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Review

# Advances in ovarian cancer diagnosis: A journey from immunoassays to immunosensors

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ABSTRACT

This review focuses on the technological advancements, challenges and trends in immunoassay technologies for ovarian cancer diagnosis. Emphasis is placed on the principles of the technologies, their merits and limitations and on the evolution from laboratory-based methods to point-of-care devices. While the current market is predominantly associated with clinical immunoassay kits, over the last decade a major thrust in development of immunosensors is evident due to their potential in point-of-care devices. Technological advancements in immunosensors, extending from labeled to label-free detection, with and without mediators, for enhancing proficiencies and reliability have been dealt with in detail. Aspects of the utilisation of nanomaterials and immobilization strategies for enhancing sensitivity and altering the detection range have also been addressed. Finally, we have discussed some distinct characteristics and limitations associated with the recently commericalised technologies used for quantitation of relevant ovarian cancer markers.



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## 1. Introduction

Ovarian cancer is the seventh most common cancer and is amongst the top five leading causes of cancer deaths in women. A recent statistical report covering the last two decades suggests that the percentage increase in cancer cases since 1990 is highest for breast cancer (166.4%) followed by ovarian cancer (153.7%) and

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the increase in death rate since 1990 is greatest for ovarian cancer (122.6%) followed by lung cancer (94.1%). These statistics highlight the need for critical evaluation of the methods employed for ovarian cancer diagnosis and prognosis. Early diagnosis of this disease can decrease mortality rates significantly. However, only 20% of the cases are diagnosed when the disease is curable because the symptoms are relatively non-specific during early stages of ovarian cancer [1]. In 2015, the estimated number of new cases of ovarian cancer in the United States was greater than 21,000 while the expected number of deaths is over 14,000 [2]. The survival rate is greater than 90% in stage I, i.e. when the cancer is localized at the primary site, however, when metastasized (Stage III) i.e. distant from the primary site, this results in a much lower survival rate of approximately 27% [3,4]. Currently, early diagnosis of cancer is limited to 16% of cases, while most cases are diagnosed at stage III (63%).

For screening of ovarian cancer, regular pelvic examination, ultrasound, magnetic resonance imaging (MRI) [5], X-ray computed tomography (CT), biopsy and blood tests for cancer-related markers are in practice. Tumor markers are the proteins present in the circulatory system and their elevated levels suggest malignant condition(s). Tumor markers such as cancer antigen 125 (CA-125), cancer antigen 19–9 (CA-19-9), alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), Creatine Kinase (CK), cancer antigen 15–3 (CA15-3), cancer antigen 72–4 (CA72-4), carcinoembryonic antigen (CEA), Human epididymis protein 4 (HE4) and others have been associated with ovarian cancer, and may be used for screening, diagnosis and prognosis [6–12]. The following sections of this review describe contemporary diagnostic techniques, ovarian cancer-associated biomarkers and advances in diagnostic tools including both conventional and recently developed immunosensor devices. Scheme 1 shows various diagnostic methods used currently, the time duration for results to reach the clinician for confirmed diagnosis (2–4 weeks) and survival rates at different stages of ovarian cancer. In contrast to these methods, point-of-care devices generate results rapidly (15–30 min) thus reducing delays in diagnosis and facilitating new approaches for early screening and detection.

## 2. Contemporary diagnostic evaluation

Ovarian cancer develops mainly from epithelial cells (90%) and the remainder (ca. 10%) from sex cord cells and germ cells. Histological examination of biopsy samples confirms malignancy with 100% specificity and sensitivity, but cannot be used as an initial screening method. Generally, transvaginal ultrasonography is used as the initial screening method for ovarian cancer, on presentation of an adnexal mass (lesion or cyst in the area surrounding the uterus—fallopian tubes and ovary) because of its availability, high resolution and lack of associated health hazards [13,14]. However, the ultrasonographic technique has very low prediction accuracy due to its inability to differentiate benign and malignant cysts and the observed higher incidence of benign cysts as compared to malignant cysts. In addition, the importance of the ultrasound operator's expertise further complicates the problem as evidenced by huge variations in reported sensitivity (85–100%) and specificity (50–100%). To date there is no single validated, accurate test for early diagnosis of ovarian cancer. Hence, the indeterminate cases from ultrasound analysis are further evaluated using Doppler ultrasound, Magnetic Resonance Imaging (MRI), contrast enhanced MRI and CT with sensitivities of 84%, 76%, 81% and 81%, respectively, and the associated specificities being 82%, 97%, 98% and 87%, respectively. Currently, none of the available tests are recommended for clinical screening of early stage ovarian cancer [10,11].

