ACCSCIENCE PUBLISHING

Tumor Discovery

REVIEW ARTICLE

Immunocytochemistry profiling of ovarian cysts: A review of its clinical utility, future direction, and challenges

Owajioniro Godstime Mordecai[1†], Yibala Ibor Oboma[2*], and Raphael Teme[3†]

[1]Department of Histopathology and Cytology, Faculty of Medical Laboratory Science, Federal University, Otuoke, Bayelsa State, Nigeria

[2]Department of Medical Laboratory Science, School of Allied Health Sciences, Kampala International University Western Campus, Kampala, Uganda

[3]Department of Histopathology/Cytopathology, Faculty of Medical Laboratory Science, Rivers State University, Port Harcourt, Rivers State, Nigeria

Abstract

Ovarian cysts are fluid-filled sacs located in or on the surface of the ovaries. They can affect women of all ages but are most commonly seen in those of reproductive age and postmenopausal women. The two primary types of ovarian cysts are functional and pathological. Due to their asymptomatic nature, ovarian cysts are frequently diagnosed at later stages, potentially limiting therapeutic options and negatively affecting patient outcomes. Early detection requires diagnostic modalities that can differentiate between benign and malignant ovarian lesions. One promising approach is the use of immunocytochemistry (ICC) in the diagnosis of ovarian cysts, a rapidly evolving method in ovarian pathology. ICC utilizes antibodies to detect and visualize specific cellular antigens (proteins) in cytology specimens, serving as an ancillary diagnostic technique for establishing empirical diagnoses, predicting biomarkers, and assessing prognosis. A standard ICC protocol entails sample preparation, fixation, permeabilization, blocking, immunolabeling, counter-staining, and microscopic imaging of stained cells. Specific biomarkers play a significant role in distinguishing between benign and malignant ovarian cysts. Commonly used biomarkers include Cancer antigen 125, Ki-67, p53, HE4, and WT1. Future directions in this field are likely to focus on multiplex ICC, the discovery of novel biomarkers, the integration of artificial intelligence with other diagnostic modalities, and improving the standardization of ICC practice. Integrating these biomarkers into ovarian pathology will enable accurate diagnosis with reduced turnaround times, overcoming the limitations of hematoxylin and eosin staining methods.

Keywords: Immunocytochemistry; Profiling; Ovarian cyst; Review

†These authors contributed equally to this work.

***Corresponding author:**
Yibala Ibor Oboma
(yibalaoboma@kiu.ac.ug)

Citation: Mordecai OG, Oboma YI, Teme R. Immunocytochemistry profiling of ovarian cysts: A review of its clinical utility, future direction, and challenges. *Tumor Discov*. 2025;4(1):14-26.
doi: 10.36922/td.5369

Received: October 21, 2024

1st revised: December 9, 2024

2nd revised: December 16, 2024

Accepted: December 16, 2024

Published online: January 9, 2025

Copyright: © 2025 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Ovarian cysts, also known as adnexal cysts, are among the plethora of gynecological challenges faced by clinicians every day. These cysts are best described as fluid-filled sacs located inside or on the epithelial surface of the ovaries. While ovarian cysts can occur in females of all ages, they are more prevalent in those of reproductive age, particularly

around menarche, due to increased endogenous hormone production.[1-3] Their asymptomatic nature complicates early detection, which could have enhanced clinical management and improved patient outcomes. Consequently, ovarian cysts are often diagnosed at a later stage, negatively impacting therapeutic options and patient outcomes. These ovarian masses can significantly affect the reproductive vibrancy of women, survival, and overall health status.[4,5] Ovarian cysts are broadly grouped into functional (physiological) and non-functional (pathological) cysts. Functional cysts include follicular and luteal cysts, while pathological cysts are further classified into benign and malignant cysts.[6] Benign cysts often resolve spontaneously and require minimal intervention, whereas malignant cysts engender prompt and aggressive treatment.[7] An accurate diagnostic distinction between benign and malignant ovarian cysts is significant for definitive patient treatment and outcomes. Immunocytochemistry (ICC) plays a key role in distinguishing between benign and malignant ovarian lesions. This diagnostic technique uses antibodies to detect and visualize specific cellular antigens (proteins) in cytology specimens, offering detailed and distinct cellular and molecular insights on tissues.[8] Clinical manifestations of ovarian cysts may include pelvic and abdominal pain, bloating, and menstrual irregularities. Complications such as ovarian torsion (twisting of the ovaries) and rupture can arise, underscoring the need for timely diagnosis and management.[9] This review explores the diagnostic role of ICC in distinguishing between benign and malignant ovarian lesions.

2. Scope and methodology of the review

The research strategy for this review involved a systematic and integrative approach encompassing literature collection, data extraction, synthesis, and analysis. This strategy ensured a thorough exploration of the topic across multiple perspectives. Key biomedical, public health, and imaging databases such as PubMed, Scopus, Web of Science, and EMBASE were used. The search strategy incorporated specific keywords and Medical Subject Headings terms related to ovarian cancer and tumor markers.

3. Pathogenesis of ovarian cysts

The pathogenesis of ovarian cysts remains poorly understood, though several established hypotheses suggest a complex interplay of hormonal, molecular, immunological, and environmental factors (Figure 1). One of the most common hypotheses involves alterations in the regulation of hormones associated with ovulation,[10] particularly disruptions in the hypothalamus-pituitary axis that impair the adequate release of luteinizing hormone. This deficiency can prevent ovulation, leading to the formation of follicular cysts. Another molecular hypothesis suggests that mutations in oncogenes, such as *KRAS* and *BRAF*, activate the MAPK signaling pathway,[11] contributing to cyst development. In addition, ovarian cysts may also arise from the exposure of ovarian surface epithelial cells to inflammatory cytokines, such as interleukin-6, and reactive oxygen species linked to ovulation or physical trauma, resulting in DNA damage.[12]

4. Classification of ovarian cysts

Ovarian cysts are classified into functional (physiological) and non-functional (pathological) cysts. The World Health Organization's histological classification includes benign, malignant, and metastatic cysts based on their histogenesis.[10] Functional cysts include follicular and luteal cysts, which are typically self-limiting and may require less therapeutic intervention. In contrast, pathological cysts, further classified into benign and malignant cysts, require prompt and aggressive therapeutic interventions due to their life-threatening potential.[7]

4.1. Physiological cysts

Functional cysts: These cysts are non-neoplastic masses or enlargements (<3 mm) in women of reproductive years. They are the most common type of cysts during the menstrual cycle and arise due to either ovulation failure or corpus luteum formation. In cases of ovulation failure, cysts that form are lined by granulosa cells, persisting for a few days to a few weeks. The corpus luteum is formed from follicular remnants after ovulation and can develop into a cyst if it fails to dissolve within 14 days. Hyper-physiological ovarian response induces the development of functional cysts, such as the follicular, corpus luteum, and theca-lutein cysts.[14,15]

Follicular cysts: These are ovarian cysts that are formed when ovulation fails to occur, causing the follicles to grow without releasing a matured ovum. Histopathologically, follicular cysts are characterized by thin walls and pale acidophilic remnants. They often contain pigmented macrophages, degenerated oocytes, and cellular debris. Follicular cysts are lined by one to four layers of cuboidal granulosa cells without luteinization[15,16] (Figure 2).

