DIAGNOSIS OF EPITHELIAL OVARIAN CANCER IN WOMEN WITH OVARIAN MASSES USING MULTIPLEX IMMUNOASSAYS AND KRESTCHMANN-SURFACE PLASMON RESONANCE

VIKNESWARY A/P RAVI KUMAR

UNIVERSITI KEBANGSAAN MALAYSIA

UKM-SPKPPP-PT(PdP)-05-AK04-BO07	No. Semakan: 02	Tarikh Kuat Kuasa: 27/01/2026
BORANG DAN SENARAI SEMAK PENYERAHAN TESIS/DISERTASI SELEPAS PEMBETULAN ***FORM AND CHECKLIST OF AMENDED THESIS/DISSERTATION SUBMISSION***		

F. KELULUSAN DEKAN/PENGARAH / *APPROVAL FROM DEAN/DIRECTOR*

Tandatangan:
Signature

Tarikh:
Date 2/7/2026

Nama dan Cap Rasmi:
Name and Official Stamp

PROFESOR DATO' DR MARINA MAT BAKI
MD (UKM), MS ORL-HNS (UKM), PhD (UCL)
Dekan Fakulti Perubatan
Pakar Perunding Kanan ORL-HNS
Universiti Kebangsaan Malaysia

G. PERAKUAN TESIS/ DISERTASI SARJANA / DOKTOR FALSAFAH (*CERTIFICATION OF MASTER'S / DOCTORAL THESIS / DISSERTATION*)

Nama penuh pengarang (*Author's Full Name*)	Vikneswary A/P Ravi Kumar		
No. Pendaftaran Pelajar (Student's Registration No.)	P119142	Sesi Akademik (*Academic Session*)	Semester 2/ Sesi 2025/2026
Tajuk Tesis / Disertasi (*Thesis / Dissertation Title*)	DIAGNOSIS OF EPITHELIAL OVARIAN CANCER IN WOMEN WITH OVARIAN MASSES USING MULTIPLEX IMMUNOASSAYS AND KRESTCHMANN-SURFACE PLASMON RESONANCE		

Semua tesis/disertasi dan penerbitan berkaitan hasil penyelidikan pelajar adalah tertakluk kepada Dasar Harta Intelek Universiti Kebangsaan Malaysia.
All theses/ dissertations and publications relating to the research work of a student are subject to the Intellectual Property Policy of Universiti Kebangsaan Malaysia.

Saya mengaku bahawa tahap akses tesis/disertasi ini sebagai (*Tandakan ✓ dalam kotak bagi maklumat yang berkaitan; tandakan satu (1) kotak di bawah sahaja):
I hereby declare the level of access for this thesis/dissertation as follows (tick ✓ the relevant box below; tick only one option).

Pilihan Tahap Akses *Access Level Selection*		Tafsiran Tahap Akses Tesis/Disertasi *Definition of Thesis/Dissertation Access Level*
√	**RAHSIA** ***(CONFIDENTIAL)***	Mengandungi maklumat rahsia sepertimana yang diperuntukan bawah Akta Rahsia Rasmi 1972. *Contains classified information as stipulated under the Official Secrets Act 1972.*
√	**TERHAD** ***(RESTRICTED)***	Mengandungi maklumat yang hanya boleh diakses oleh pihak yang mempunyai kebenaran atau keperluan tertentu untuk mengetahuinya yang ditentukan oleh organisasi/ badan di mana penyelidikan dijalankan. *Contains information that may only be accessed by parties with specific authorization*

UNIVERSITI KEBANGSAAN MALAYSIA	UKM-SPKPPP-PT(PdP)-05-AK04-BO07	No. Semakan: 02	Tarikh Kuat Kuasa: 27/01/2026
	BORANG DAN SENARAI SEMAK PENYERAHAN TESIS/DISERTASI SELEPAS PEMBETULAN ***FORM AND CHECKLIST OF AMENDED THESIS/DISSERTATION SUBMISSION***		

		or a defined need to know, as determined by the organization or body where the research is conducted.
√	**AKSES TERBUKA** ***(OPEN ACCESS)***	Tesis/disertasi versi akhir dalam format PDF yang boleh diakses secara percuma secara dalam talian dan bebas dari isu hak cipta dan pelesenan *The final version of the thesis/dissertation in PDF format that is freely accessible online and free from copyright and licensing issues.* Kementerian Pendidikan Tinggi melalui surat bertarikh 27 Mac 2025 memutuskan bahawa kebenaran akses tesis / disertasi dalam borang serahan akses terbuka ditentukan oleh penyelia (bukan pelajar). *The Ministry of Higher Education, through a letter dated 27 March 2025, has decided that permission for thesis / dissertation access in the open access submission form shall be determined by the supervisor (not the student).*
√	**EMBARGO**	Tempoh masa di mana akses kepada tesis/disertasi disekat (ditutup) selama dua (2) tahun atas sebab-sebab tertentu sebagai contoh kerahsiaan atau hak cipta. Tesis/disertasi akan berstatus akses terbuka selepas tamat tempoh *embargo.* *The period during which access to the thesis/dissertation is restricted for two (2) years due to specific reasons, such as confidentiality or copyright. The thesis/dissertation will be granted open-access status upon the expiry of the embargo period.*

PENGESAHAN PELAJAR ***(STUDENT VERIFICATION)***	**PENGESAHAN PENYELIA** ***(SUPERVISOR VERIFICATION)***
Vikneswary Ravi Kumar **TANDATANGAN PELAJAR** ***(STUDENT'S SIGNATURE)***	Dr Nirmala CL Kampan (MMC No. 38695 & NSR No. 1218/09) Consultant Obstetrician & Gynaecologist and Gynae-Oncologist Pantai Hospital Kuala Lumpur **TANDATANGAN PENYELIA / PENGERUSI JK SISWAZAH** ***(SUPERVISOR'S / CHAIRPERSON SUPERVISION COMMITTEE SIGNATURE)***
Nama Pelajar *(Student name)*: Vikneswary A/P Ravi Kumar No. Pendaftaran Pelajar *(Student's Registration No.)* : P119142 Tarikh: 19/05/2026 *(Date)*	Nama Penyelia / Pengerusi JK Siswazah : *(Supervisor's / Chairperson Supervision Committee Name)* Professor Dr Nirmala Chandralega (Main Supervisor) Tarikh: 19/05/2026 *(Date)*

DIAGNOSIS OF EPITHELIAL OVARIAN CANCER IN WOMEN WITH OVARIAN MASSES USING MULTIPLEX IMMUNOASSAYS AND KRESTCHMANN-SURFACE PLASMON RESONANCE

VIKNESWARY A/P RAVI KUMAR

THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF MASTER OF MEDICAL SCIENCE

FACULTY OF MEDICINE
UNIVERSITI KEBANGSAAN MALAYSIA
KUALA LUMPUR

2026

DIOGNOSA KANSER OVARI EPITELIAL DI KALANGAN WANITA DENGAN KETUMBUHAN OVARI MENGGUNAKAN MULTIPLEKS IMMUNOESEI DAN KRETSCHMANN RESONAN PLASMON PERMUKAAN

VIKNESWARY A/P RAVI KUMAR

TESIS YANG DIKEMUKAKAN UNTUK MEMPEROLEH
IJAZAH SARJANA SAINS PERUBATAN

FAKULTI PERUBATAN
UNIVERSITI KEBANGSAAN MALAYSIA
KUALA LUMPUR

2026

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

26 June 2026

VIKNESWARY A/P RAVI KUMAR
P119142

ACKNOWLEDGMENT

By the grace of God alone I stand here today carried by His mercy, guided by His light, and strengthened through every moment I felt like giving up. This thesis is, above all, a testament to His greatness.

To my late father you left us in the middle of this journey, and that loss was the heaviest thing I have ever carried. I struggled, but I felt your quiet guidance every step of the way. This is for you. It has always been for you.

I am deeply grateful to my three supervisors for their guidance and mentorship that went far beyond academic instruction. My sincerest thanks go to my main supervisor, Professor Dr. Nirmala Chandralega Kampan, for her unwavering encouragement, invaluable guidance, and belief in me throughout my research journey. I am equally thankful to my co-supervisors, Professor Dr. Susthitha Menon (Prof. Susi) of Institute of Microengineering and Nanoelectronics (IMEN), UKM, for her patience and the invaluable opportunities she provided, and Dr. Nor Haslinda Abd Aziz (Obstetrics and Gynaecology Departmen (O&G dept), HCTM) for her steady and thoughtful supervision. Together, their mentorship was instrumental in the successful completion of this thesis.

To my beloved mother, Mdm. Kala Devi my heart. You sacrificed more than I will ever fully understand so that I could have this opportunity. Every time I felt exhausted and overwhelmed, I thought of you of your quiet strength, your unconditional love, and the way you never once stopped believing in me. I hope this makes you proud. You deserve every bit of this joy and more.

To my dear siblings, Binosha Ravi Kumar and Saravanan Ravi Kumar thank you for being my safe place. For the phone calls, the laughter, the reassurance, and for never letting me feel alone in this. Having you by my side means the world to me.

My heartfelt thanks go to all my wonderful postgraduate colleagues Muzhill, Nazzary, Dharvind from Faculty perubatan, UKM (O&G dept, HCTM) and Dr. Akmar (IMEN, UKM), as well as many others who have contributed directly or indirectly to my work. You turned what could have been an isolating experience into one of the most meaningful chapters of my life. The shared struggles, the spontaneous conversations, the quiet companionship in the lab I will treasure all of it. Thank you for making UKM feel like home.

I would also like to acknowledge the dedicated staff at the Pathology Department, O&G Department, Faculty of Medicine, and IMEN (UKM) for their technical support, and the Faculty of Medicine, UKM, for funding this project. To everyone who contributed in any way a kind word, a helping hand, a moment of encouragement nothing was too small to matter. You all mattered, enormously.

