The ROC 'n' role of the multiplex assay for early detection of ovarian cancer

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The sensitivity and specificity of CA125 for early detection of ovarian cancer is improved when analysed in combination with novel biomarkers, although further validation studies are required to confirm the clinical utility of the multiplex assay.

Summary

In order to overcome the significant mortality associated with ovarian cancer, a highly sensitive and specific screening test is urgently needed. CA125 is used to assess response to chemotherapy, detect recurrence and distinguish malignant from benign disease; however, this marker is elevated in only 50%–60% of stage I ovarian cancers, making it inadequate for early detection of malignancy. Here, we discuss **Visintin et al.'s** attempt to validate a novel multiplex assay that uses a panel of six serum biomarkers – leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor and CA125 (**Diagnostic markers for early detection of ovarian cancer** *Clin Cancer Res* 14:1065–1072). The study, included 362 healthy controls and 156 patients with newly diagnosed ovarian cancer. The final model yielded 95.3% sensitivity, 99.4% specificity, a positive predictive value of 99.3% and a negative predictive value of 99.2%. These results indicate potential utility of this assay for early detection of ovarian cancer, although further validation is needed in a sample set representative of the general population.

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Ovarian cancer is the deadliest gynaecologic malignancy in the US, with an estimated 15,520 deaths in 2008. This high mortality reflects the poorly understood preclinical state of ovarian cancer and the fact that its nonspecific symptoms are typically unrecognised in the earliest stages of disease. Almost 70% of patients present with advanced-stage disease; however, the five-year survival for women with either stage I or II ovarian cancer is good (92% for localised disease versus 30% for advanced disease), as is the survival for those with small-volume advanced-stage disease following optimal cytoreduction.[1] The search for biomarkers that detect ovarian cancer before an increase in tumour burden is justified by these facts.

In order to be adopted as a screening strategy, most researchers agree that a biomarker must achieve a minimum positive predictive value (PPV) of 10% along with a minimum specificity of 99.6%.[2] Historically, CA125 has proven useful in ovarian cancer for assessing response to chemotherapy, detecting disease recurrence and distinguishing malignant from benign masses. More recently, serum and tissue expression of CA125 has been linked to prognosis, particularly in late-stage ovarian cancer.[3] Nonetheless, elevated CA125 levels are noted in only 50%–60% of patients with stage I disease.[4] New modalities are therefore needed in order to improve the likelihood of early detection of ovarian cancer. Jacobs and colleagues examined the merit of a multimodal

approach to screening for ovarian cancer by incorporating bimanual examination and ultrasonography with CA125 testing; however, this combined approach yielded a PPV of only 21%.[5] In addition, the majority of women with screen-detected ovarian cancer were diagnosed with advanced disease, highlighting the need for early detection.[5] Other researchers have evaluated the benefit of combining CA125 with novel biomarkers in an effort to improve the sensitivity and specificity of CA125 measurement alone. One such example is the combination of CA125 and human epididymis protein 4, resulting in 76.4% sensitivity and 95% specificity, which was better than either biomarker alone.[6] Nevertheless, there remains room for improvement.

In a recent study, Visintin et al. validated a panel of six serum biomarkers (leptin, prolactin, osteopontin, insulin-like growth factor II, macrophage inhibitory factor and CA125) that showed differential expression in disease-free individuals and patients with ovarian cancer on microarray analysis.[7] This study serves as a follow-up to a similar study in which a panel of four novel biomarkers (leptin, prolactin, osteopontin and insulin-like growth factor II) exhibited 95% sensitivity, 95% specificity, 95% PPV and 94% negative predictive value for detection of ovarian cancer.[8] Although the accuracy of this combination of four biomarkers is a considerable improvement on current screening methods given the low prevalence of ovarian carcinoma, there is still need for a test with greater specificity. Consequently, these investigators added CA125 and macrophage inhibitory factor to their four-plex assay in an attempt to further improve specificity. The authors evaluated the serum concentration of the six markers in a training set (181 controls and 113 patients with newly diagnosed ovarian cancer) and validation set (181 controls and 43 patients with newly diagnosed ovarian cancer).[7] The area under the receiver operating characteristic (ROC) curve was used to calculate the sensitivity at 95% specificity for each marker, and four models were used to combine markers in the training and test sets. This analysis yielded a final model that combined observations from both sets to result in a sensitivity of 95.3%, specificity of 99.4%, PPV of 99.3% and negative predictive value of 99.2%.[7]

The authors should be commended for the various strengths of this study, which include the design of a diagnostic panel that permits simultaneous measure of multiple markers by the use of a relatively small volume of patient serum. The evaluation of only six biomarkers is a feasible alternative to a single measurement of CA125, and the inclusion of both patients with early-stage disease and those with advanced cancer shows the utility of the assay for early detection. There are, however, a few limitations. Both study sets represent populations that are enriched for ovarian cancer, with the prevalence of ovarian cancer being slightly higher in the training set than in the validation set (21% versus 19%, respectively). The sensitivity and specificity of the assay decreased in the validation set compared with the training set. Although the combined assay remains a better test than detection of CA125 alone, one must be aware that there could be further decline in the specificity of the assay as sample size increases, given that the prevalence of ovarian cancer in the validation set was considerably higher than in the general population. The authors attempted to validate the assay with a unique validation population. Nevertheless, the final model involved combination of the test and training sets, making it imperative that there should be further validation before use of this assay in a clinical setting. Also, although this assay distinguishes between patients with epithelial ovarian cancer and healthy controls, it may not be specific to ovarian cancer, thereby potentially decreasing its utility in a clinical setting. Furthermore, CA125 levels are known to be elevated in certain benign gynaecologic diseases, which may further affect the accuracy of this screening modality. The authors matched cases to controls only on the basis of stage and histologic grade, so other baseline differences might have affected the assay results. Finally, several questions arise concerning sample handling and processing that could ultimately affect specimen quality and assay reproducibility and reliability.

In conclusion, this study provides a potential viable alternative to screening for CA125 alone for the diagnosis of ovarian cancer. Nevertheless, further prospective multi-institutional evaluation will be required to validate this six-plex assay as a feasible tool for diagnosis and screening of ovarian cancer in the general population.

Details of the references cited in this article can be accessed at www.cancerworld.org/magazine

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