Corpus luteum cysts: These are ovarian cysts that occur after successful ovulation, when the follicle releases the ovum, leading to the formation of corpus luteum from the follicular remnants (Figure 3). The corpus luteum is responsible for secreting progesterone. If fluid accumulates inside the corpus luteum and it fails to dissolve within 14 days of no pregnancy or 14 weeks after pregnancy, it enlarges into a cyst. These cysts are characterized by thick walls and are lined by many layers of luteinized granulosa

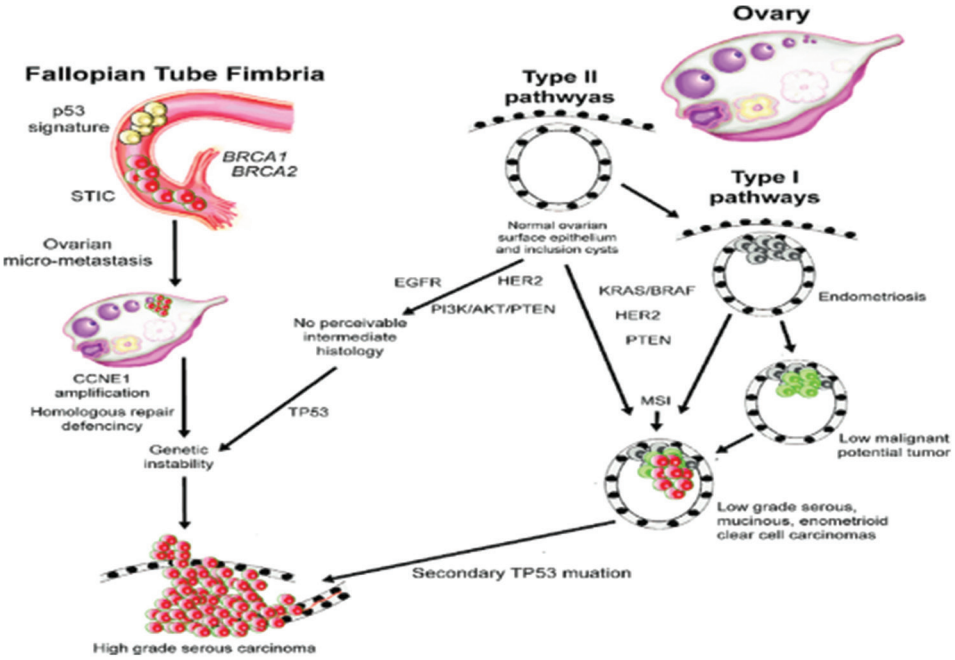


Figure 1. Pathogenesis of ovarian lesions[13]

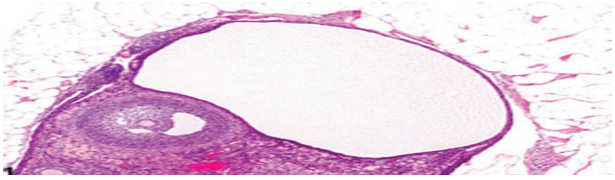


Figure 2. Follicular cysts.[23] Magnification ×100.

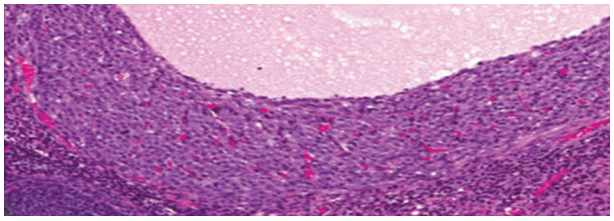


Figure 3. Corpus luteum cyst.[23] Magnification ×100.

cells (polygonal cells), containing abundant eosinophilic luteal cells with fine vacuoles.[3,16]

Theca-lutein cysts: These are ovarian cysts or luteinized follicle cysts that arise from hyperstimulation, leading to increased production of human chorionic gonadotropin hormone. Theca-lutein cysts usually occur during pregnancy, in women with gestational trophoblastic disease, multiple gestation, or ovarian hyperstimulation. These cysts are multilocular, thin-walled, and lined by luteinized theca cells exhibiting abundant stroma and hyperplasia.[16,17]

4.2. Pathological cysts

Benign ovarian cysts: These cysts are non-functional and can be self-limiting. Common types of benign ovarian cysts include serous and mucinous cystadenomas, dermoid cysts, polycystic ovarian syndrome, endometriotic cysts, and paraovarian cysts.[14,18] Histologically, serous cysts are lined by a monolayer of ciliated columnar epithelial cells, while the mucinous cysts are lined by epithelial cells that produce mucin.[19]

Mucinous cystadenoma: These cysts are benign ovarian cysts primarily resulting from the metaplasia of germinal epithelial cells. Occasionally, they can originate from a Brenner tumor or teratoma, typically preceded by mucinous transformation of the epithelium. Mucinous cystadenomas appear as translucent, ovoid masses with smooth capsules. Microscopically, they are lined by tall, secretory epithelium coupled with goblet cells and are characterized by thick walls and multiloculated structures.[14,19]

Serous cystadenoma: These cysts are notably more common compared to mucinous cystadenomas. The cystic fluid is characterized as thin, watery, and yellow-tinged with a smooth surface. Microscopically, distinguishing features of these cysts include psammoma bodies, which are calcified granules resulting from the degeneration of papillary implants. Serous cystadenomas are lined by a monolayer of ciliated columnar epithelial cells.[19]

Dermoid cyst: These cysts are a type of neoplastic cyst that originates from young ova, hypothesized to be

triggered by parthenogenetic processes in younger women. Gross examination indicates that they are thick-walled, opaque, and whitish (Figure 4). The contents of dermoid cysts include hair, cartilage, bone, and a significant amount of sebaceous greasy fluid. Histomorphologically, these cysts present with thick walls composed of ectodermal, mesodermal, and endodermal tissues.[7,14]

5. Malignant ovarian cysts

Malignant ovarian cysts are less common and primarily of histological origin, with various subtypes, as shown in Figure 5. Epithelial malignant ovarian cysts are the most prevalent, accounting for 90% of total cases. The incidence of these cysts is particularly high among postmenopausal women, especially those aged 60 – 70 years.[14] Malignant ovarian cysts include epithelial ovarian cancer, stromal tumors, and germ cell tumors.[13,20] Epithelial ovarian malignant lesions are subdivided into serous carcinoma, mucinous carcinoma, endometrioid, and clear cell carcinoma. Malignant germ cell tumors encompass embryonal carcinoma, immature teratoma, polyembryoma, and endodermal sinus tumors. Risk factors for malignancy include age, family history, genetic predispositions (such as *BRCA* mutations), and certain reproductive histories.[20,21] Histopathologically, malignant ovarian cysts exhibit complex papillae, necrotic foci, cystic spaces, neuroepithelial cells in solid sheets, and Call-Exner bodies.[22]

6. ICC

ICC is a diagnostic technique that involves applying antibodies to detect and visualize cellular antigens (proteins) of interest in cytology specimens, offering detailed cellular and molecular information on tissues.[8] This technique is particularly relevant in diagnostic cytopathology, offering improved sensitivity and specificity for detecting benign and malignant lesions compared to traditional hematoxylin and eosin staining. ICC can be performed using either direct or indirect methods, with the latter requiring a secondary antibody coupling. According to Kirbiš *et al*.,[24] ICC is considered an invaluable diagnostic technique for establishing empirical diagnosis, predicting biomarkers and prognosis, and determining the origin of tumors.[8,24] It can be applied to various cytology preparations, including direct smears, liquid-based preparations, cell blocks, cytospins, and cell cultures. Many institutions prefer using cell blocks due to their advantage of producing several identical sections for cases requiring a panel of ICC stains. In addition, most biomarkers have been validated on formalin-fixed paraffin-embedded (FFPE) tissue, eliminating the need for separate validation.[25-27] With the discovery of specific biomarkers, ICC has demonstrated the capability to distinguish between benign and malignant ovarian cysts.[28]

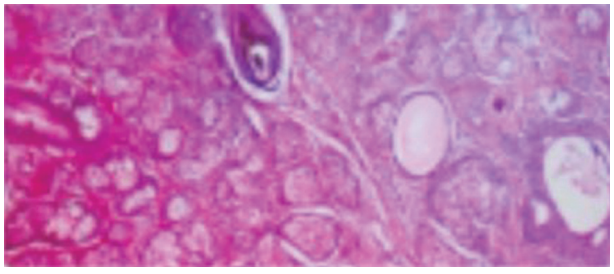


Figure 4. Dermoid cyst.[13] Magnification ×100.