ABSTRAK

Kanser ovari epitelial (EOC) mempunyai kadar kematian yang tinggi, sebahagian besarnya disebabkan oleh pengesanan lewat serta keterbatasan kaedah diagnostik semasa dalam membezakan jisim ovari malignan dan benigna. Kajian ini bertujuan untuk menilai prestasi diagnostik pelbagai biomarker serum dalam membezakan kes malignan daripada benigna, mengenal pasti biomarker utama berprestasi tinggi untuk diintegrasikan ke dalam model multi-biomarker, serta menilai kebolehlaksanaan biosensor K-SPR tanpa label bagi pengesanan biomarker secara pantas dan kos efektif di tempat rawatan. Sebanyak 34 sampel serum (benigna n = 18, malignan n = 16) telah dianalisis menggunakan immunoesei multipleks untuk mengukur kepekatan sepuluh biomarker (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, dan MIF), dan analisis lengkung ciri operasi penerima (ROC) dilakukan untuk menilai prestasi diagnostik serta membangunkan panel multi-biomarker. Seterusnya, subset sampel (n = 8) dianalisis menggunakan biosensor resonans plasmon permukaan berasaskan konfigurasi Kretschmann tanpa label (K-SPR) bagi menilai prestasi pengesanan linear biomarker terpilih. Keputusan menunjukkan bahawa CA125 (peningkatan ~7.8 kali ganda) dan HE4 muncul sebagai biomarker paling dominan. HE4 menunjukkan kekhususan 100% tetapi sensitiviti sederhana (60%) (AUC = 0.951). Bagi CA125, penggunaan nilai ambang 60 U/mL meningkatkan sensitiviti (93.75%) dan ketepatan (97.00%) berbanding ambang konvensional 35 U/mL (sensitiviti 68.18%, ketepatan 79.00%). OPN dan biomarker keradangan (IL-6, MIF, TNFR2) menunjukkan prestasi sederhana (AUC ≥ 0.70), menyokong peranan pelengkap, manakala CCL11, PRL, VCAM1 dan IL-2Rα menunjukkan prestasi diagnostik yang lemah. Panel CA125 + HE4 + IL-6 menunjukkan prestasi yang kukuh dan stabil (AUC = 0.979), manakala panel CA125 + HE4 + IL-6 + MIF mencapai klasifikasi sempurna (AUC = 1.000) tetapi menunjukkan kestabilan yang lebih rendah semasa pengesahan, mencadangkan kemungkinan overfitting. Dalam sampel serum K-SPR, CA125 membezakan sampel benigna (<60 U/mL) daripada malignan (96.1–716.36 U/mL) dengan lineariti yang tinggi (R^2 > 0.94). HE4 pula menunjukkan diskriminasi yang jelas antara sampel benigna (<46 pM) dan malignan (>61.55 pM) pada ambang 60 pM, serta menunjukkan lineariti yang kukuh (R^2 ≈ 0.95). Kesimpulannya, panel CA125 + HE4 + IL-6 menunjukkan kebolehpercayaan tertinggi dalam membezakan kanser ovari, manakala biosensor K-SPR bersepadu menunjukkan potensi yang tinggi sebagai alat diagnostik. Secara keseluruhan, gabungan pelbagai biomarker dengan teknologi biosensor ini berpotensi meningkatkan pengesanan EOC dengan kaedah yang lebih pantas, kos efektif dan mudah berbanding kaedah semasa. Walau bagaimanapun, pengesahan lanjut dalam kohort pesakit yang lebih besar diperlukan sebelum ia dapat digunakan secara meluas dalam amalan klinikal.

ABSTRACT

Epithelial ovarian cancer (EOC) has a high mortality rate, largely attributable to late detection and limitations in current diagnostic methods for distinguishing between malignant and benign ovarian masses. This study aimed to evaluate the diagnostic performance of multiple serum biomarkers in differentiating malignant from benign ovarian masses, to identify high-performing anchor biomarkers for integration into a multi-biomarker model, and to assess the feasibility of a label-free K-SPR biosensor for rapid and cost-effective point-of-care biomarker detection. A total of 34 serum samples (benign n = 18, malignant n = 16) were analysed using multiplex immunoassay to measure ten biomarkers (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF), and receiver operating characteristic (ROC) analysis was performed to evaluate their diagnostic performance and develop multi-biomarker panels. Subsequently, a subset of samples (n = 8) was analysed using a label-free Kretschmann-based surface plasmon resonance (K-SPR) biosensor to assess the linear detection performance of selected biomarkers. The results showed that CA125 (~7.8-fold increase) and HE4 emerged as the strongest biomarkers. HE4 demonstrated 100% specificity but moderate sensitivity (60%) (AUC = 0.951). For CA125, the 60 U/mL cut-off improved sensitivity (93.75%) and accuracy (97.00%) compared to the conventional 35 U/mL threshold (68.18% sensitivity, 79.00% accuracy). OPN and inflammatory markers (IL-6, MIF, TNFR2) showed moderate performance (AUC ≥ 0.70), supporting a complementary role, while CCL11, PRL, VCAM1, and IL-2Rα demonstrated poor diagnostic performance. The CA125 + HE4 + IL-6 panel demonstrated strong and stable performance (AUC = 0.979), while the CA125 + HE4 + IL-6 + MIF panel achieved perfect classification (AUC = 1.000) but exhibited lower stability upon validation, indicating possible overfitting. In K-SPR serum samples, CA125 differentiated benign (<60 U/mL) from malignant (96.1–716.36 U/mL) samples with strong linearity (R^2>0.94). HE4 showed clear discrimination between benign (<46 pM) and malignant (>61.55 pM) samples at a 60 pM threshold, also exhibiting robust linearity ($R^2 \approx 0.95$). In conclusion, the CA125 + HE4 + IL-6 panel demonstrated the highest reliability for distinguishing ovarian cancer, while the integrated K-SPR biosensor showed strong potential as a diagnostic tool. Overall, combining multiple biomarkers with this biosensor technology may improve EOC detection, offering a faster, more affordable, and simpler alternative to current methods. However, further validation in larger patient cohorts is required before it can be widely implemented in clinical practice.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

11-MUA	11-mercaptoundecanoic acid
3 HEMA-MATrp	3-(Hydroxyethyl methacrylate)-N-methacryloyl-L-tryptophan
5-PL	Five-Parameter Logistic
670 & 785 nm	Dual-wavelength
AAb	AutoAb
Ab	Antibodies
AC	Adenocarcinoma
ACTH	Adrenocorticotropic Hormone
Ag	Ag
Ag	Antigens
Ag	Silver
Ag–Ab	Antigen-Antibody
Anti-HE4	Anti-HE4 antibody
Anti-MUC16	Anti-Mucin 16 antibody
Au	Gold
AuAg	Gold-Silver
AUC	Area Under Curve
BMI	Body Mass Index
BRCA	Breast Cancer
BSA	Bovine Serum Albumin
BT	Brenner Tumour
C-6	Mucin 16 (MUC16/CA125), a cell surface–associated glycoprotein (C-6)
CA125	Cancer Antigen 125
CA19.9	Carbohydrate Ag 19.9
CCL11	C-C Motif Chemokine Ligand-11/Eotaxin-1
CEA	Carcinoembryonic Ag

cfDNA	cell-free DNA
CLIA	Chemiluminescence Immunoassay
Clone 3F9	Anti-HE4 monoclonal antibody (clone 3F9)
CT	Computed Tomography
CVA	Cerebrovascular Acid
cVCAM1	circulating vascular cell adhesion molecule 1
DIP	*Dana Impak Perdana*
DNA	Deoxyribonucleic Acid
EC	Endometrioid Carcinoma
ECL	Electrochemiluminescence
ECLIA	Electrochemiluminescence Immunoassay
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EGFR	Epidermal Growth Factor Receptor
EIS	Electrochemical Impedance Spectroscopy
ELISA	Enzyme-Linked Immunosorbent Assay
EOC	Epithelial Ovarian Cancer
ERK	Extracellular signal-Regulated Kinase
FDA	U.S. Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
FP	False Positives
G-CSF	Granulocyte Colony-Stimulating Factor
GMR	Giant Magnetoresistive
HA	Hospital Ampang
HCl	Hydrochloride
HCTM	Hospital Canselor Tuanku Muhriz
HE4	Human Epididymis Protein 4
HEK293	Human Embryonic Kidney 293 cells
HGSOC	High-Grade Serous Ovarian Cancer
HIF-1α	Hypoxia-inducible factor-1α

HPE	Histopathological Examination
ID	Identification
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IL-1β	Interleukin-1 beta
IL2Rα	Interleukin-2 Receptor Alpha
IL6	Interleukin-6
IL8	Interleukin-8
IL9	Interleukin 9
IMEN	Institute of Microengineering and Nanoelectronics
IP-10	Induced Protein 10
JAK/STAT	Janus Kinase/Signal Transducer And Activator Of Transcription
JNK/AP-1	Jun N-terminal Kinase/ Activator Protein-1.
K-SPR	Kretschmann-based Surface Plasmon Resonance
LGSC	Low-Grade Serous Carcinoma
LOD	limit of Detection
LSPR	Localized SPR
Lym	Lymphocytes
mAb	monoclonal Ab
MAPK	Mitogen-Activated Protein Kinase
MC	Mucinous Cystadenoma
MCA	Mucinous Adenocarcinoma
MCC	Mucinous Carcinoma
MedCalc	MedCalc Statistical Software
MFI	Median Fluorescence Intensity
MHC	Major Histocompatibility Complex
MIF	Migration Inhibitory Factor
MIP	Molecularly Imprinted Polymer

MIP-1β	Macrophage Inflammatory Protein
Mixed CCC	Mixed Clear Cell
MLR	multiple Logistic Regression
MMP	Matrix Metalloproteinases
MNCR	Malaysian National Cancer Registry
MREC	Medical Research and Ethics Committee
MRI	Magnetic Resonance Imaging
MUC16	Mucin-16
n	Sample size
N/A	Not Available
NEOCs	Non-Epithelial Ovarian Cancers
NEU	Neutrophil
NF-κB	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NHS	N-hydroxysuccinimide
NP	Nanoparticles
NPV	Negative Predictive Value
NS	Nanoscale
ns	non-significant
NSAIDS	Non-Steroidal Anti-Inflammatory Drugs
O&G	Obstetrics and Gynaecology
OC	Ovarian cancer
OCC	Ovarian Clear Cell Carcinoma
OPN	Osteopontin
pAb	Polyclonal Ab
PBS	Phosphate Buffered Saline
PCA	Principal Component Analysis
PD-L1	Programmed Death Ligand 1
PE	Phycoerythrin

PI3K/AKT	Phosphatidylinositol 3-Kinase/Protein Kinase B
PLT	Platelet:
POC	Point-Of-Care
PPV	Positive Predictive Value
PRL	Prolactin
PRLR	Prolactin Receptor
Py	Pyrrole
QCM	Quartz Crystal Microbalance
R2	R2 r squared
R2	R2 r squared
RI	Refractive Index
RMI	Risk of Malignancy Index
RNA	RNA Ribonucleic Acid
RNA	RNA Ribonucleic Acid
ROC	ROC receiver Operating Characteristic
ROC-AUC	Receiver Operating Characteristic – Area Under the Curve
ROMA	Risk of Ovarian Malignancy Algorithm
ROS	Reactive Oxygen Species
RT	Room Temperature
SAM	Self-Assembled Monolayer
SAW	Surface Acoustic Wave
SC	Serous Cystadenoma
SD	Standard Deviation
SDS	Sodium Dodecyl Sulfate
SEER	Surveillance, Epidemiology, and End Results
SEM	Scanning Electron Microscope
SERS	Surface-Enhanced Raman Scattering
SHAP	SHapley Additive exPlanations
SimplePlex™	ProteinSimple, Bio-Techne

