Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors

Dobilas, Arturas

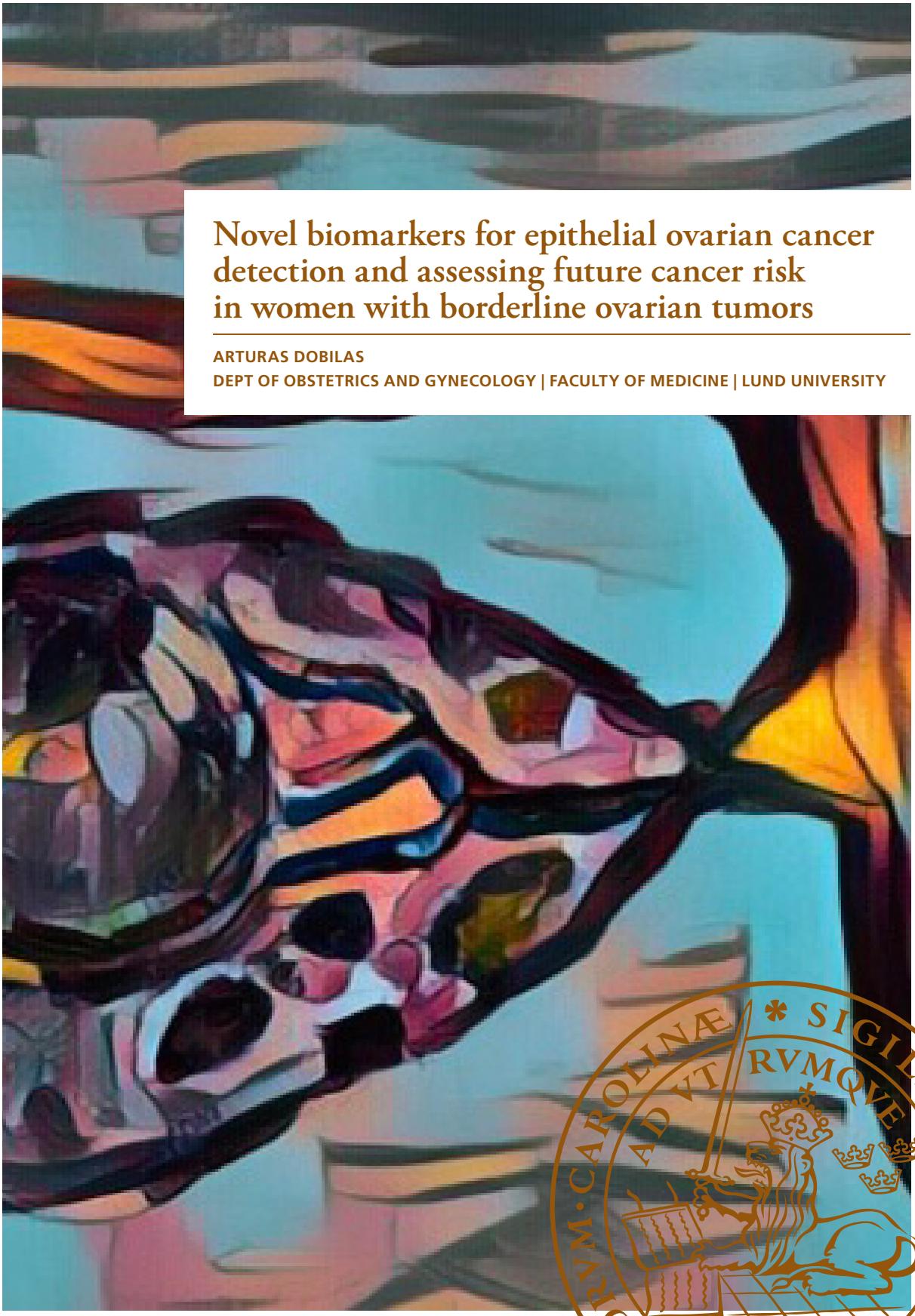
2023

Document Version:
Publisher's PDF, also known as Version of record

Link to publication

Citation for published version (APA):
Dobilas, A. (2023). *Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors*. [Doctoral Thesis (compilation), Department of Clinical Sciences, Lund]. Lund University, Faculty of Medicine.