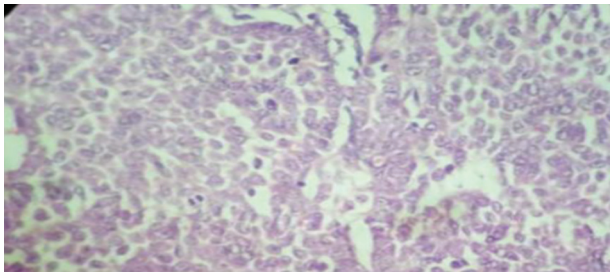


Figure 5. Malignant ovarian cancer.[13] Magnification ×100.

7. Principles and application of ICC

ICC relies on the complementary binding of antibodies to target proteins, known as antigens, in cells from pathological specimens. This technique involves labeling antibodies with enzymes, optionally counterstained with hematoxylin and eosin, and visualizing the results using a light microscope. ICC merges principles from immunology, cytochemistry, and histology to identify specific cellular structures. The fundamental principle of ICC is the identification, visualization, and localization of specific antigens based on a satisfactory signal-to-noise ratio. Signal amplification and the reduction of non-specific background staining are crucial strategies for achieving reliable results, which are invaluable for routine practices. The reaction involves primary and, in some cases, secondary antibodies, blockers, enzymes, and enzyme or fluorescent labels.[29] An antibody is an immunoglobulin molecule produced in response to the presence of an antigen. The choice of antibody for ICC is dependent on the specificity and sensitivity of the antibody-antigen binding reaction. Since antibodies are not visible under light or electron microscope, they must be labeled for visualization. This technique has widespread applications in diagnostic cytopathology, research, and therapeutic monitoring. In the context of ovarian cysts, ICC can identify specific biomarkers associated with benign and malignant lesions, offering critical information for diagnosis and clinical management. Profiling ovarian cysts involves using an antibody panel that targets specific biomarkers associated with benign and malignant cells, such as Cancer

antigen 125 (CA125), Ki-67, and p53. The expression of these biomarkers is analyzed using imaging techniques to distinguish between benign and malignant lesions.[29,30]

8. ICC versus traditional diagnostic methods

A standard ICC protocol entails several key steps: sample preparation, fixation, permeabilization, blocking, immunolabeling, counterstaining, and microscopic imaging of stained cells. Each step requires optimization, as experimental variables can significantly impact the overall staining outcome.[31-33] Traditional diagnostic methods for ovarian cysts include ultrasound, computed tomography (CT) scans, and magnetic resonance imaging (MRI). While these techniques provide essential cytomorphological information about the cyst's appearance and size, they are ineffective in distinguishing between benign and malignant cysts. Studies indicate that the sensitivity and specificity of these traditional diagnostic techniques range between 60% and 70%.[34] In contrast, ICC has shown improved sensitivity and specificity for differentiating benign from malignant cysts. A study by Li *et al*.[35] showed that a panel of antibodies targeting CA125, p53, and Ki-67 yielded a sensitivity of 93% and a specificity of 95% in distinguishing between benign and malignant cysts. Similarly, Lee *et al*.[36] demonstrated that ICC yielded a sensitivity of 91% and a specificity of 94% for detecting malignant ovarian cysts.

9. Advantages and limitations of ICC

The use of ICC in ovarian cyst profiling offers several advantages over traditional diagnostic techniques. It is a non-invasive, time-saving, and cost-effective technique.[37] ICC allows for accurate antigen localization and offers improved specificity and sensitivity in distinguishing between benign and malignant cysts, thereby reducing the likelihood of unnecessary surgical interventions. In addition, it facilitates the detection of specific biomarkers associated with benign and malignant lesions, which is crucial for therapeutic planning. It is often applied in both clinical and basic research.[38] However, ICC has limitations. One of the biggest limitations of ICC is the difficulty in standardizing the procedure due to the variations in specimen preparations, unlike ICC, which uses FFPE tissue sections. This variability can affect the interpretation of results, leading to diagnostic variability across different laboratories. Moreover, the choice of antibody panels in immunocytochemical profiling may differ among laboratories, contributing to diagnostic variability. A false negative result may occur due to inadequate samples or low cellularity.[39]

10. Markers in ovarian pathology

Tumor markers have emerged as critical diagnostic tools for characterizing and differentiating between tumor types. Biomarkers are biological substances or structures (antigens) produced by tumors and can be measured to detect the presence and stages of suspected tumors.[40] Various markers play significant roles in understanding ovarian pathology.

CA125: CA125 is encoded by the *MUC16* gene. It is one of the earliest known biomarkers of ovarian pathology. In healthy women, CA125 levels are typically below 36 units/mL.[40-42] Elevated serum levels of CA125 can indicate the presence of ovarian masses and other pathologies, such as uterine fibroid, endometriosis, pelvic inflammatory disease, and liver cirrhosis.[43] While approximately 80% of women with advanced epithelial ovarian cancer exhibit elevated levels of CA125, about 50% of cases with early-stage ovarian cancer also showed increased levels of CA125. This drawback disqualifies CA125 as a marker for ovarian cancer screening.[44] When used independently, CA125 has a sensitivity of 60% and specificity of 90%, with higher sensitivity and specificity observed in postmenopausal women as compared to premenopausal women. Integrating CA125 into the risk malignancy index algorithm (a risk assessment of ovarian cancer in clinical settings) enhanced its sensitivity to 87% and specificity to 97% for the detection of ovarian cancer.[45] However, CA125 is not suitable for early-stage detection of ovarian cancer. It offers a good diagnostic value for epithelial ovarian cancer, although its level is also elevated in benign ovarian cysts.[45]

Kallikreins (KLKs): KLKs are part of a 15-member *KLK* human gene family located on chromosome 19q. Their diagnostic and prognostic utility in cancer has been demonstrated in many studies. Twelve out of 15 members are upregulated in ovarian cancer, and these biomarkers are expressed in the endocrine epithelium. They are regulated by cancer hormones and are released and detected in various body fluids.[46,47] A study measuring the pre-operative serum levels of human KLK10 in 318 participants found significantly elevated results in 146 patients with ovarian cancer. The specificity and sensitivity of KLKs were reported to be 75% and 77%, respectively.[48]

Osteopontin (OPN): OPN is a glycophosphoprotein expressed in ovarian cancer, as well as in cervical, gastric, endometrial, hepatocellular, prostate, and breast cancers. Its secretion is primarily attributed to activated T-lymphocytes, leukocytes, and macrophages found at sites of inflammation and within the extracellular matrix. OPN, first discovered in 2001 from RNA isolated from ovarian cancer cell lines,[49] is associated with tumor progression, invasion, and metastasis. A study showed that OPN levels are more elevated in epithelial ovarian cancer compared to ovarian cyst.[50] Moreover, the study also revealed that

OPN plays a prognostic role by predicting the progression of disease in late-stage epithelial ovarian cancer with a specificity of 34% and sensitivity of 81%. The possibility of measuring OPN in urine samples of patients with high-grade ovarian cancer presents an opportunity for early diagnosis of ovarian cancer.[50]

Bikunin: Bikunin is a glycoprotein with several functions, known to mediate the suppression of invasion and metastasis of cancer cells. It can be measured in the tissue and plasma of patients with malignant conditions and serves as a prognostic marker for benign and malignant ovarian pathologies. A study reported that high levels of pre-operative bikunin are indicative of ovarian cancer prognosis.[51] This finding was consolidated by Tanaka *et al*.,[52] who analyzed bikunin protein levels in the plasma of ovarian cancer patients (n = 327), benign ovarian cyst patients (n = 200), and healthy controls (n = 200). The study concluded that bikunin is a useful prognostic marker of ovarian pathology, demonstrating 70% specificity and 75% sensitivity.