Sn	Sensitivity
Sp	Specificity
SPE	Screen-Printed Electrode
SPR	Surface Plasmon Resonance
SPRi	Surface Plasmon Resonance imaging
SPs	Surface Plasmons
STAT3	Signal Transducer And Activator Of Transcription 3
SWV	Square Wave Voltammetry
TGF-β1	Transforming Growth Factor Beta-1
TIR	Total Internal Reflection
TME	Tumour Microenvironment
TNFR2	Tumour Necrosis Factor Receptor 2
TNF-α	Tumour Necrosis Factor-alpha
Tregs	Regulatory T cell
TVUS	Transvaginal Ultrasound
Tweak	TNF-like weak inducer of apoptosis
U	Unit
UKM	Universiti Kebangsaan Malaysia
VCAM1	Vascular Cell Adhesion Protein-1
VEGF	Vascular Endothelial Growth Factor
WAP	Whey Acidic Protein
WBC	White Blood Cell
WFDC2	WAP Four-Disulfide Core Domain 2
WVA	Weak Value Amplification
xMAP	Luminex Multi-Analyte Profiling
xPONENT	Luminex xPONENT Software
α4β1(VLA-4)	Very Late Ag-4

LIST OF SYMBOLS

%	Percent
®	Registered Trademark
°C	Degree Celsius
µg	Microgram
µg/mL	Microgram per millilitre
µL	Microlitre
µM	Micromolar
µm	Micrometer
a.u.	Reflectivity
fM	Femtomolar
g	Gram
Kd	Dissociation Constant)
kDa	Kilodaltons
kg	Kilograms
kg/m²	Kilograms per square meter
log_2	Logarithm to the base 2 transformation
m	Metres
M	Molar
min	Minutes
mL	Millilitre
mm	Millimetres
mM	Millimolar
N	Number of samples
ng/mL	Nanogram per millilitre
nm	Nanometer
nM	Nanomolar
pg	Picogram

pg/mL	Picogram per millilitre
pM	Picomolar
r	Pearson correlation coefficient
R&D	Research and Development
R^2	Coefficient of Determination
rpm	Revolutions Per Minute
Sn	Sensitivity
S_{SPR}	Sensitivity in terms of detecting molecular binding
S_{SPR}	Sensitivity in SPR
™	Trademark
U/mL	Unit per millilitre
v/v	Volume per volume
vs	Versus
w/v	Weight per volume
x	Times
Δ	Delta angle
Δθ (°)	Delta angle (in degrees)
Δλmax	Maximum wavelength shift
θ	Incident angle
θ_{SPR}	Resonance angle

CHAPTER I

INTRODUCTION

1.1 OUTLINE OF CHAPTER

This chapter presents an overview of the research background, with a primary focus on epithelial ovarian cancer (EOC). Special emphasis is placed on the detection of multiple biomarkers using advanced biosensing technologies, particularly the Kretschmann-Surface Plasmon Resonance (K-SPR) biosensor. The study explores the role of specific biomarkers in the development of EOC and evaluates their potential use in diagnosis. Although these biomarkers are considered crucial, there is currently limited knowledge regarding their expression patterns in malignant compared to benign conditions. This research aims to bridge that gap. In addition to the background, this chapter outlines the problem statement, research questions, objectives, hypotheses, and the novel contributions of the study. It also highlights the significance of the research. The chapter concludes with a summary emphasizing the study's potential impact and importance.

1.2 RESEARCH BACKGROUND

Ovarian cancer (OC) is an increasingly prevalent gynecological malignancy and ranks as the fifth most commonly diagnosed cancer among women worldwide (Akinyemi et al., 2025). In Malaysia, OC is among the top ten most common cancers affecting women (Hashim et al., 2021; Leong et al., 2022). Globally, an estimated 300,000 new OC cases and 185,000 related deaths were reported in 2020 (Rajapaksha et al., 2024). The Malaysian National Cancer Registry (MNCR) from 2017 to 2021 has documented 2,513 newly diagnosed OC cases, with 54.4% identified at an advanced stage, mainly

due to the absence of noticeable early symptoms (National Cancer Registry, 2024). The estimated lifetime risk of developing OC among Malaysian women is 1.6%.

Among the different histological subtypes, epithelial ovarian cancer (EOC) is the most lethal, accounting for over 95% of ovarian cancer cases worldwide (Atallah et al., 2023). Standard treatment for EOC typically involves a combination of surgery, chemotherapy, and targeted therapies; however, clinical outcomes vary considerably due to inter-individual differences in age, tumour grade, and disease stage. A major clinical challenge remains the frequent diagnosis of EOC at advanced stages, largely attributable to vague early symptoms and limitations in early-stage classification. This highlights the urgent need for rapid and accurate diagnostic tools capable of reliably distinguishing malignant from benign ovarian masses (Zhang et al., 2024).

Currently, transvaginal ultrasound (TVUS) is widely used as a first-line diagnostic tool for ovarian cancer, demonstrating moderate to high sensitivity (52–84.9%) and high specificity (84–98.5%). Its diagnostic performance improves substantially when combined with serum CA-125, achieving reported sensitivity and specificity of 89.4% and 99.8%, respectively (Solis Cano et al., 2021; Robertson, 2009). Advanced imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI) further aid tumour characterisation and disease staging; however, CT shows limited sensitivity for detecting lymph node involvement and residual disease. Although MRI offers high diagnostic accuracy (sensitivity up to 98%, specificity 83%), its widespread use is constrained by cost and accessibility (Forstner, 2020; El-Ameen et al., 2020). In addition, FDG-PET/CT enhances the detection of disease progression and recurrence but exhibits variable accuracy for lymph node and peritoneal involvement and reduced sensitivity in early-stage disease, underscoring the need for complementary diagnostic approaches (Rusu et al., 2021; Alenazi et al., 2022).

In parallel with imaging, serum biomarker detection remains central to ovarian cancer diagnostics. Traditional antibody-based immunoassays, including enzyme-linked immunosorbent assay (ELISA), electrochemiluminescence immunoassay (ECLIA), and magnetic-bead assays, are widely used for the measurement of Cancer

Antigen 125 (CA125) and human epididymis protein 4 (HE4). Both biomarkers are FDA cleared for clinical risk assessment and management of ovarian cancer, but are not intended for use as sole diagnostic markers. (Boylan et al., 2017; Woo et al., 2020; Premera et al., 2023). However, the single-plex nature of these assays necessitates separate testing for each analyte, thereby limiting throughput and efficiency (Zhang et al., 2019).

The use of CA125 and HE4 as core biomarkers ensures clinical relevance, regulatory alignment, and translational feasibility, enabling the diagnostic performance of additional biomarkers to be benchmarked against established standards of care. In contrast, reliance solely on less-studied biomarkers would limit clinical interpretability, as such markers often lack standardized cut-off values, regulatory acceptance, and defined clinical workflows. To address these limitations, multivariate index assays integrating CA125, HE4, and additional clinical parameters have been developed, including the Risk of Ovarian Malignancy Algorithm (ROMA), Risk of Malignancy Index (RMI), OVA1, and Overa, all of which have received FDA approval for clinical triage and referral of women with adnexal masses (Jacobs et al., 1990; Moore et al., 2010; Wang et al., 2014; Premera et al., 2023).

Pooled analyses report that ROMA achieves sensitivities ranging from>85–97% with specificities of>80–91%, while RMI demonstrates sensitivities exceeding 87% and specificities above 80%, outperforming CA125 alone (Jacobs et al., 1990; Moore et al., 2010; Wang et al., 2014). In addition, the triple-screen assay combining CA125, HE4, and a symptom index achieved a sensitivity of 79%, specificity of 91%, positive predictive value (PPV) of 83%, and negative predictive value (NPV) of 89% (Goff et al., 2011).

Despite these advances, multivariate index assays are not intended as definitive diagnostic tools but primarily function as triage or referral tests. Their clinical utility is constrained by tumour heterogeneity, insufficient biomarker specificity, and biological overlap with benign conditions, which limit their suitability for population-based screening and reliable early-stage diagnosis (Zhang et al., 2019; Menon et al., 2021). These limitations have driven increasing interest in expanding

biomarker panels beyond tumour-associated antigens to better capture the biological complexity of EOC.

Although CA125 and HE4 can be detected in serum during the early stages of ovarian cancer and may show elevation prior to clinical diagnosis in some patients, their sensitivity for early-stage disease remains limited when used as single markers. This limitation arises from tumour heterogeneity and overlaps with benign gynaecological and inflammatory conditions, whereby CA125 may remain within normal ranges in a substantial proportion of early-stage EOC cases, and HE4, despite higher specificity, also fails to reliably detect all early tumours. Consequently, combining CA125 and HE4 with additional biomarkers reflecting immune modulation, inflammation, and tumour microenvironment (TME) activity has emerged as a promising strategy to improve diagnostic accuracy.

Emerging biomarkers, including inflammatory cytokines, chemokines, adhesion molecules, and hormonal regulators, have demonstrated significantly improved diagnostic performance when analysed in combination with established tumour markers such as CA125 and HE4. Accumulating evidence indicates that multi-biomarker panels integrating tumour burden, immune response, and TME–related markers consistently outperform single-marker approaches for EOC detection. For example, a microsphere-based multiplex panel comprising CA125, chemokine CCL11, interleukin-2 receptor alpha (IL2Rα), vascular cell adhesion protein-1 (VCAM1), and migration inhibitory factor (MIF) achieved 98.2% sensitivity and 98.7% specificity for early-stage disease discrimination (Muinao et al., 2019).

Similarly, panels integrating CA125, HE4, CEA, and VCAM1 reported sensitivities of 86% with specificities of 98% (Yurkovetsky et al., 2010), while CA125, HE4, and CEA achieved an AUC of 0.972 for malignant versus benign classification (Chen et al., 2018). Inflammatory-driven combinations have shown particularly strong performance, with IL6-based panels incorporating CA125 and HE4 yielding AUC values exceeding 0.9 (Kampan et al., 2020), and the dual-marker combination tumour necrosis factor receptor-2 (TNFR2) and interleukin-6 (IL6) demonstrating perfect classification (AUC = 1.000) between malignant and non-

malignant cases (Kampan et al., 2023). Additional pathway-diverse panels combining CA125, HE4, osteopontin (OPN), prolactin (PRL), and MIF reported AUC values up to 0.96, further supporting the benefit of integrating tumour antigens with immune, adhesion, and signalling biomarkers (Walker et al., 2021; Watrowski et al., 2022).

Based on this evidence, a ten-biomarker panel comprising CA125, HE4, IL6, IL2Rα, TNFR2, CCL11, MIF, VCAM1, PRL, and OPN was selected based on established diagnostic relevance in EOC, representation of complementary biological pathways, and proven performance in prior multiplex immunoassay studies. Rather than introducing new biomarkers, the panel integrates tumour markers, inflammatory and immunosuppressive mediators, adhesion/angiogenesis factors, and hormonal signaling proteins to capture the biological heterogeneity of EOC (Al-Faze et al. 2024; Hanzek et al. 2023; Kumar & Ward. 2014; Kampan et al. 2023; Varricchi et al. 2018; Wang & Huang 2020; Ross & Cantrell, 2018; Song et al., 2023; Clendenen et al. 2013; Guo et al. 2019; Mitchell et al. 2002). To support early detection and interpretation, these biomarkers are classified into functional categories that reflect their roles in tumorigenesis, immune modulation, and cellular signalling (Figure 1.1), providing a structured framework for multi-biomarker analysis.