## 3. Tumor markers

Tumor markers are biomolecules that are expressed or over-expressed by tumor cells or other normal cells in response to the presence of a tumor. They are present in body fluids and tissues and can be proteins, hormones, genes, and modifications of DNA or RNA [15]. The elevated levels of these markers can be used to monitor disease progression and therapeutic response in addition to detection of the tumor itself [16,17]. Prevalent tumor markers for primary or secondary ovarian cancer diagnosis are CA125, HE4, AFP, β-hCG, CA72-4, CA15-3 and Creatine Kinase (CK-BB). These tumor markers are at elevated levels in 85% of the cases at stage II/III and 20–25% cases at stage I [3,12,18]. Table 1 lists various ovarian cancer marker types, their primary site of origin, secondary site, where sampled (blood/urine) and their threshold values in healthy individuals. HE4 is over-expressed in 93% epithelial cell ovarian cancers. However, it is not expressed in mucinous and germ-cell ovarian cancers. In comparison to CA125, HE4 is less frequently expressed in benign ovarian diseases [14,19]. CA72-4 and CA15-3 have prognostic relevance, i.e. increasing levels of these markers are associated with residual tumor cells [17].

In addition to these, CK-BB activity has been reported to be enhanced in more than 66% of ovarian cancers. However, there is no clear association of CK-BB levels and clinical stage, histological grade or size of residual tumor [20]. During pregnancy elevated levels of CA125, AFP and β-hCG are found resulting in false positive results for ovarian cancer [11,17]. CEA and β-hCG are found to be increased above threshold levels in individuals taking marijuana and smoking cigarettes. CA125 has the maximum reported sensitivity (73%) followed by HE4 (57%) while other tumor markers such as CA15-3, CA72-4 and transthyretin have sensitivities ranging between 35 and 50% and that of CA19-9 is as low as 23% [17,18]. The aforementioned problems of low sensitivity and false positives demonstrate the need for developing multi-analyte assays, i.e. combination of tumor markers to identify specific tumor type with higher confidence levels. This has led to the development of immunosensing arrays for detection of tumor markers with sensitivities and specificity greater than 90% [17].

Currently available techniques for detection and quantification of tumor markers include enzyme-linked immunosorbent assay (ELISA) [22], radioimmunoassay (RIA) [23], chemiluminescence-based immunoassay (CLIA) [24], fluoro-immunoassay (FIA) [25] and electrochemiluminescence-based immunoassay [26]. Table 2 shows sensitivity and limit of detection (LOD) of immunoassay-based diagnostic kits developed for various ovarian cancer biomarkers. As observed, RIA-based diagnostic assays for ovarian cancer have good sensitivity with the lower detection limit for CEA being 0.3 ng/mL while that of CA125 is 0.7 U/mL. Although RIA has extreme sensitivity (detection limit as low as few picograms) and excellent precision, associated risks of radiation hazards limits its use. The ELISA-based methods on the other hand are safe and easy to use but detection limits are sometimes poorer (1.7 U/mL for CA125) than RIA-based methods [27]. However, with improved ELISA techniques using biotinylated anti-CA125 antibody and streptavidin-tagged HRP the sensitivity was enhanced to 0.6 U/mL. Since ELISA is cost-effective and has few associated health hazards it is a widely accepted technique for diagnostic purposes. Further development in immunoassay technology lead to FIA-based methods using flourophore labels rather than enzyme labels to eliminate additional steps associated with enzyme substrate addition [28–30]. FIA-based immunoassays showed increased sensitivity and lower detection limits e.g. the lower limit of detection of CA125 using ELISA was 1.7 U/mL while a FIA-based method could detect 0.23 U/mL which is comparable to the RIA-based method. These conventional immunoassays have sub-nanomolar sensitivities and are extensively used. However, the associated