Vascular endothelial growth factor (VEGF): VEGF is a protein that enhances vascular permeability and plays a role in regulating physiological and pathological angiogenesis, as well as tumorigenesis. Elevated levels of VEGF levels have been observed in ovarian cancer patients. In a study by Matsuzaki *et al*.,[53] 12 out of 18 tumor samples from late-stage serous epithelial ovarian cancer patients showed strong positivity for VEGF, while six revealed low to negative expression of VEGF. The study reported that the levels of VEGF sensitivity and specificity were 79% and 74%, respectively.[53]

Human epididymis protein 4 (HE4): HE4 was first discovered as a biomarker of ovarian cancer in 1999. It is a peptide protease inhibitor that plays a critical role in the innate immune response of epithelial tissues and belongs to the whey acidic four-disulfide core (WFDC) protein family. HE4 was identified in the epithelium of the distal epididymis.[54] Since its discovery, HE4 has been observed to be significantly elevated in ovarian cancer and endometrial cancer, with a specificity of 95% and a sensitivity of 73%. Interestingly, studies indicated that HE4 protein is not expressed on the surface epithelium of the ovaries; however, its expression is found in 100% of human endometrioid epithelial ovarian cancer cases and slightly less in serous ovarian carcinoma.[55]

Creatine kinase B (CKB): CKB is known for its function in the metabolism of vertebral cells and exhibits unregulated expression in some cancers. CKB is a cytosolic form of creatine kinase. Studies have documented elevated expression of CKB in ovarian cancer. Research indicates that CKB activity is significantly higher in pre-operative serum in women with ovarian cancer compared to women with benign ovarian cysts. Notably, CKB expression is high in early-stage ovarian tumors, making it a potential biomarker for early detection of ovarian cancer.[56,57]

Human prostasin (PSN): PSN is located on chromosome 16p11.2 and is a 40 kDa, trypsin-like proteinase responsible for activating epithelial sodium channels. PSN has been designated as a novel biomarker for ovarian cancer, demonstrating a sensitivity of 51% and specificity of 94%.[58] However, a study by Tamir *et al*.[59] reported a sensitivity of 92% and specificity of 94% for detecting ovarian cancer when PSN was combined with CA125. Clinically, PSN serves as a differential diagnostic biomarker for ovarian cancer.

Mesothelin: Mesothelin was discovered at the National Cancer Institute, United States, in 1996. It is found in the mesothelial cells lining the peritoneum, pleura, and pericardium. It is an antigen with tumor differentiation properties, exhibiting a widespread expression in tumors, particularly in 70% of ovarian cancers. Tumor cells expressing mesothelin showed a greater tendency to migrate and metastasize.[60] As a potential novel biomarker for ovarian cancer, higher levels of mesothelin are associated with poor overall survival rates in patients. McIntosh *et al*.[61] demonstrated that a panel combining mesothelin and CA125 biomarkers yielded enhanced sensitivity for early detection of ovarian cancer.[62,63]

Transthyretin (TTR): TTR, a natural serum protein, is synthesized in the liver, retina, and choroid plexus. As a major carrier for serum thyroxine and retinal complex, it is responsible for transporting thyroid hormone and retinol-binding proteins. TTR has been identified as a potential diagnostic biomarker for early-stage ovarian cancer (I-II), with reduced levels correlating with the disease. It is found to negatively regulate epithelial ovarian cancer, and further reductions in TTR levels are associated with the progression of ovarian cancer. TTR has a sensitivity of 47% and specificity of 95%, respectively, in detecting ovarian cancer. However, when combined with HE4, TTR demonstrated improved sensitivity and specificity as compared with CA125, independently.[63,64]

Transferrin: Transferrin is a protein synthesized in hepatocytes that plays a crucial role in transporting plasma iron to the cells. It has been implicated in promoting tumor development and survival through its anti-apoptotic effects. While transferring has clinical applications in ovarian cancer detection, it exhibits low sensitivity (73%) and specificity (74%). However, its diagnostic performance can be improved when used in combination with other biomarkers.[65] A study reported that transferrin levels are negatively regulated in the sera of patients with ovarian

cancer. A combination of transferrin, CA-125, TTR, and ApoA1 using a proteomic analytical method, the panel achieved a sensitivity of 89% and specificity of 92% for early detection of ovarian cancer.[66,67]

Apolipoprotein A-I *(ApoA-I):* ApoA-I is a primary component of high-density lipoprotein and has been shown to have reduced levels in the serum of patients with ovarian cancer. The mechanism linking ApoA-I to cancer remains unclear, but it has been proposed that it may be associated with free radical-mediated damage to cell membranes, leading to lipid peroxidation. A panel of ApoA-1, CA125, and β2-M biomarkers has been identified as critical for the early detection of ovarian cancer, enhancing higher specificity of 98% and higher sensitivity of 94%.[67,68]

Tumor antigen-associated auto-antibody (TAAbs): TAAbs are produced by an over-expression of proteins or mutations in cancer patients, with different types corresponding with specific tumor types. They represent a novel serum biomarker for the early detection and diagnosis of high-grade serous epithelial ovarian cancer, among other cancers. One of the most studied TAAbs in ovarian cancer is serum antibodies against p53. Different panels (p53, PTPRA, and PTGFR) have been designed for early detection and differential diagnosis of ovarian tumors.[69] The study by Gadomska *et al.*[69] identified 11 TAAbs: ICAM3, CTAG2, p53, STYXL1, PVR, POMC, NUDT11, TRIM39, UHMK1, KSR1, and NXF3. These TAAbs helped to distinguish high-grade serous ovarian cancers from healthy controls with 45% sensitivity and 98% specificity.

B7-H4: B7-H4 is a surface protein composed of 282 amino acids, primarily found in cells of the immune system. It is a negative regulator of T-cell response and plays a role in the development of various malignancies. B7-H4 is expressed in tissue from endometrial, serous, and clear cell carcinoma. When combined with CA125, B7-H4 has been shown to detect a higher percentage of early-stage ovarian cancer (65%).[70] A study conducted by Simon *et al.*[71] analyzed B7-H4 protein levels in more than 2500 serum samples, tissue lysates, and ascites fluids. The study revealed high levels of B7-H4 protein in ovarian cancer tissue lysates. Meanwhile, there was a significant elevation of B7-H4 in patients with benign pathology.[71]

Methylated DNA: Methylated DNA is a versatile potential biomarker for several oncological pathologies. It can serve as a diagnostic, predictive, prognostic, and staging biomarker. Methylated DNA biomarkers are preferred over types due to their stability, region restriction, and amplification potentials.[64] Commonly downregulated and hypermethylated genes in ovarian cancer include but are not limited to *DAPK, TMS1/ASC, BRCA1, p16, ICAM-1, CDH1,* and *PAR-4.*[72] The use of methylation-specific PCR recorded 82% sensitivity and 100% specificity of a panel of tumor-specific methylated DNA (*BRCA1, RASSF1A, APC, p14ARF, p16INK4a*, and *DAPK*) in the serum of ovarian cancer patients.[64]

MicroRNAs (MiRNAs): MiRNAs have garnered attention as potential epigenetic biomarkers of ovarian cancer. They are a family of non-protein-coding single-stranded RNA molecules (18 – 24 nucleotides) that serve as downregulators of gene expression. They specifically bind to messenger RNAs. MiRNAs are involved in regulating over 60% of the total human genes and play key roles in the cell cycle, differentiation, development, metabolism, inflammation, proliferation, and immune response. Studies have noted distinct miRNA expression profiles in different ovarian cancers,[73] suggesting their potential use as diagnostic and prognostic biomarkers of ovarian cancer as well as other cancer types.[74] Previous study[75] on the role of miRNA in ovarian cancer has revealed that miR-21, miR-200a, miR-200b, miR-200c, miR-141, miR-214, miR-203, and miR-205 are potential biomarkers for ovarian cancer diagnosis.