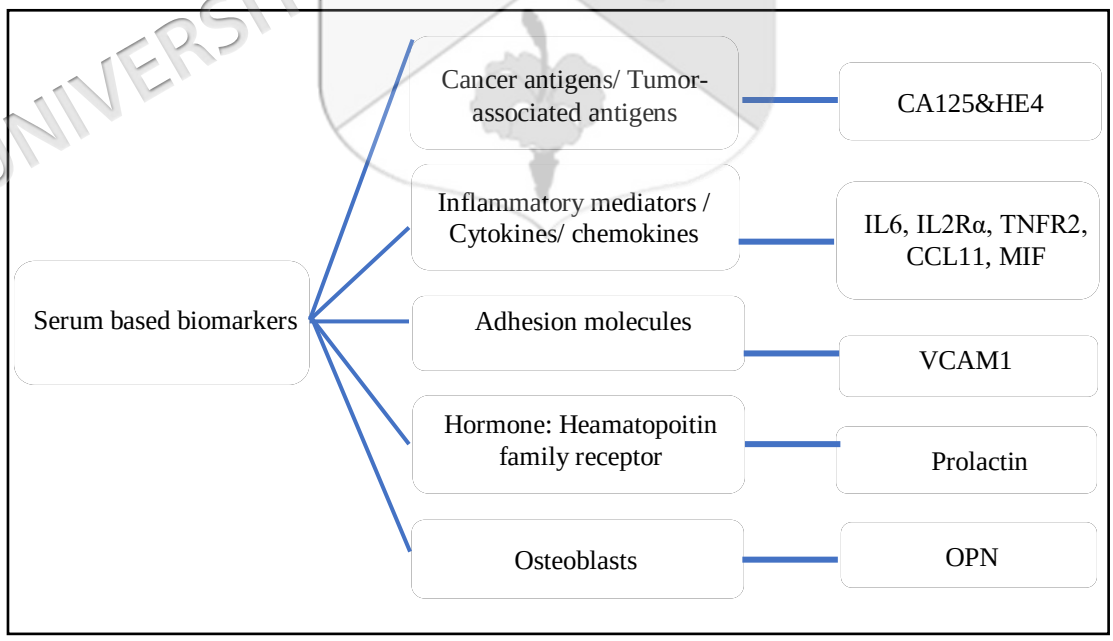

Figure 1.1 List of selected soluble protein biomarkers with their biological group

Advances in immunosensing technologies have enabled the rapid and efficient analysis of multiple protein biomarkers, driving increasing interest in multiplex detection strategies for complex diseases such as EOC. Importantly, the novelty of the present study lies not in the discovery of new biomarkers, but in establishing an integrated diagnostic workflow that combines high-throughput multiplex immunoassay analysis with a targeted biosensor-based platform and evaluates its performance in a Malaysian clinical cohort. By assessing diagnostic accuracy, platform compatibility, and population-specific biomarker behaviour within a single cohort, this work addresses translational feasibility rather than biomarker novelty, aligning with current clinical diagnostic needs. Collectively, existing evidence demonstrates that integrated multi-biomarker panels consistently outperform single-marker approaches in sensitivity and specificity, supporting their potential to enhance diagnostic accuracy and clinical decision-making in EOC.

In parallel with these developments, surface plasmon resonance (SPR) technology has gained attention as a promising biosensing platform due to its label-free detection, real-time monitoring, and high analytical sensitivity. Early SPR research in Malaysia focused on improving sensor sensitivity through material and structural optimization, with Fouad et al. (2016) demonstrating enhanced refractive index sensitivity using gold–dielectric multilayer configurations. Subsequent studies shifted toward chemical, biochemical, and biological sensing, including SPR-based approaches for dengue diagnostics (Zainuddin et al., 2018), reflecting growing biomedical relevance. Doctoral-level work further confirmed the technical maturity of SPR surface engineering in Malaysia, although applications largely remained within material science and environmental or chemical sensing rather than clinical diagnostics (Mustaqim et al., 2022).

Kretschmann-based SPR (K-SPR) platforms have been refined for quantitative biomedical sensing, with Menon et al. (2019a, 2019b) reporting high-sensitivity and reproducible detection of clinically relevant analytes such as urea and glucose using gold and Au–Cr thin films. Building upon this validated approach, the present study employed K-SPR measurements at dual wavelengths of 670 nm and 785 nm, corresponding to the visible red and near-infrared regions where gold thin films

support stable plasmon excitation and sensitive biomolecular detection. Consistent with Menon et al. (2019), linear regression analysis confirmed a proportional relationship between SPR response and biomarker concentration, enabling accurate quantification and reliable differentiation between malignant and benign cases. The selected wavelengths were adopted based on instrumental availability and prior local validation within the established Malaysian K-SPR system, ensuring reliable refractive index sensitivity, methodological consistency, and comparability with previous Malaysian studies. Furthermore, benchmarking K-SPR findings against multiplex immunoassay results obtained from the same clinical cohort strengthened the translational relevance and analytical reliability of the platform.

Collectively, these studies illustrate the progressive evolution of SPR research in Malaysia from sensor design and sensitivity enhancement toward chemical, biochemical, and early biomedical applications. Nevertheless, the majority of Malaysian SPR studies have concentrated on analytical optimisation, material development, or single-analyte detection, with comparatively few investigations evaluating structured clinical diagnostic workflows using patient-derived serum samples.

Beyond the local context, international studies have demonstrated the translational feasibility of integrating biosensor technologies into clinical workflows. Notably, Klein et al. (2018) developed a multiplexed giant magnetoresistive (GMR) biosensor prototype capable of detecting CA125, HE4, and IL6, with the objective of enabling point-of-care (POC) detection of ovarian cancer. Building on this foundation, subsequent investigations between 2020 and 2023 reported multiple-array surface plasmon resonance imaging (SPRi) biosensors capable of detecting CA125, HE4, carcinoembryonic antigen (CEA), IL6, and aromatase in real serum samples from women with ovarian cancer and endometrial cysts. These studies, including that of Szymanska et al. (2023), further support the development of miniaturised, highly sensitive platforms suitable for bedside and POC applications.

Against this background, the present study aims to establish a structured and clinically relevant diagnostic workflow for EOC by integrating serum biomarker

analysis across two complementary analytical platforms. Specifically, the study employs: (i) a high-throughput multiplex immunoassay (Luminex) to evaluate the diagnostic performance of a ten-biomarker(CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF) serum panel; and (ii) a targeted K-SPR biosensing approach was employed to assess the analytical feasibility and discriminatory capability of two clinically established anchor biomarkers, defined as lead biomarkers selected for subsequent biosensor validation based on their strong diagnostic performance.,

Although the multiplex immunoassay quantified all ten biomarkers, the K-SPR evaluation was restricted to two analytes to align with the platform's dual-channel capacity, thereby precluding direct one-to-one cross-validation of the full biomarker panel. This limitation reflects a hardware constraint rather than a methodological shortcoming. Accordingly, the study prioritised the translation of the most diagnostically robust biomarkers to the biosensor platform to preserve analytical reliability and clinical relevance. Future advancements in higher-channel or multiplexed SPR systems may enable comprehensive cross-platform validation of the entire biomarker panel.

This study focused on clinically established biomarkers, namely CA125 and HE4, which have been extensively validated and remain central to ovarian cancer diagnostics. To the best of our knowledge, no prior study has directly compared multiplex immunoassay detection with K-SPR biosensor performance within the same clinical framework and patient cohort. Addressing this gap, the present study evaluated the capability of the K-SPR platform to differentiate between benign and malignant ovarian conditions within a Malaysian cohort. The selection of CA125 and HE4 was based on their strong clinical validation, established diagnostic utility, and suitability for biosensor-based detection.

It is important to acknowledge that the K-SPR analysis was limited to these two biomarkers due to technical constraints, including limited reagent availability and the two-channel configuration of the current system, which restricts simultaneous multi-analyte detection. Therefore, this study should be interpreted as a proof-of-

concept investigation, aimed at evaluating the analytical feasibility and diagnostic potential of K-SPR within a clinically relevant context.

The investigation was intentionally designed as a sequential and integrated diagnostic development framework, rather than as two independent feasibility studies. Both analytical phases were guided by a unified objective: to enhance discrimination between malignant and benign ovarian masses and to translate validated biomarkers toward potential POC application. The use of the same serum samples across both platforms ensured biological consistency, preserved dataset continuity, and maintained a shared clinical context.

The study progresses through interconnected phases within a unified diagnostic pipeline. Phase I employs a multiplex immunoassay to quantify serum biomarkers and evaluate their individual and combined diagnostic performance. Phase II applies multivariable modelling to optimise biomarker panels and identify statistically robust anchor biomarkers based on discriminatory strength and clinical relevance. These validated anchor biomarkers, identified from the same serum cohort sample, are subsequently assessed in Phase III using the K-SPR platform to evaluate analytical performance, concentration-dependent linearity, signal response behaviour, and their ability to discriminate between malignant and benign cases.

Demonstrating linear detection is fundamental to K-SPR performance, as proportional sensor responses to biomarker concentration changes enable accurate quantification within clinically relevant ranges and reliable discrimination between malignant and benign ovarian conditions. This capability is essential for translating biosensor technology into a clinically applicable diagnostic tool.

By benchmarking K-SPR analytical behaviour directly against multiplex immunoassay findings within the same clinical framework, methodological continuity and translational coherence are maintained. The dual-platform approach therefore reflects a deliberate and complementary translational strategy rather than methodological inconsistency. Accordingly, this thesis represents a continuous diagnostic development pipeline from biomarker stratification and panel optimisation

to biosensor-based validation rather than two disconnected feasibility studies. Its primary contribution lies in local clinical validation and population-specific translational feasibility assessment of K-SPR technology.

Overall, this work establishes a coherent and clinically oriented diagnostic framework for EOC that integrates multi-biomarker strategies with biosensor-based evaluation to support rapid, accurate, and translatable detection. By demonstrating analytical feasibility, diagnostic relevance, and workflow compatibility within a Malaysian clinical setting, the study addresses an important translational gap and supports the development of population-validated, POC-adaptable diagnostic approaches, particularly in resource-limited environments.