Total number of authors:
1



Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors

ARTURAS DOBILAS
DEPT OF OBSTETRICS AND GYNECOLOGY | FACULTY OF MEDICINE | LUND UNIVERSITY

Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors

Arturas Dobilas

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at the Department of Obstetrics and Gynaecology, Lund,
October 20, 2023, at 09:00 am.

Faculty opponent
Docent Ulrika Ottander, Umeå, Sweden

Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation

Date of issue: 2023-10-20

Author(s): Arturas Dobilas

Sponsoring organization:

Title and subtitle: Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors

Abstract:

Epithelial ovarian carcinoma (EOC) is the most deadly gynecological malignancy. Owing to delayed diagnosis as a result of vague symptoms, the five-year survival rate of patients with advanced-stage EOC is approximately 30%. The "golden standards" to diagnose ovarian cysts and pelvic malignancies of unknown origin include the biomarker cancer antigen (CA) 125 and transvaginal ultrasound. Our understanding of the etiology and heterogeneity of ovarian cancer has been altered by new information on mutations specific to EOC.

This thesis aims to examine the existing and new biomarkers and algorithms for EOC detection in a diverse population of women with ovarian tumors. This has been achieved through the analysis and comparison of two research papers (I and II). To explore innovative approaches for diagnosing gynecological cancer, a specific aspect of my thesis centers on the application of circulating tumor DNA (ctDNA). The main aim of the fourth study is to investigate the potential risk of subsequent cancers among women with prior borderline ovarian tumors (BOTs).

Study I: This study analyzed peripheral blood samples from 199 patients with adnexal masses. It found higher levels of histone H3 citrullinated DNA (H3Cit-DNA) and double-stranded DNA (dsDNA) in women with borderline or malignant ovarian tumors compared to benign tumors. Elevated CA125 levels were also observed in both borderline and ovarian cancer cases, with a significant impact on overall survival.

Study II: Preoperative blood samples were collected from 116 EOC patients, diagnosed with epithelial ovarian cancer (EOC) and scheduled to undergo primary debulking surgery. In total, 177 protein biomarkers were analyzed. The combined use of cross-validation and least absolute shrinkage and selection operator (LASSO) regression was used to determine the optimal prediction models for OS. A predictive model combining age and specific biomarkers demonstrated a significant association with worse overall survival. The inclusion of CA125 and HE4 further improved predictive accuracy. Combining age, stage, and biomarkers enhanced the predictive capability for overall survival.

Study III: This study performed targeted sequencing on tumor tissues from 41 ovarian tumor patients, identifying somatic mutations in 24 tumors. Circulating tumor DNA (ctDNA) levels in preoperative plasma were measured using SAGAsafe digital PCR (dPCR). The concentration of mutant ctDNA in plasma correlated with advanced tumor stage and reduced overall survival.

Study IV: A retrospective cohort study examined 4,998 women diagnosed with serous and mucinous BOTs between 1995 and 2018. These women exhibited increased risks of various non-ovarian cancers, including colon, rectum, small intestine, cervix, endometrium, pancreas, upper aerodigestive tract, lung, kidney, and bladder cancers. Thyroid cancer incidence was higher in women with serous borderline ovarian tumors. The risk of lung and pancreatic cancers increased after the first year following a BOT diagnosis.

In conclusion, our first study found no association between NETs markers and ovarian tumors. However, a second study that explored the performance of plasma protein biomarkers in predicting overall survival (OS), showed promising results. Specifically, the addition of biomarkers, especially NT-3, to the panel improved OS prediction. The third study indicated that ctDNA in preoperative plasma could serve as a valuable predictive biomarker for tumor staging and prognosis in patients with ovarian cancer. Lastly, a Swedish population-based study revealed an elevated risk of multiple malignancies, including lung and pancreatic cancers, beyond the first year of diagnosis in patients with borderline ovarian tumors (BOTs). This finding suggests a potentially shared etiology of these cancers.

