCOG & BSGE 2011. Management of Suspected Ovarian Masses in Premenopausal Women. *Green-top Guideline.* Royal College of Obstetrics and Gynaecology.

SMITH, L. H., MORRIS, C. R., YASMEEN, S., PARIKH-PATEL, A., CRESS, R. D. & ROMANO, P. S. 2005. Ovarian cancer: can we make the clinical diagnosis earlier? *Cancer,* 104**,** 1398-407.

SOLETORMOS, G., DUFFY, M. J., OTHMAN ABU HASSAN, S., VERHEIJEN, R. H., THOLANDER, B., BAST, R. C., JR., GAARENSTROOM, K. N., STURGEON, C. M., BONFRER, J. M., PETERSEN, P. H., TROONEN, H., CARLOTORRE, G., KANTY KULPA, J., TUXEN, M. K. & MOLINA, R. 2016. Clinical Use of Cancer Biomarkers in Epithelial Ovarian Cancer: Updated Guidelines From the European Group on Tumor Markers. *Int J Gynecol Cancer,* 26**,** 43-51.

SUNDAR, S., NEAL, R. D. & KEHOE, S. 2015. Diagnosis of ovarian cancer. *Bmj,* 351**,** h4443.

WEI, S. U., LI, H. & ZHANG, B. 2016. The diagnostic value of serum HE4 and CA-125 and ROMA index in ovarian cancer. *Biomed Rep,* 5**,** 41-44.

YU, S., YANG, H. J., XIE, S. Q. & BAO, Y. X. 2012. Diagnostic value of HE4 for ovarian cancer: a meta-analysis. *Clin Chem Lab Med,* 50**,** 1439-46.

ZHU, C. S., PINSKY, P. F., CRAMER, D. W., RANSOHOFF, D. F., HARTGE, P., PFEIFFER, R. M., URBAN, N., MOR, G., BAST, R. C., JR., MOORE, L. E., LOKSHIN, A. E., MCINTOSH, M. W., SKATES, S. J., VITONIS, A., ZHANG, Z., WARD, D. C., SYMANOWSKI, J. T., LOMAKIN, A., FUNG, E. T., SLUSS, P. M., SCHOLLER, N., LU, K. H., MARRANGONI, A. M., PATRIOTIS, C., SRIVASTAVA, S., BUYS, S. S. & BERG, C. D. 2011. A framework for evaluating biomarkers for early detection: validation of biomarker panels for ovarian cancer. *Cancer Prev Res (Phila),* 4**,** 375-83.