1.3 STATEMENT OF THE PROBLEM

Accurately differentiating malignant from benign ovarian masses in EOC remains a significant clinical challenge, largely due to the limitations of existing diagnostic methods and reliance on single biomarkers such as CA125. While multi-biomarker combinations have shown potential to enhance diagnostic accuracy, these strategies remain insufficiently validated within Malaysian populations. Although multiplex immunoassays are valuable for research, their high cost and limited feasibility in routine clinical use remain major constraints. Conversely, K-SPR biosensors provide a promising alternative, serving as a rapid and highly analytical sensitive bedside tool for EOC biomarker detection. However, their clinical use remains underexplored locally. As a result, a critical gap exists in developing a cost-effective, clinically relevant, and locally validated diagnostic workflow that integrates multi-biomarker strategies with biosensor-based platforms to support early and accurate EOC detection in the Malaysian healthcare setting.

1.4 RESEARCH OBJECTIVE

To reliably detect potential biomarkers (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF) that can discriminate between women with malignant and benign ovarian masses, the study objectives are outlined as follows:

1. To determine the differences in serum concentrations of ten protein biomarkers between malignant and benign ovarian masses using multiplex immunoassay and to evaluate their individual diagnostic performance (sensitivity, specificity, accuracy, and ROC–AUC).

2. To develop and analyse multi-biomarker panels using multivariable modelling to identify high-performing anchor biomarkers for subsequent evaluation within a biosensor-based detection platform.

3. To evaluate the linear detection performance of the K-SPR platform using selected anchor biomarkers to distinguish malignant from benign ovarian conditions and to assess its point-of-care (POC) potential.

1.5 RESEARCH HYPOTHESIS

We hypothesise that there are present differences in EOC biomarkers (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF) detection in a panel that can be exploited for improved diagnostic discrimination using both multiplex immunoassay and biosensor-based platforms.

1. There are significant differences in serum concentrations of the selected protein biomarkers between malignant and benign ovarian masses, and at least one biomarker demonstrates significant diagnostic discrimination (based on sensitivity, specificity, accuracy, and ROC–AUC).

2. There is significant diagnostic improvement achieved by multi-biomarker panels developed through multivariable modelling compared to individual biomarkers, and at least one biomarker demonstrates sufficient discriminatory strength to be selected as an anchor candidate for subsequent biosensor-based evaluation.

3. The K-SPR biosensor platform exhibits significant linear detection responses for selected anchor biomarkers and generates distinguishable sensor signals between malignant and benign ovarian conditions, supporting its feasibility as a potential point-of-care (POC) diagnostic tool

1.6 RESEARCH QUESTION

1. Are there statistically significant differences in the serum concentrations of ten protein biomarkers (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF) between malignant and benign ovarian masses, and do these biomarkers demonstrate clinically meaningful diagnostic performance as measured by sensitivity, specificity, accuracy, and ROC–AUC?

2. Does the multi-biomarker panels developed through multivariable modelling improve differentiation between malignant and benign ovarian masses, and which biomarkers exhibit adequate diagnostic performance to be selected as anchor candidates for subsequent biosensor-based validation?

3. Does the K-SPR biosensor platform demonstrate reliable linear detection performance for selected anchor biomarkers, enabling discrimination between malignant and benign ovarian conditions and supporting its potential application as a point-of-care (POC) diagnostic tool?

1.7 NOVELTIES

The novelty of this study lies primarily in its local validation and translational evaluation of K-SPR biosensor technology for EOC detection, rather than the development of new biomarkers or novel sensing platforms. This work represents one of the first systematic investigations of a ten-biomarker panel (CA125, HE4, IL-6, TNFR2, CCL11, IL-2Rα, VCAM1, PRL, OPN, and MIF) in Malaysian women with ovarian masses, addressing an important population-specific evidence gap. The study's structured, two-phase workflow integrates multiplex immunoassay-based biomarker profiling with targeted biosensor assessment to establish analytical feasibility, linear detection performance, and clinical relevance. By benchmarking K-SPR performance against established immunoassay results, the study demonstrates the practical potential of SPR-based detection in a real-world clinical context. This approach provides early translational validation, bridging conventional laboratory diagnostics with prospective POC applications, and highlights the utility of locally generated data for guiding the implementation of rapid, label-free biosensor technologies in population-specific settings.

1.8 SIGNIFICANCE OF THE RESEARCH

EOC remains difficult to diagnose early due to its asymptomatic presentation and the limited sensitivity of single biomarkers such as CA125. This study addresses these challenges by evaluating a multiplex serum biomarker panel to enhance discrimination between malignant and benign ovarian masses and by demonstrating the analytical feasibility of K-SPR biosensing for clinically established anchor biomarkers. Benchmarking K-SPR against multiplex immunoassays strengthens its translational relevance as a label-free, cost-effective diagnostic platform. Validation within a Malaysian clinical cohort fills an important population-specific evidence gap and supports the development of accessible, POC adaptable diagnostic strategies that may enable earlier detection, improved clinical decision-making, and reduced healthcare burden from late-stage EOC.

REFERENCES

Abuzinadah, N., Umer, M., Posa, S. K., Alarfaj, A. A., Alabdulqader, E. A., Kim, T.-H., Alsubai, S.,&Ashraf, I. (2023). Improved prediction of ovarian cancer using ensemble classifier and Shapley explainable AI. Diagnostics, 13(21), Article 3387.

Agarwal, A., Pandey, A.,&Prajapati, G. (2021). Demographic profile and outcomes of advanced epithelial ovarian cancer. International Journal of Medical and Biomedical Studies, 5(5), 152–157.

Alenazi, A. M., Alkhalaf, A. K. A., Alkhateeb, S. A. S., Alshammari, B. M., Alharbi, D. A., Alangari, A., Alkhatani, S., Fadan, O. Y., Alanzi, A. M., Alanazi, N. H., Seraj, M. A., Alkhunaizi, F. K., & Albugami, D. M. (2022). Advances in biomarker discovery and radiological techniques for early detection of ovarian cancer: A comprehensive review. International Journal of Health Sciences, 6(S10), 1614–1632.

Al-Faze, R., Ahmed, H. A., El-Atawy, M. A., Zagloul, H., Alshammari, E. M., Jaremko, M., Emwas, A. H., Nabil, G. M.,&Hanna, D. H. (2024). Mitochondrial dysfunction route as a possible biomarker and therapy target for human cancer. Biomedical Journal, 48(1), 12–22.

Andryukov, B. G., Bazhenov, N. N., Romashko, R. V., Zaporozhets, T. S., & Efimov, T. A. (2020). Label-free biosensors for laboratory-based diagnostics of infections: Current achievements and new trends. Biosensors (Basel), 10(2), Article 11.

Araguillín-López, R. D., Méndez-Cevallos, A. D.,&Costa-Vera, C. (2020). Detailed modeling of surface plasmon resonance spectrometer response for accurate correction. Sensors, 20(14), 3905–3917.

Arora, R., Yadav, P.,&Sharma, S. (2024). Ovarian physiology and hormonal regulation in female reproductive health. Journal of Ovarian Research, 17(1), 45. https://doi.org/10.1186/s13048-024-01234-5

Atallah, G. A. K., Teik, C. K., Mokhtar, M. N., Zin, R. R. M., Shafiee, M. N.,&Aziz, N. A. (2023). Predicting prognosis and platinum resistance in ovarian cancer: Role of immunohistochemistry biomarkers. International Journal of Molecular Sciences, 24(3), 2156–2173.

Azizah, A. M., Nor Saleha, I. T., Noor Hashimah, A., Asmah, Z. A., & Mastulu, W. (2016). Malaysian National Cancer Registry Report 2007–2011: Malaysia Cancer Statistics, Data and Figure. National Cancer Institute, Ministry of Health Malaysia.

Barr, C. E., Njoku, K., Owens, G. L.,&Crosbie, E. J. (2022). Urine CA125 and HE4 for the detection of ovarian cancer in symptomatic women. Diagnostics, 12(2), Article 367.

Bartnik, K., Janik, M., Koba, M., Rygiel, T., Musolf, P., & Śmietana, M. J. (2025). Biosensing solutions for protein measurement in blood-derived media: A review. Measurement, 253, 117756.

Baugh, J. A.,&Donnelly, S. C. (2003). Macrophage migration inhibitory factor: A neuroendocrine modulator of chronic inflammation. Endocrinology, 179(1), 15–23.

Bee, C., Abdiche, Y. N., Stone, D. M., Collier, S., Lindquist, K. C., Pinkerton, A. C., Pons, J., & Rajpal, A. (2012). Exploring the dynamic range of the kinetic exclusion assay in characterizing antigen–antibody interactions. PLoS ONE, 7(4), e36261. https://doi.org/10.1371/journal.pone.0036261

Bellassai, N., D'Agata, R., Jungbluth, V., & Spoto, G. (2019). Surface plasmon resonance for biomarker detection: Advances in non-invasive cancer diagnosis. Frontiers in Chemistry, 7, 570.

Boylan, K. L. M., Geller, M. A., Koopmeiners, J. S., Starr, T. K., Skubitz, A. P. N.,&Griffin, T. J. (2017). A multiplex platform for the identification of ovarian cancer biomarkers. Clinical Proteomics, 14(1), 34–46.

Bozkurt, M., Yumru, A. E., & Aral, İ. (2013). Evaluation of the importance of the serum levels of CA-125, CA15-3, CA-19-9, carcinoembryonic antigen and alpha fetoprotein for distinguishing benign and malignant adnexal masses and contribution of different test combinations to diagnostic accuracy. European Gynaecological Oncology, 34(6), 540–544.

Brusko, T. M., Wasserfall, C. H., Hulme, M. A., Cabrera, R., Schatz, D., & Atkinson, M. A. (2009). Influence of membrane CD25 stability on T lymphocyte activity: Implications for immunoregulation. PLoS One, 4(11), e7980.

Cao, J., Sun, Y., Kong, Y., & Qian, W. (2019). The sensitivity of grating-based SPR sensors with wavelength interrogation. Sensors (Basel), 19(2), 405.

Case, K., Tran, L., Yang, M., Zheng, H., Kuhtreiber, W. M., & Faustman, D. L. (2020). TNFR2 blockade alone or in combination with PD-1 blockade shows therapeutic efficacy in murine cancer models. Journal of Leukocyte Biology, 107(6), 981–991.

Charkhchi, P., Cybulski, C., Gronwald, J., Wong, F. O., Narod, S. A.,&Akbari, M. R. (2020). CA125 and ovarian cancer: A comprehensive review. Cancers (Basel), 12(12), 3730.

Chen, F., Shen, J., Wang, J., Cai, P.,&Huang, Y. (2018). Clinical analysis of four serum tumor markers in 458 patients with ovarian tumors: Diagnostic value of the combined use of HE4, CA125, CA19-9, and CEA in ovarian tumors. Cancer Management and Research, 10, 1313–1318.

Clendenen, T. V., Arslan, A. A., Lokshin, A. E., Liu, M., Lundin, E., Koenig, K. L., Berrino, F., Hallmans, G., Idahl, A., Krogh, V., Lukanova, A., Marrangoni, A., Muti, P., Nolen, B. M., Ohlson, N., Shore, R. E., Sieri, S., & Zeleniuch-

Jacquotte, A. (2013). Circulating prolactin levels and risk of epithelial ovarian cancer. Cancer Causes&Control, 24(4), 741–748.