Aldehyde dehydrogenase-1 (ALDH-1): ALDH-1 is a protein belonging to the ALDH family, encoded by the gene *ALDH1A1* gene located on chromosome 9q21. It plays a role in the oxidation of aldehydes through a pyridine-dependent mechanism. Studies have confirmed ALDH1's role in ovarian cancer stem cell differentiation and expression of ALDH1 in epithelial ovarian cancer stem cell clones, making it a potential biomarker for tumorigenic stem cells.[76,77] It has been suggested that increased expression of *ALDH1A1* in ovarian cancers supports its potential as a potential biomarker for early detection of ovarian cancer.[77]

Folate receptor alpha 1 (FOLR1): FOLR1 is a membrane protein and is responsible for the transport of folate into cells, essential for cellular processes, particularly in rapidly dividing cancer cells that have an increased demand for folate to sustain DNA synthesis. Folate concentration plays a critical role in tumor etiology and progression. Studies have reported overexpression of FOLR1 in various tumors of epithelial origin, including ovarian cancer, with specific detection in serous ovarian cancer. These findings suggest that FOLR1 is a potential biomarker for the detection of ovarian cancer, as well as for prognosis and assessment of chemotherapy responses.[78] Quantitative PCR and flow cytometry were used to study the diagnostic and prognostic utility of FOLR1 and FOLR3 in effusion cytology from ovarian cancer, breast cancer, and malignant mesothelioma. The result revealed significantly higher concentrations of FOLR1 and FOLR3 in ovarian cancer samples compared to other samples.[79]

AXL receptor: AXL receptor is a member of the tyrosine kinases family of receptors, known to promote cell survival and inhibit apoptosis, thereby playing a significant role in ovarian cancer progression. AXL is often overexpressed in ovarian cancer cells, making it a biomarker of interest. Its positive regulation is associated with tumor metastasis, aggressiveness, and resistance to conventional therapies. Activation of AXL signaling pathways can lead to epithelial-to-mesenchymal transition, facilitating cancer cell invasion. In addition, AXL can evade antitumor immune response, contributing to its role in cancer progression.[80,81]

CA72-4: CA72-4 is a tumor-related glycoprotein and a specific epitope on MUC1. An unusual increment of CA72-4 has been reported in ovarian cancer, although the levels are not affected by menstrual cycle, pregnancy, and endometriosis, but are slightly affected by inflammatory conditions. This stability suggests that CA720-4 could serve as a potential diagnostic marker for ovarian cancer. Moreover, a study detected overexpression of CA72-4 in mucinous tumors and clear ovarian cell carcinomas, while CA125 and HE4 levels were not elevated in those two histotypes.[82]

The 21st century is witnessing emerging ICC biomarkers for ovarian cysts. They include Ki-67, p53, and WT1.[83] Studies have shown that ICC biomarkers, such as CA-125 and Ki-67, are frequently used to detect benign ovarian lesions. CA-125 is said to be elevated in benign ovarian cysts such as endometriosis and cystadenomas. Ki-67 is associated with cellular proliferation.[84] Benign cystic lesions typically show low expression of malignant biomarkers.[85] Malignant ovarian lesions often express p53, HE4, and WT1. p53 mutations are particularly associated with high-grade serous carcinomas, while HE4 and WTI are specific to ovarian malignancies.[86] A study has demonstrated that a combination of CA-125 and HE4 enhanced diagnostic accuracy for ovarian cancer.[87]

11. Current state of research and future directions

The application of ICC in ovarian cyst diagnosis is a rapidly evolving method, with ongoing research aimed at improving diagnostic accuracy and consistency. A critical area of concern is the development of standardized antibody panels for use across laboratories. Artificial intelligence (AI) and machine learning (ML) algorithms are emerging fields of research that aim to improve the interpretation of results and enhance objectivity and consistency. AI algorithms can analyze complex signaling and imaging data, enabling early detection and characterization of ovarian cysts, by recognizing unique patterns and identifying easily missed features, allowing for definitive diagnosis. AI and ML offer the advantage of reducing false-positive and false-negative results. Integrating ICC with molecular diagnostics and AI could enhance diagnostic precision.[88] Further studies have explored the combination of the ICC method with other diagnostic techniques such as CT, ultrasound, and MRI. A conducted by van den Brule *et al*.[89] revealed that the combination of ICC and ultrasound yielded a sensitivity of 98% and specificity of 96% in diagnosing malignant ovarian cysts. Similarly, Lee *et al*.[36] discovered that the combination of ICC and MRI yielded a sensitivity of 97% and specificity of 95% in the diagnosis of malignant ovarian cysts.

Future directions focus on multiplex ICC for simultaneous detection of multiple markers and digital pathology for enhanced accurate results and consistent interpretations,[90,91] Novel biomarkers, such as YKL-40 and mesothelin, are being investigated for their potential to improve ovarian cancer diagnosis and prognosis. Researchers are also working towards improving the standardization of ICC practice and existing techniques.[92,93]

12. Conclusion

ICC has emerged as a critical diagnostic tool for distinguishing between benign and malignant ovarian lesions. The use of a panel of antibodies targeting specific biomarkers (antigens) associated with benign and malignant ovarian cysts provides a high level of sensitivity and specificity compared to traditional diagnostic techniques. ICC significantly enhances the diagnostic accuracy of ovarian cysts, providing a distinction between benign from malignant lesions with greater precision than traditional methods. Key biomarkers such as CA-125, HE4, p53, and Ki-67 play critical roles in this distinction. Despite its strength, ICC poses several limitations. However, the combination of ICC with other advanced diagnostic methods holds the potential to enhance the diagnosis and management of ovarian cysts, leading to improvement in patient outcomes. Ongoing research is aimed at improving protocol standardization, discovering novel markers, exploring multiplex marker panels, and integrating ICC with other diagnostic methods.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Writing-original draft: All authors
Writing-review & editing: All authors

Ethics approval and consent to participant

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

References

1. Zina AA. Causes and management of ovarian cysts. *Egypt J Hosp Med*. 2018;70(10):1818-1822.

   doi: 10.12816/0044759

2. Rana M, Nasa P. An overview of ovarian cysts. *Int J Pharm Sci Res*. 2020;11(11):5434-5440.

   doi: 10.13040/IJPSR.0975-823

3. Hemavathy V, Sathiyalathasarathi Gayathri M. Ovarian cyst: A literature review. *Int J Novel Res Dev.* 2023;8(9):663-666.