Cohen, L., & Walt, D. R. (2019). Highly sensitive and multiplexed protein measurements using digital immunoassays. Annual Review of Analytical Chemistry, 12, 345–363.

Colombo, N., Sessa, C., du Bois, A., Ledermann, J., McCluggage, W. G., McNeish, I., Morice, P., Pignata, S., Ray-Coquard, I., Vergote, I., Baert, T., Belaroussi, I., Dashora, A., Olbrecht, S., Planchamp, F., & Querleu, D., on behalf of the ESMO–ESGO Ovarian Cancer Consensus Conference Working Group. (2019). ESMO–ESGO consensus conference recommendations on ovarian cancer: Pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. Annals of Oncology, 30(5), 672–705.

Cybula, M., & Biecek, M. (2022). Patient-derived tumor models are attractive tools to repurpose drugs for ovarian cancer treatment: Pre-clinical updates. Oncology, 12, 100324.

Damborsky, P., Svitel, J., & Katrlik, J. (2016). Optical biosensors. Essays Biochemistry, 60(1), 91–100.

Das, S.,&others. (2023). Surface plasmon resonance (SPR) sensor for cancer biomarker detection. Biosensors and Bioelectronics, 222, 115021.

Davar, R., & Yazdizadeh, M. (2022). Identification of a panel of biomarkers for the early detection of ovarian cancer. Ovarian Research, 15(1), 56.

Dochez, V., Caillon, H., Vaucel, E., Dimet, J., Winer, N., & Ducarme, G. (2019). Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. Ovarian Research, 12(1), 28.

Duan, D., Zhang, J., Sun, Y., et al. (2014). Plasma adhesion molecules and ovarian cancer risk and progression. International Journal of Cancer, 135(4), 921–929.

Duan, R.,&Xi, M. (2020). A novel label-free biosensor for detection of HE4 in urine based on localized surface plasmon resonance and protein G directional fixed. Nanomaterials, 2020, 8613240.

Eddin, F.,&Kang, F. (2020). The principle of nanomaterials-based surface plasmon resonance biosensors and its potential for dopamine detection. Molecules, 25(12), 2878.

El-Ameen, N. F., Eissawy, M. G., Mohsen, L. A. M. S., Nada, O. M., & Beshreda, G. M. (2020). MR diffusion versus MR perfusion in patients with ovarian tumors; how far could we get? Egyptian Journal of Radiology and Nuclear Medicine, 51, 35

Fan, L., Luo, G., Yi, T., Wang, Q., Wang, D., Zhang, G., Jiang, X., & Guo, X. (2017). Diagnostic value of urinary-to-serum human epididymis protein 4 ratio in ovarian cancer. Biomarker Research, 5, 13.

Ferraro, S., Braga, F., Lanzoni, M., Boracchi, P., Biganzoli, E. M., & Panteghini, M. (2013). Serum human epididymis protein 4 vs CA125 for ovarian cancer diagnosis: A systematic review. Journal of Clinical Pathology, 66(4), 273–281.

Florczak, A., Królikowska, A., Mazurek, M., Woźniak, S., & Domagała, Z. (2025). Most relevant genetic factors in ovarian cancer development. AIMS Molecular Science, 12(1), 1–26.

Forstner, R. (2020). Early detection of ovarian cancer. European Radiology, 30, 5370–5373.

Fouad, H., Zainuddin, A. A., Zainal Abidin, Z. H.,&Zainal, N. (2016). Enhancement of surface plasmon resonance sensor sensitivity using gold–dielectric multilayer structures. Optics Communications, 370, 17–21.

Funston, G., Hamilton, W., Abel, G., Crosbie, E. J., Rous, B., & Walter, F. M. (2020). The diagnostic performance of CA125 for the detection of ovarian and non-ovarian cancer in primary care: A population-based cohort study. PLoS Medicine, 17(10), e1003295.

Gasiorowska, E., Kluz, T., Lipski, D., Warchoł, W., Tykarski, A.,&Nowak-Markwitz, E. (2019). Human epididymis protein 4 (HE4) reference limits in Polish population of healthy women, pregnant women, and women with benign ovarian tumors. Disease Markers, 2019, 3890906.

Gaudreault, J., Nguyen, C. F., Crescenzo, G. D., Durocher, Y.,&Henry, O. (2021). On the use of surface plasmon resonance-based biosensors for advanced bioprocess monitoring. Processes, 9(11), 1932–1945.

Ghaffari, S.,&Rezaei, N. (2023). Eosinophils in the tumor microenvironment: Implications for cancer immunotherapy. Translational Medicine, 21(1), 551–562.

Giampaolino, P., Foreste, V., Della Corte, L., Di Filippo, C., Iorio, G., & Bifulco, G. (2020). Role of biomarkers for early detection of ovarian cancer recurrence. Gland Surgery, 9(3), 1032–1043.

Goff, B. A., Mandel, L., Drescher, C. W., Urban, N., Gough, S., Schurman, K. M., Patras, J., Mahony, B. S., Andersen, M. R., & Liao, S. Y. (2011). Development of an ovarian cancer symptom index: Possibilities for earlier detection. Cancer, 117(4), 739–746.

Guo, J., Yang, W. L., Pak, D., Celestino, J., Lu, K. H., & Ning, J. (2019). Osteopontin, macrophage migration inhibitory factor and anti-interleukin-8 autoantibodies complement CA125 for detection of early stage ovarian cancer. Cancers (Basel), 11(5), 596–608.

Gür, S. D., Bakhshpour, M., & Denizli, A. (2022). Nanoscale SPR sensor for the ultrasensitive detection of the ovarian cancer marker carbohydrate antigen 125. New Journal of Chemistry, 46(15), 7263–7270.

Habel, A., Weili, X., Hadj Ahmed, M., Stayoussef, M., Bouaziz, H., Ayadi, M., Mezlini, A., Larbi, A., & Yaacoubi-Loueslati, B. (2023). Immune checkpoints as potential theragnostic biomarkers for epithelial ovarian cancer. International Journal of Biological Markers, 38(3–4), 203–213.

Hanczar, B., Hua, J., Sima, C., Weinstein, J., Bittner, M., Dougherty, E. R. (2010). Small-sample precision of ROC-based diagnostic tests. BMC Bioinformatics, 11, 286.

Hanžek, A., Ducongé, F., Siatka, C.,&Duc, A.-C. E. (2023). Identification and characterization of aptamers targeting ovarian cancer biomarker human epididymis protein 4 for the application in urine. Cancers (Basel), 15(2), 452–463.

Hasenburg, A., Du, E., Vosshagen, F., Obermayr, E., Geroldinger, A., Zeillinger, R., et al. (2021). Biomarker-based early detection of epithelial ovarian cancer based on a five-protein signature in women's plasma: A prospective trial. BMC Cancer, 21(1), 1–8.

Hashim, N., Shet, D., & Mohd Zain, N. (2021). Women's knowledge of ovarian cancer and its determinant factors. Quantum Journal of Medical and Health Sciences, 1(5), 37–47.

Hong, R., Li, S., Li, D., Yang, W., Fan, K., Liu, C., et al. (2022). A review of biosensors for detecting tumor markers in breast cancer. Life (Basel), 12(3), 412–428.

Huang, B., Yu, F., & Zare, R. N. (2005). Surface plasmon resonance: A tool for studying protein–protein interactions in complex media. Analytical Chemistry, 77(21), 6990–6996.

Huang, W., Suominen, H., Liu, T., Rice, G., Salomon, C.,&Barnard, A. S. (2023). Explainable discovery of disease biomarkers: The case of ovarian cancer to illustrate the best practice in machine learning and Shapley analysis. Biomedical Informatics, 141, 104365.

Huo, Q., Xu, C., Shao, Y., Yu, Q., Huang, L., Liu, Y.,&Bao, H. (2021). Free CA125 promotes ovarian cancer cell migration and tumor metastasis by binding Mesothelin to reduce DKK1 expression and activate the SGK3/FOXO3 pathway. International Journal of Biological Sciences, 17(2), 574–588.

Jacobs, I., Oram, D., Fairbanks, J., Turner, J., Frost, C., & Grudzinskas, J. G. (1990). A risk of malignancy index incorporating CA-125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. British Journal of Obstetrics and Gynaecology, 97(10), 922–929

Jeon, H. B., et al. (2025). Enhancing ELISA sensitivity: From surface engineering to synthetic biology. Analytical Biochemistry, 659, 115092.

Kamal, R., Hamed, S., Mansour, S., Mounir, Y., & Abdel Sallam, S. (2018). Ovarian cancer screening—Ultrasound: Impact on ovarian cancer mortality. British Journal of Radiology, 91(1089), 20170571.

Kampan, N. C., Kartikasari, A. E. R., Deceneux, C., Madondo, M. T., McNally, O. M., Flanagan, K. L., Aziz, N. A., Stephens, A. N., Reynolds, J., Quinn, M. A., & Plebanski, M. (2023). Combining TNFR2-expressing Tregs and IL-6 as superior diagnostic biomarkers for high-grade serous ovarian cancer masses. Cancers (Basel), 15(3), 612–625.

Kampan, N. C., Madondo, M. T.,&Reynolds, J. (2020). Pre-operative sera interleukin-6 in the diagnosis of high-grade serous ovarian cancer. Scientific Reports, 10(1), 2213–2221.

Kampan, N. C., Madondo, M. T.,&Reynolds, J. (2020). Pre-operative sera interleukin-6 in the diagnosis of high-grade serous ovarian cancer. Scientific Reports, 10(1), 2213.

Kang, K. N., Eun, Y., et al. (2022). Multiple biomarkers are more accurate than a combination of carbohydrate antigen 125 and human epididymis protein 4 for ovarian cancer screening. Ovarian Research, 15(1), 24–32.

Kausaite-Minkstimiene, A., et al. (2022). Surface plasmon resonance immunosensor with antibody-functionalized magnetoplasmonic nanoparticles for ultrasensitive quantification of the CD5 biomarker. Biosensors and Bioelectronics, 200(1), 113902.

Khan, T. M., Tahir, H., Adil, Q., Baig, M. R., Jaber, A. A. S., Khaliel, A. M.,&Mohammed, Z. M. (2024). Female-specific cancers in Malaysia: A comprehensive analysis of three decades. Archives of Pharmacy Practice, 15(1), 109–120.

Kivrak, E., & Kara, P. (2025). Simultaneous detection of ovarian cancer related miRNA biomarkers with carboxylated graphene oxide modified electrochemical biosensor platform. Bioelectrochemistry, 161, 108806.

Klein, D. M., et al. (2018). Development of a multiplexed giant magnetoresistive biosensor array prototype to quantify ovarian cancer biomarkers. Biosensors and Bioelectronics, 117, 659–666.

Koleva, V., Shefket, S., & Stoencheva, S. (2025). Clinical significance of human epididymis protein 4 (HE4), cancer antigen 125 (CA125), the risk of ovarian malignancy algorithm (ROMA), and Copenhagen index (CPH-I) for the diagnosis of endometrial carcinoma. Folia Medica, 67(1), e143849.

Kumar, J., & Ward, A. C. (2014). Role of the interleukin 6 receptor family in epithelial ovarian cancer and its clinical implications. Biochimica et Biophysica Acta (BBA) – Reviews on Cancer, 1845(2), 117–125.

Kumar, V. R., Kampan, N. C., Abd Aziz, N. H., Teik, C. K., Shafiee, M. N.,&Menon, P. S. (2023). Recent advances in surface plasmon resonance (SPR) technology for detecting ovarian cancer biomarkers. Cancers (Basel), 15(23), 5820–5835.

Kumari, S. (2018). Serum biomarker-based algorithms in diagnosis of ovarian cancer: A review. Gynecologic Oncology, 29(4), e56.

Leandersson, P., Kalapotharakos, G., Henic, E., Borgfeldt, H., Petzold, M., Høyer-Hansen, G.,&Borgfeldt, C. (2016). A biomarker panel increases the diagnostic performance for epithelial ovarian cancer type I and II in young women. Anticancer Research, 36(3), 957–965.

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

Lee, S. A., & Link, S. (2021). Chemical interface damping of surface plasmon resonances. Accounts of Chemical Research, 54(8), 1950–1960.

Leong, E., et al. (2022). Incidence, mortality and survival analysis of epithelial ovarian cancer in Brunei Darussalam. Asian Pacific Journal of Cancer Prevention, 23(6), 1911–1917.

Levin, G., Brezinov, Y., Tzur, Y., Bar-Noy, T., Nourmoussavi Brodeur, M., Salvador, S., Lau, S., & Gotlieb, W. (2024). Association between BMI and oncologic outcomes in epithelial ovarian cancer: A predictors-matched case-control study. Archives of Gynecology and Obstetrics, 310(1), 587–593.

Levina, V., Nolen, B. M., Marrangoni, A. M., Cheng, P., Marks, J. R., Szczepanski, M. J., Szajnik, M. E., Gorelik, E., & Lokshin, A. E. (2009). Role of eotaxin-1 signaling in ovarian cancer. Clinical Cancer Research, 15(8), 2647–2656.

Lheureux, S., Braunstein, M., & Oza, A. M. (2019). Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA: A Cancer Journal for Clinicians, 69(4), 280–304.

Li, H., Wu, M., Wu, Z., Liang, J., Wang, L., Yang, X., Lin, Z.,&Li, J. (2022). Prognostic value of preoperative soluble interleukin 2 receptor alpha as a novel immune biomarker in epithelial ovarian cancer. Cancer Immunology, Immunotherapy, 71(6), 1519–1530.

Li, L., et al. (2024). Targeting TNFR2 for cancer immunotherapy: Recent advances and future directions. Cancer Letters, 589(1), 216684.

Lycke, M., Kristjansdottir, B., & Sundfeldt, K. (2020). Clinical implementation of novel diagnostic biomarkers for epithelial ovarian cancer – Can we improve diagnosis? Journal of Ovarian Research, 13, 16.

Mariani, S., & Minunni, M. (2014). Surface plasmon resonance applications in clinical analysis. Analytical and Bioanalytical Chemistry, 406(9–10), 2303–2323.

Masson, J. F. (2017). Surface plasmon resonance clinical biosensors for medical diagnostics. ACS Sensors, 2(1), 16–30.

Matsas, A., Troupis, T., Kontzoglou, K., Eleftheriades, M., Christopoulos, P., et al. (2023). Tumor markers and their diagnostic significance in ovarian cancer. Life, 13(8), 1669.

Medler, J., et al. (2022). Tumor necrosis factor receptor 2 (TNFR2): An emerging target in cancer therapy. Oncology, 12(8), 876543–876552.

Menon, P. S. M. (2020). Multilayer CVD-graphene and MoS_2 ethanol sensing and characterization using Kretschmann-based SPR. Sensors, 2020(2), 122–133.

Menon, P. S. M., Budi, J., Jamil, N. A. W. C., Nugroho, H. S., Gan, S. M., Abidin, N. F. Z., et al. (2019b). Refractive index and sensing of glucose molarities determined using Au-Cr K-SPR at 670/785 nm wavelength. Sains Malaysiana, 48(6), 1259–1265.

Menon, P. S., Said, F. A., Mei, G. S., & Yahaya, M. (2019a). High-sensitivity Au-based Kretschmann surface plasmon resonance sensor for urea detection. Sains Malaysiana, 48(6), 1179–1185.

Menon, U., Karpinskyj, C., & Gentry-Maharaj, A. (2021). Ovarian cancer prevention and screening. Obstetrics and Gynecology, 137(3), 529–539.

Mitchell, R. A., Liao, H., Chesney, J., Fingerle-Rowson, G., Baugh, J., David, J., & Bucala, R. (2002). Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: Regulatory role in the innate immune response. National Academy of Sciences, 99(1), 345–350.

Mokhtar, N. M., et al. (2012). Human epididymis protein 4 reference intervals in a multiethnic Asian women population. Clinical Laboratory Analysis, 26(1), 31–36.

Montagnana, M., Benati, M., & Danese, E. (2017). Circulating biomarkers in epithelial ovarian cancer diagnosis: From present to future perspective. Annals of Translational Medicine, 5(13), 276–284.

Moore, R. G., et al. (2011). Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstetrics&Gynecology, 118(2), 280–288.

Moore, R. G., Miller, M. C., Steinhoff, M. M., Skates, S. J., Lu, K. H., Lambert-Messerlian, G.,&Bast, R. C. (2010). Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. American Journal of Obstetrics and Gynecology, 203(4), 344.e1–344.e8.

Muinao, T., Bhattacharyya, D. R., & Pal, M. (2019). Multi-biomarker panel signature as the key to diagnosis of ovarian cancer. Heliyon, 5(12), e02826.

Mukkamalla, S. K. R., Kankanala, V. L., & Zubair, M. (2024). Carcinoembryonic antigen. StatPearls, 12(4), 155–168.

Mustaqim, M. A. (2022). Development of surface plasmon resonance sensors for chemical and biological detection (Doctoral dissertation). Universiti Kebangsaan Malaysia.

Naresh, V.,&Lee, N. (2021). A review on biosensors and recent development of nanostructured materials-enabled biosensors. Sensors (Basel), 21(4), 1102–1118.*

National Cancer Registry Department, Institut Kanser Negara. (2024). Summary of Malaysia National Cancer Registry Report 2017–2021. Ministry of Health Malaysia. https://nci.moh.gov.my/images/pdf_folder/SUMMARY-OF-MALAYSIA- NATIONAL-CANCER-REGISTRY-REPORT-2017-2021.pdf

National Cancer Registry Department, Ministry of Health Malaysia. (2019). Malaysia National Cancer Registry Report (MNCR) 2012–2016. National Cancer Institute, Ministry of Health Malaysia. https://www.moh.gov.my/moh/resources/Penerbitan/Laporan/Umum/2012-2016%20(MNCRR)/MNCR_2012-2016_FINAL_(PUBLISHED_2019).pdf

Nemati, M., Ebrahimi, A., & Abdollahi, M. (2020). Monoclonal versus polyclonal antibodies: Differences and applications in biomedical research and diagnostics. Journal of Immunological Methods, 478, 112714.

Nogueira-Rodrigues, A., Giannicchini, G. V.,&Alvarez Secord, A. (2024). Real-world challenges and disparities in the systemic treatment of ovarian cancer. Gynecologic Oncology, 185, 180–185.

Nurrohman, D. T., Wang, Y. H., & Chiu, N. F. (2020). Exploring graphene and MoS_2 chips based surface plasmon resonance biosensors for diagnostic applications. Chemistry, 8, 728–742.

Partheen, K., Kristjansdottir, B., & Sundfeldt, K. (2011). Evaluation of ovarian cancer biomarkers HE4 and CA-125 in women presenting with a suspicious cystic ovarian mass. Journal of Gynecologic Oncology, 22(4), 244–252.

Piliarik, M., & Homola, J. (2009). Surface plasmon resonance (SPR) sensors: Approaching their limits? Optics Express, 17(19), 16505–16517.

Premera. (2023). Multimarker serum testing related to ovarian cancer. Available at: https://www.premera.com/medicalpolicies/2.04.62.pdf (Accessed 1 February 2023).

Puiu, M., & Bala, C. (2016). SPR and SPR imaging: Recent trends in developing nanodevices for detection and real-time monitoring of biomolecular events. Sensors (Basel), 16(6), 870–882.

Qian, J., LeSavage, B. L., Hubka, K. M., Ma, C., Natarajan, S., Eggold, J. T., Xiao, Y., Fuh, K. C., Krishnan, V., Enejder, A., Heilshorn, S. C., Dorigo, O., &

Rankin, E. B. (2021). Cancer-associated mesothelial cells promote ovarian cancer chemoresistance through paracrine osteopontin signaling. Clinical Investigation, 131(16), e147852

Rajapaksha, W., Khetan, R., Johnson, I. R. D., Blencowe, A., Garg, S., Albrecht, H., & Gillam, T. A. (2024). Future theranostic strategies: Emerging ovarian cancer biomarkers to bridge the gap between diagnosis and treatment. Frontiers in Drug Delivery, 4, 1339936.

Razi, S., Ghoncheh, M., Mohammadian-Hafshejani, A., Aziznejhad, H., Mohammadian, M., & Salehiniya, H. (2016). The incidence and mortality of ovarian cancer and their relationship with the Human Development Index in Asia. Ecancermedicalscience, 10, 628.

Rebelo, T. S. C. R., Costa-Rama, E., Seguro, I. M. Z., Pacheco, J. G., Nouws, H. P. A., Cordeiro, M. N. D. S., & Delerue-Matos, C. (2019). Molecularly imprinted polymer SPE sensor for analysis of CA-125 on serum. Analytica Chimica Acta, 1082, 126–135.

Robertson, D. M. (2009). Screening for the early detection of ovarian cancer. Women's Health, 5(4), 327–340.

Rohani-Rasaf, M., Abdollahi, M., Jazayeri, S., Kalantari, N., & Asadi-Lari, M. (2013). Correlation of cancer incidence with diet, smoking and socio-economic position across 22 districts of Tehran in 2008. Asian Pacific Journal of Cancer Prevention, 14(3), 1669–1676

Ross, S. H.,&Cantrell, D. A. (2018). Signaling and function of interleukin-2 in T lymphocytes. Annual Review of Immunology, 36, 411–433.

Rossing, M. A., Wicklund, K. G., Cushing-Haugen, K. L.,&Weiss, N. S. (2010). Predictive value of symptoms for early detection of ovarian cancer. Journal of the National Cancer Institute, 102(4), 222–229.