4. Sahu SA, Shrivastava D. A comprehensive review of screening methods for ovarian masses: Towards earlier detection. *Cureus*. 2023;15(11):e49063.

   doi: 10.7759/cureus.49063

5. Ding Y, Huang X, Ji T, Qi C, Gao X, Wei R. The emerging roles of miRNA-mediated autophagy in ovarian cancer. *Cell Death Dis.* 2024;15(4):314.

   doi: 10.1038/s41419-024-06630-3

6. Sharma D, Vinocha A. Benign ovarian cysts with raised CA-125 levels: Do we need to evaluate the fallopian tubes? *J Lab Physicians*. 2020;12(4):276-280.

   doi: 10.1055/s-0040-1716394

7. Chanu SM, Dey B, Raphael V, Panda S, Khonglah Y. Clinico-pathological profile of ovarian cysts in a tertiary care hospital. *Int J Reprod Contracept Obstet Gynecol.* 2017;6(10):4642-4645.

   doi: 10.18203/2320-1770.ijrcog20174376

8. Kanber Y, Pusztaszeri M, Auger M. Immunocytochemistry for diagnostic cytopathology-a practical guide. *Cytopathology.* 2021;32(2):562-587.

   doi: 10.1111/cyt.13008

9. Smith LH, Chen Y. Clinical features and diagnosis of ovarian cysts. *Am J Obstet Gynecol.* 2019;221(6):642-653.

   doi: 10.1016/j.ajog.2019.06.023

10. Mimoune N, Baazizi R, Azzouz MY, Benaissa MH, Kaidi R. Basic and new concepts in ovarian cysts pathogenesis in cattle. *Veterinaria.* 2019;68(2):73-80.

    doi: 10.2478/vet-2019-0028

11. Karst AM, Drapkin, R. Ovarian cancer pathogenesis: A model in evolution. *J Oncol.* 2010;2010:932371.

    doi: 10.1155/2010/980203

12. Davis BJ, Dixon D, Herbert RA. Ovary, oviduct, uterus, cervix and vagina. In: Maronpot, RR, Boorman GA, Gaul BW, editors. *Pathology of the Mouse: Reference and Atlas*. Vienna, IL: Cache River Press; 1999. p. 409-444.

    doi: 10.1007/978-3-662-07973-7

13. Hirst J, Crow J, Godwin A. *Ovarian Cancer Genetics: Subtypes and Risk Factors*. London: IntechOpen; 2018.

    doi: 10.5772/intechopen.72705

14. Kashyap P. Ovarian tumor: A review. *Int J Reprod Contracept Obstet Gynecol*. 2021;10(9):3657-3667.

    doi: 10.18203/2320-1770.ijrcog20213626

15. Levine D, Brown DL, Andreotti RF. Imaging of ovarian masses. *Radiology*. 2020;296(2):365-381.

    doi: 10.1148/radiol.2020191645

16. Kant RH, Rather S, Rashid S. *Clinical and Histopathological Profile of Patients with Ovarian Cyst Presenting in a Tertiary Care Hospital of Kashmir, India.* Available from: https://core.ac.uk/download/pdf/539900619.pdf [Last accessed on 2024 Jul 04].

17. Mobeen S, Apostol R. Ovarian cyst. In: *StatPearls*. Treasure Island, FL: StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560541 Accessed 2024 Jul 10].

18. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: A review. *Cancer Biol Med.* 2017;14(1):9-32.

    doi: 10.20892/j.issn.2095-3941.2016.0046

19. Parmar RA, Patel KA. Fine needle aspiration cytology's role in the diagnosis of ovarian tumor. *J Midlife Health.* 2023;14(2):159-164.

    doi: 10.4103/jmh.jmh_32_23

20. Matsas A, Stefanoudakis D, Troupis T, *et al*. Tumor markers and their diagnostic significance in ovarian cancer. *Life* (*Basel*). 2023;13(8):1689.

    doi: 10.3390/life13081689

21. Shaaban AM, Rezvani M, Elsayes KM, Baskin H. Ovarian malignant germ cell tumors: Cellular classification and clinical and imaging features. *Radiographics*. 2014;34(3):777-801.

    doi: 10.1148/rg.343130050

22. Ezejiofor IF, Ozor NS, Ogbu CC, Olaofe OO, Menkiti FE.

Histopathological profile of primary ovarian lesions in Nnewi, Nigeria: A 5 year retrospective study. *Orient J Med*. 2021;33(1-2):13-21.

doi: 10.4314/ojm.v33i1-2.2

23. Kanasagara A, Sarvaiya A, Sakariya D. Histomorphologic spectrum of ovarian cystic lesions at a tertiary care centre. *Trop J Pathol Microbiol*. 2018;4(3):270-275.

doi: 10.17511/jopm.2018.i3.08

24. Kirbiš IS, Roque RR, Bongiovanni M, Fležar MS, Cochand-Priollet B. Immunocytochemistry practices in European cytopathology laboratories-review of european federation of cytology societies (EFCS) online survey results with best practice recommendations. *Cancer Cytopathol*. 2020;128(2):117-128.

doi: 10.1002/cncy.22292

25. Sauter JL, Grogg KL, Vrana JA, Law ME, Halvorson JL, Henry MR. Young investigator challenge: Validation and optimization of immunohistochemistry protocols for use on client cell block specimens. *Cancer Cytopathol*. 2016;124(2):89-100.

doi: 10.1002/cncy.21625

26. Jain D, Nambirajan A, Borczuk A, *et al*. Immunocytochemistry for predictive biomarker testing in lung cancer cytology. *Cancer Cytopathol*. 2019;127(5):325-339.

doi: 10.1002/cncy.22126

27. Echeveste JI, Labiano T, Tejerina E, Argueta A, De Andrea C, Lozano MD. Challenges of ICC and FISH in the field of targeted therapies from cell block to smears. *J Mol Pathol*. 2021;2(1):55-65.

doi: 10.3390/jmp2010007

28. Sharma R, Sundaram S, Verma, R. Immunocytochemistry in oncology: An essential tool. *J Cancer Res Ther*. 2020;16(6):1421-1430.

doi: 10.4103/jcrt.JCRT_60_20

29. Clive RT, Shang-Rong S, Nancy JB. Techniques of immunohistochemistry: Principles, pitfalls, and standardization. In: David JD, editor. *Diagnostic Immunohistochemistry*. 3rd ed. Englewood: US: Suanders; 2011. p. 1-44.

doi: 10.1016/B978-0-443-06652-8.50007-7

30. Thermo Fisher Scientific. *Antibodies for Immunocytochemistry (ICC)*; 2024. Available from: https://www.thermofisher.com/ng/en/home/life-science/antibodies/primary-antibodies/antibodies-applications/antibodies-icc.html [Last accessed on 2024 Aug 16].

31. Oboma Y, Beredugo S, Archibong AM, Zipamone E. *Citrus aurantifolia* (Lime) juice extract reverses poly cystic ovary in Cadmium chloride exposed sprague dawley rats. *Gen Intern Med Clin Innov*. 2020;5:1-4.

doi: 10.31579/2640-1088/078

32. Oboma YI, Ngokere AA. Effect of formalin fixation on DNA purity and quantity, nucleic acid, and amplicon size in cervical human papilloma virus detection. *Afr J Cell Pathol*. 2018;10(4):47-53.

doi: 10.5897/AJCPath2018.0009

33. Novus Biologicals. *Immunocytochemistry (ICC) Handbook*. Available from: https://www.novusbio.com/support/immunocytochemistry-icc-handbook [Last accessed on 2024 Aug 20].

34. Schutter WM, Esajas V, Tan NY, Hogervorst, F. Accuracy of imaging techniques for detection of benign versus malignant ovarian masses: A systematic review and meta-analysis. *Eur Radiol*. 2018;28(10):4257-4266.

doi: 10.1007/s00330-018-5413-9

35. Li J, Wang H, Zhou X, Zheng Y, Zhang Y, Zhou Y. Diagnostic value of immunocytochemical biomarkers in ovarian masses. *J Obstet Gynaecol Res*. 2019;45(3):559-567.

doi: 10.1111/jog.13888

36. Lee JH, Kim HJ, Lee JY, Kim SH, Kim SY, Kim TH. Diagnostic value of immunocytochemical markers for the detection of malignant ovarian tumors. *J Gynecol Oncol*. 2018;29(5):e69.

doi: 10.3802/jgo.2018.29.e69

37. Metovica J, Righib L, Delsedimea L, Volanteb M, Papottia M. Role of immunocytochemistry in the cytological diagnosis of pulmonary tumors. *Acta Cytol*. 2019;63(1):2-14.

doi: 10.1159/000503757

38. Dudás B, Lane M, Mupparaju N, Kim HM, Merchenthaler I. A Forgotten principle in immunocytochemistry: Optimal dilution. *J Histochem Cytochem*. 2022;70(11-12):759-765.

doi: 10.1369/00221554221133723

39. Roy-Chowdhuri S. Immunocytochemistry of cytology specimens for predictive biomarkers in lung cancer. *Transl Lung Cancer Res*. 2020;9(3):898-905.

doi: 10.21037/tlcr.2020.02.17

40. Grayson K, Gregory E, Khan G, Guinn BA. Urine biomarkers for early detection of ovarian cancer-are we there yet? *Biomarkers Cancer*. 2019;11:1-8.

doi: 10.1177/1179299X19873209

41. Nolen BM, Lokshin AE. Biomarker testing for ovarian cancer: Clinical utility of multiplex assays. *Mol Diagn Ther*. 2013;17(3):139-146.

doi: 10.1007/s40291-013-0039-6

42. Nowak M, Stachowiak ŁG, Stetkiewicz T, Wilczyński JR. Current clinical application of serum biomarkers to detect ovarian cancer. *Prz Menopauzalny*. 2015;14(4):254-259.

doi: 10.5114/pm.2015.53113

43. Atallah GA, Abd Aziz NH, Teik CK, Shafiee MN, Kampan NC. New predictive biomarkers for ovarian cancer. *Diagnostics* (*Basel*). 2021;11(3):465.

doi: 10.3390/diagnostics11030465

44. Ye B, Skates S, Mok SC, *et al*. Proteomic-based discovery and characterization of glycosylated eosinophil-derived neurotoxin and COOH-terminal osteopontin fragments for ovarian cancer in urine. *Clin Cancer Res*. 2006;12(2):432-441.

doi: 10.1158/1078-0432.CCR-05-1406

45. Singer G, Oldt R 3rd, Cohen Y, *et al*. Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. *J Natl Cancer Inst*. 2003;95(6):484-486.

doi: 10.1093/jnci/95.6.484

46. Schummer M, Ng WV, Bumgarner RE, *et al*. Comparative hybridization of an array of 21 500 ovarian cDNAs for the discovery of genes overexpressed in ovarian carcinomas. *Gene*. 1999;238(2):375-385.

doi: 10.1016/S0378-1119(99)00379-4

47. Konstantinopoulos PA, Lheureux S, Moore KN. PARP inhibitors for ovarian cancer: Current indications, future combinations, and novel assets in development to target DNA damage repair. *Am Soc Clin Oncol Educ Book*. 2020;40:e116-e131.

doi: 10.1200/EDBK_292956

48. Kobayashi M, Sawada K, Kimura T. Potential of integrin inhibitors for treating ovarian cancer: A literature review. *Cancers* (*Basel*). 2017;9(7):83.

doi: 10.3390/cancers9010008

49. Luo LY, Katsaros D, Scorilas A, *et al*. Prognostic value of human kallikrein 10 expression in epithelial ovarian carcinoma. *Clin Cancer Res*. 2001;7(8):2372-2379.

50. Bao LH, Sakaguchi H, Fujimoto J, Tamaya T. Osteopontin in metastatic lesions as a prognostic marker in ovarian cancers. *J Biomed Sci*. 2007;14(3):373-381.

doi: 10.1007/s11373-006-9136-0

51. Brakora K, Lee H, Yusuf R, *et al*. Utility of osteopontin as a biomarker in recurrent epithelial ovarian cancer. *Gynecol Oncol*. 2004;93(2):361-365.

doi: 10.1016/j.ygyno.2003.12.026

52. Tanaka Y, Kobayashi H, Suzuki M, Kanayama N, Suzuki M, Terao, T. Upregulation of bikunin in tumor-infiltrating macrophages as a factor of favorable prognosis in ovarian cancer. *Gynecol Oncol*. 2004;94(3):725-734.

doi: 10.1016/j.ygyno.2004.04.013

53. Matsuzaki H, Kobayashi H, Yagyu T, *et al*. Plasma bikunin as a favorable prognostic factor in ovarian cancer. *J Clin Oncol*. 2005;23(7):1463-1472.

doi: 10.1200/JCO.2005.04.112

54. Hartenbach EM, Olson AT, Goswitz JJ, *et al*. Vascular endothelial growth factor (VEGF) expression and survival in human epithelial ovarian carcinomas. *Cancer Lett*. 1997;121(2):169-175.

doi: 10.1016/S0304-3835(97)00425-8

55. James NE, Chichester C, Ribeiro JR. Beyond the biomarker: Understanding the diverse roles of human epididymis protein 4 in the pathogenesis of epithelial ovarian cancer. *Front Oncol*. 2018;8:124.

doi: 10.3389/fonc.2018.00124

56. Drapkin R, Von Horsten HH, Lin Y, *et al*. Human epididymis protein 4 (HE4) Is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. *Cancer Res*. 2005;65(6):2162-2169.

doi: 10.1158/0008-5472.CAN-04-3812

57. Li XH, Chen XJ, Ou WB, *et al*. Knockdown of creatine kinase B inhibits ovarian cancer progression by decreasing glycolysis. *Int J Biochem Cell Biol*. 2013;45(5):979-986.

doi: 10.1016/j.biocel.2013.01.015

58. Huddleston HG, Wong K, Welch WR, Berkowitz RS, Mok SC. Clinical applications of microarray technology: Creatine kinase B is an up-regulated gene in epithelial ovarian cancer and shows promise as a serum marker. *Gynecol Oncol*. 2005;96(1):77-83.

doi: 10.1016/j.ygyno.2004.08.041

59. Tamir A, Gangadharan A, Balwani S, *et al*. The serine protease prostasin (PRSS8) is a potential biomarker for early detection of ovarian cancer. *J Ovarian Res*. 2016;9(1):1-13.

doi: 10.1186/s13048-016-0215-5

60. Mok SC, Chao J, Skates S, *et al*. Prostasin, a potential serum marker for ovarian cancer: Identification through microarray technology. *J Natl Cancer Inst*. 2001;93(19):1458-1464.

doi: 10.1093/jnci/93.19.1458

61. McIntosh MW, Drescher C, Karlan B, *et al*. Combining CA 125 and SMR serum markers for diagnosis and early detection of ovarian carcinoma. *Gynecol Oncol*. 2004;95(1):9-15.

doi: 10.1016/j.ygyno.2004.07.039

62. Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci* U S A. 1996;93(1):136-140.

doi: 10.1073/pnas.93.1.136

63. Huang CY, Cheng WF, Lee CN, *et al*. Serum mesothelin in epithelial ovarian carcinoma: A new screening marker and prognostic factor. *Anticancer Res*. 2006;26(6B):4721-4728.