Ruiz, R., Galvez-Nino, M., Castro-Mollo, M. W.,&Flores, G. J. (2024). Clinical and outcome patterns in epithelial ovarian cancer (EOC) within a Latin American cohort. Quality Care/Health Services Research, 59(2), 145–156

Rusu, G., Achimaș-Cadariu, P., Piciu, A., Piciu, S. C., Căinap, C., & Piciu, D. (2021). A comparative study between 18F-FDG PET/CT and conventional imaging in the evaluation of progressive disease and recurrence in ovarian carcinoma. Healthcare, 9(6), 666.

Sakaguchi, S., Yamaguchi, T., Nomura, T.,&Ono, M. (2008). Regulatory T cells and immune tolerance. Cell, 133(5), 775–787.

Salazar, C., Campbell, I. G., Gorringe, K. L., & Cho, K. R. (2018). When is "type I" ovarian cancer not "type I"? Indications of an out-dated dichotomy. Molecular Oncology, 12(2), 161–177.

Sameer, Q. (2022). The potential implementation of biosensors for the diagnosis of biomarkers of various cancer. Biointerface Research in Applied Chemistry, 12(3), 3586–3598.

Scalici, J. M., Arapovic, S., Saks, E. J., Atkins, K. A., Petroni, G., Duska, L. R.,&Slack-Davis, J. K. (2017). Mesothelium expression of vascular cell adhesion molecule-1 (VCAM-1) is associated with an unfavorable prognosis in epithelial ovarian cancer (EOC). Cancer, 123(6), 977–984.

Shang, Z. B., Wang, J., Kuai, S. G., Zhang, Y. Y., Ou, Q. F., Pei, H.,&Huang, L. H. (2018). Serum macrophage migration inhibitory factor as a biomarker of active pulmonary tuberculosis. Annals of Laboratory Medicine, 38(1), 9–16.

Sharma, S., Raghav, R., O'Kennedy, R.,&Srivastava, S. (2016). Advances in ovarian cancer diagnosis: A journey from immunoassays to immunosensors. Enzyme and Microbial Technology, 89, 15–30.

Shi, J.-X., Qu, Q., Ye, H., Wang, P., Wang, K.-J.,&Zhang, J.-Y. (2015). Tumor associated antigens or anti-TAA autoantibodies as biomarkers in the diagnosis of ovarian cancer: A systematic review with meta-analysis. Expert Review of Molecular Diagnostics, 15(6), 829–852.

Singh, A., & Sachan, M. (2019). Epigenetic biomarkers in the management of ovarian cancer: Current perspectives. Cell and Developmental Biology, 7, 182.

Singh, A., Sharma, P., & Gupta, N. (2023). Monoclonal antibodies: Production, engineering, and therapeutic applications. Biotechnology Advances, 65, 108141.

Solis Cano, D. G., Cervantes Flores, H. A., de los Santos Farrera, O., Guzman Martinez, N. B., & Soria Céspedes, D. (2021). Sensitivity and specificity of ultrasonography using ovarian-adnexal reporting and data system classification versus pathology findings for ovarian cancer. Cureus, 13(9), e1764

Song, J., Sokoll, L. J., Zhang, Z.,&Chan, D. W. (2023). VCAM-1 complements CA-125 in detecting recurrent ovarian cancer. Clinical Proteomics, 20(1), 25.

Spindel, S.,&Sapsford, K. E. (2014). Evaluation of optical detection platforms for multiplexed detection of proteins and the need for point-of-care biosensors for clinical use. Sensors, 14(12), 22313–22341.

SPRpages. (n.d.). SPR instruments. Retrieved March 2026, from https://www.sprpages.nl/spr-instruments

Steitz, A. M., et al. (2025). Elucidating the mechanistic role of ovarian cancer biomarkers: Lessons learnt from affinity-proteomics. Proteomics, 25(4), e2200175.

Steyerberg, E. W. (2019). Clinical Prediction Models: A Practical Approach to Development, Validation, and Updating (2nd ed.). Springer.

Suwansa-ard, S., Kanatharana, P., Asawatreratanakul, P., Wongkittisuksa, B., Limsakul, C., & Thavarungkul, P. (2009). Comparison of surface plasmon resonance and capacitive immunosensors for cancer antigen 125 detection in human serum samples. Biosensors and Bioelectronics, 24(12), 3436–3441.

Szymańska, B., Ławrowska, Z., Hermanowicz-Szamatowicz, K., & Gorodkiewicz, E. (2023). A multiple-array SPRi biosensor as a tool for detection of gynecological-oncological diseases. Biosensors, 13(2), 210.

Szymańska, B., Ławrowska, Z., Hermanowicz-Szamatowicz, K., & Gorodkiewicz, E. (2020). A biosensor for determination of the circulating biomarker CA125/MUC16 by surface plasmon resonance imaging. Sensors, 20(2), 375.

Szymańska, B., Zelazowska-Rutkowska, B., Hermanowicz-Szamatowicz, K., & Gorodkiewicz, E. (2021). An SPRi biosensor for determination of the ovarian cancer marker HE4 in human plasma. Sensors, 21(10), 3485.

Tian, Y., Wang, C., Cheng, L., Zhang, A., Liu, W., Guo, L., Ye, H., Huang, Y., Chen, J., Wen, X., Xing, Y., Zheng, G., Sun, Z., Li, H., Zhang, P., Liu, W., Chen, Y., Zhang, Z., Xu, Y., Huo, Y., & Ou, O. (2015). Determination of reference intervals of serum levels of human epididymis protein 4 (HE4) in Chinese women. Journal of Ovarian Research, 8, 72.

Tighe, P. J. (2015). ELISA in the multiplex era: Potentials and pitfalls. Methods, 82, 1–3.

Tighe, P., Negm, O., Todd, I.,&Fairclough, L. (2013). Utility, reliability and reproducibility of immunoassay multiplex kits. Methods, 61(1), 23–29.

Ugurlu, M., et al. (2024). Ovarian cancer awareness of women in Turkey: A cross-sectional study. European Journal of Obstetrics&Gynecology and Reproductive Biology, 295, 10–16.

Varricchi, G., Galdiero, M. R., Loffredo, S., Lucarini, V., Marone, G., Mattei, F., & Schiavoni, G. (2018). Eosinophils: The unsung heroes in cancer? OncoImmunology, 7(2), e1393134–e1393146.

Walker, C., Nguyen, T.-M., Jessel, S., Alvero, A. B., Silasi, D.-A., Rutherford, T., Draghici, S., & Mor, G. (2021). Automated assay of a four-protein biomarker panel for improved detection of ovarian cancer. Cancers, 13(2), 325.

Wang, B., Liang, G., Meng, L., Li, H., Song, Z., Xu, Y., He, Y., Duan, D., Shi, Q., Guan, T.,&Gong, Y. (2025). A dual immunosensor based on optical weak value amplification for simultaneous detection of CA125 and HE4. Sensors, 25(11), 3347.

Wang, H., Wang, X., Wang, J., Fu, W., & Yao, C. (2016). A SPR biosensor based on signal amplification using antibody-QD conjugates for quantitative determination of multiple tumor markers. Scientific Reports, 6, Article 33140.

Wang, J., Gao, J., Yao, H., Wu, Z., Wang, M.,&Qi, J. (2014). Diagnostic accuracy of serum HE4, CA125 and ROMA algorithm in ovarian cancer: A meta-analysis. Tumor Biology, 35, 6127–6138.

Wang, R.,&Huang, K. (2020). CCL11 increases the proportion of CD4+CD25+Foxp3+ Treg cells and the production of IL-2 and TGF-β by CD4+ T cells via the STAT5 signaling pathway. Molecular Medicine Reports, 21(6), 2522–2532.

Watrowski, R., Obermayr, E., Wallisch, C., Aust, S., Concin, N., Braicu, E. I., Van Gorp, T., Hasenburg, A., Sehouli, J., Vergote, I., & Zeillinger, R. (2022). Biomarker-based models for preoperative assessment of adnexal mass: A multicenter validation study. Cancers (Basel), 14(7), 1780–1795.

Woo, S. Y.,&Kim, S. (2020). Determination of cutoff values for biomarkers in clinical studies. Precision and Future Medicine, 4, 2–8.

Xu, Y., Zhong, R., He, J., Ding, R., Lin, H., Deng, Y., et al. (2016). Modification of cut-off values for HE4, CA125 and the ROMA algorithm for early-stage epithelial ovarian cancer detection: Results from 1021 cases in South China. Clinical Biochemistry, 49(1-2), 32-40.

Yen, L., Henao-Díaz, A., Zimmerman, J., & Giménez-Lirola, L. (2025). Considerations on the stability of IgG antibody in clinical specimens. Journal of Veterinary Diagnostic Investigation, 37, 1–14.

Yi, R., Zhang, Z., Liu, C.,&Qi, Z. (2020). Gold-silver alloy film-based surface plasmon resonance sensor for biomarker detection. Materials Science and Engineering C: Materials for Biological Applications, 116, 111126–111134

Yildiz-Uludag, H. (2012). Cancer biomarker detection in serum samples using surface plasmon resonance and quartz crystal microbalance sensors with nanoparticle signal amplification. Analytical Chemistry, 84(14), 5898–5904.

Yuan, J., Duan, R., Yang, H., Luo, X.,&Xi, M. (2012). Detection of serum human epididymis secretory protein 4 in patients with ovarian cancer using a label-free biosensor based on localized surface plasmon resonance. International Journal of Nanomedicine, 7, 2921–2928.

Yurkovetsky, Z., Skates, S., Lomakin, A., Nolen, B., Pulsipher, T., Modugno, F., et al. (2010). Development of a multimarker assay for early detection of ovarian cancer. Clinical Oncology, 28(13), 2159–2166.

Zainuddin, A. A., Abdul Razak, K., Rahman, N. A.,&Fouad, H. (2018). Surface plasmon resonance biosensor for dengue virus detection. Sensors and Actuators B: Chemical, 273, 1523–1530.

Zhang, H., Huo, Q., Huang, L., Cheng, Y., Liu, Y.,&Bao, H. (2019). Neutrophil-to-lymphocyte ratio in ovarian cancer patients with low CA125 concentration. BioMed Research International, 2019, Article 8107906.

Zhang, Y., Wu, C., Huang, Y., Kang, J., Yao, S., Chen, Y., Gu, L.,&Yang, G. (2024). The impact of laterality on the incidence and prognosis of epithelial ovarian cancer. European Journal of Surgical Oncology, 50(9), 108475.

Zheng, H., & Gao, Y. (2012). Serum HE4 as a useful biomarker in discriminating ovarian cancer from benign pelvic disease. International Journal of Gynecological Cancer, 22(6), 1000–1005.

Zou, Z., Chen, Y., Sun, R., Shi, B., Jiang, L., & Huang, F. (2025). Ti(3)C (2)/Fe(3)O(4) - based surface plasmon resonance imaging biosensor for efficient separation and sensitive detection of CA125 in serum. Mikrochim Acta, 192(3), 168.