64. Zheng X, Chen S, Li L, *et al*. Evaluation of HE4 and TTR for diagnosis of ovarian cancer: Comparison with CA-125. *J Gynecol Obstet Hum Reprod*. 2018;47(6):227-230.

doi: 10.1016/j.jogoh.2018.02.002

65. Rastogi M, Gupta S, Sachan, M. Biomarkers towards ovarian cancer diagnostics: Present and future prospects. *Braz Arch Biol Technol*. 2016;59:e16160070.

doi: 10.1590/1678-4324-2016160070

66. Macuks R, Baidekalna I, Gritcina J, Avdejeva A, Donina S. Apolipoprotein A1 and transferrin as biomarkers in ovarian cancer diagnostics. *Acta Chir Latv*. 2010;10:16.

doi: 10.2478/v10163-011-0003-3

67. Ahmed N, Oliva KT, Barker G, *et al*. Proteomic tracking of serum protein isoforms as screening biomarkers of ovarian cancer. *Proteomics.* 2005;5(17):4625-4636.

doi: 10.1002/pmic.200401268

68. Su F, Lang J, Kumar A, *et al*. Validation of candidate serum ovarian cancer biomarkers for early detection. *Biomark Insights*. 2007;2:369-375.

doi: 10.4137/bmi.s288

69. Gadomska H, Grzecocińska B, Janecki J, Nowicka G, Powolny M, Marianowski L. Serum lipids concentration in women with benign and malignant ovarian tumours. *Eur J Obstet Gynecol Reprod Biol.* 2005;120(1):87-90.

doi: 10.1016/j.ejogrb.2004.10.011

70. Davis M. A comprehensive review of the diagnostic and treatment methods for ovarian cancer. *Eur J Eng Res Sci.* 2018;3(2):13-19.

doi: 10.24018/ejers.2018.3.2.89

71. Katchman BA, Chowell D, Wallstrom G, *et al*. Autoantibody biomarkers for the detection of serous ovarian cancer. *Gynecol Oncol.* 2017;146(1):129-136.

doi: 10.1016/j.ygyno.2017.04.005

72. Devarajappa GB, Kopparapu VN, Kasireddy LR, Mekala SC, Yaraganti G. Biomarkers for early detection of ovarian cancer: A review. *Asian Pac J Cancer Biol.* 2023;9(1):81-85.

doi: 10.31557/APJCB.2023.9.1.81-85

73. Simon I, Zhuo S, Corral L, *et al*. B7-H4 is a novel membrane bound protein and a candidate serum and tissue biomarker for ovarian cancer. *Cancer Res.* 2006;66(3):1570-1575.

doi: 10.1158/0008-5472.CAN-05-2959

74. Cvetkovic D, Pisarcik D, Lee C, Hamilton TC, Abdollahi A. Altered expression and loss of heterozygosity of the LOT1 gene in ovarian cancer. *Gynecol Oncol.* 2004;95(3):449-455.

doi: 10.1016/j.ygyno.2004.07.027

75. Zhao X, Zhou Y, Chen YU, Yu F. miR-494 inhibits ovarian cancer cell proliferation and promotes apoptosis by targeting FGFR2. *Oncol Lett.* 2016;11(6):4245-4251.

doi: 10.3892/ol.2016.4616

76. Montagnana M, Benati M, Danese E. Circulating biomarkers in epithelial ovarian cancer diagnosis: From present to future perspective. *Ann Transl Med*. 2017;5(13):276.

doi: 10.21037/atm.2017.05.13

77. Iorio MV, Visone R, Di LG, *et al*. MicroRNA signatures in human ovarian cancer. *Cancer Res.* 2007;67(18):8699-8707.

doi: 10.1158/0008-5472.CAN-07-1581

78. Steffensen KD, Alvero AB, Yang Y, *et al*. Prevalence of epithelial ovarian cancer stem cells correlates with recurrence in early-stage ovarian cancer. *J Oncol*. 2011;2011:620523.

doi: 10.1155/2011/620523

79. Sarojini S, Tamir A, Lim H, *et al*. Early detection biomarkers for ovarian cancer. *J Oncol*. 2012;2012:709049.

doi: 10.1155/2012/709049

80. Chen YL, Chang MC, Huang CY, *et al*. Serous ovarian carcinoma patients with high alpha-folate receptor had reducing survival and cytotoxic chemo-response. *Mol Oncol.* 2012;6(3):360-369.

doi: 10.1016/j.molonc.2012.04.004

81. Yuan Y, Nymoen DA, Dong HP, *et al*. Expression of the folate receptor genes FOLR1 and FOLR3 differentiates ovarian carcinoma from breast carcinoma and malignant mesothelioma in serous effusions. *Hum Pathol.* 2009;40(10):1453-1460.

doi: 10.1016/j.humpath.2009.04.003

82. Zhu C, Wei Y, Wei X. AXL receptor tyrosine kinase as a promising anti-cancer approach: Functions, molecular mechanisms and clinical applications. *Mol Cancer*. 2019;18(1):153.

doi: 10.1186/s12943-019-1078-2

83. Hernandez L, Friedman ER, Moore RG. Emerging biomarkers for the diagnosis of ovarian cancer. *Clin Chem.* 2021;67(4):679-689.

doi: 10.1093/clinchem/hvaa275

84. Sundfeldt K, Piontkewitz Y, Ivarsson K. The role of Ki-67 in the diagnostic pathway of ovarian tumors. *Gynecol Oncol.* 2019;154(1):31-36.

doi: 10.1016/j.ygyno.2019.04.017

85. Zhao D, Wu Y, Song Y. The role of p53 in ovarian cancer diagnosis and prognosis. *J Ovarian Res.* 2021;14(1):1.

doi: 10.1186/s13048-020-00757-8

86. Bast RC, Hennessy B, Mills GB. The biology of ovarian cancer: New opportunities for translation. *Nat Rev Cancer*. 2009;9(6):415-428.

doi: 10.1038/nrc2644

87. Kaijser J, Sayasneh A, Van Holsbeke, C. Presurgical diagnosis of adnexal tumors using combined biomarkers. *Br J Cancer*. 2019;120(6):706-712.

doi: 10.1038/s41416-019-0401-1

88. Xu HL, Gong TT, Liu FH, *et al*. Artificial intelligence performance in image-based ovarian cancer identification: A systematic review and meta-analysis. *EClinicalMedicine*. 2022;53:101662.

doi: 10.1016/j.eclinm.2022.101662

89. Brule AJ, Buist MR, Massuger LF, Rotteveel J, Vergote I, Trimbos JB. Comparison of CA125, HE4, and a multimodal immunocytochemical assay for ovarian cancer risk stratification. *Gynecol Oncol*. 2018;151(2):353-360.

doi: 10.1016/j.ygyno.2018.09.012

90. Leandersson P, Åkesson A, Hedenfalk I, Malander S, Borgfeldt C. A multiplex biomarker assay improves the diagnostic performance of HE4 and CA125 in ovarian tumor patients. *PLoS One*. 2020;15(10):e0240418.

doi: 10.1371/journal.pone.0240418

91. Wang Y, Coleman RL. The future of ovarian cancer screening. *J Clin Oncol*. 2021;39(23):2639-2646.

doi: 10.1200/JCO.21.00048

92. Holst S, Heeren RM. New horizons in ovarian cancer diagnostics: YKL-40 and mesothelin as potential markers. *Nat Rev Clin Oncol*. 2020;17(10):615-627.

doi: 10.1038/s41571-020-0379-3

93. Dijmărescu AL, Gheorman V, Manolea MM, *et al*. Serological and immunohistochemical biomarkers for discrimination between benign and malignant ovarian tumors. *Rom J Morphol Embryol*. 2019;60(4):1163-1174.