Key words:

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language: English

ISSN and key title: 1652-8220

ISBN: 978-91-8021-462-9

Recipient's notes

Number of pages: 69

Price

Security classification

Novel biomarkers for epithelial ovarian cancer detection and assessing future cancer risk in women with borderline ovarian tumors

Arturas Dobilas

LUND
UNIVERSITY

Faculty of Medicine Doctoral Dissertation Series

Department of Obstetrics and Gynecology

ISBN 978-91-8021-462-9

ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University

Lund 2023

Media-Tryck is a Nordic Swan Ecolabel certified provider of printed material. Read more about our environmental work at www.mediatryck.lu.se

MADE IN SWEDEN

Anyone who has never made a mistake has never tried anything new

Albert Einstein

Table of Contents

Abstract 8
Populärvetenskaplig sammanfattning 11
Santrauka lietuvių kalba 12
List of original papers 13
Abbreviations 14

Introduction 16

Epidemiology 16
Mortality 17
Etiology 18
Tumor characteristics 20
Ovarian cancer staging 21
Risk factors for epithelial ovarian cancer 23
Protective factors 24
Diagnosis 25
Biomarkers 26
Algorithms 28
Risk of Ovarian Malignancy Algorithm (ROMA) 28
Risk of Malignancy Index (RMI) 28
Ultrasound 29
Computed tomography (CT), positron emission tomography-CT, and magnetic resonance imaging (MRI) 30
Prognostic factors 31

Aims of the thesis 32

Material and methods 33

Paper I 33
Paper II 34
Paper III 35
Paper IV 36

Ethical considerations **39**

Results **40**

Paper I 40

Paper II 41

Paper III 45

Paper IV 47

Discussion **49**

Conclusions **55**

Future perspectives **56**

Acknowledgments **58**

References **59**

Abstract

Epithelial ovarian carcinoma (EOC) is the most deadly gynecological malignancy. Owing to delayed diagnosis as a result of vague symptoms, the five-year survival rate of patients with advanced-stage EOC is approximately 30%. The "golden standards" to diagnose ovarian cysts and pelvic malignancies of unknown origin include the biomarker cancer antigen (CA) 125 and transvaginal ultrasound, but early detection is still difficult and specificity is low. Our understanding of the etiology and heterogeneity of ovarian cancer has been altered by new information on mutations specific to EOC. Mutations occurring within ovarian tumors can be detected in liquid biopsies obtained from various compartments.

This thesis aims to examine the existing and new biomarkers and algorithms for EOC detection in a diverse population of women with ovarian tumors. This has been achieved through the analysis and comparison of two research papers (I and II). To explore innovative approaches for diagnosing gynecological cancer, a specific aspect of my thesis centers on the application of circulating tumor DNA (ctDNA) and somatic mutations. Specifically, mutation-specific markers are analyzed in liquid biopsies obtained from plasma (as discussed in Paper III). The main aim of the fourth study is to investigate the potential risk of subsequent cancers among women with prior borderline ovarian tumors (BOTs) concerning the general female population in Sweden (Paper IV).

Study I: Peripheral blood samples were obtained from 199 patients admitted for primary surgery for adnexal masses. The patients were categorized based on the type and stage of their tumors. The plasma levels of histone H3 citrullinated DNA (H3Cit-DNA), double-stranded DNA (dsDNA), and CA125 were performed. Results: Women with borderline or malignant ovarian tumors had higher plasma levels of H3Cit- DNA and dsDNA than those with benign ovarian tumors. Higher concentrations of CA125 were observed in both borderline and ovarian cancer cohorts, with a statistically significant linear trend ($p_{trend}<0.001$). The Cox regression analysis showed a significant association between CA125 levels dichotomized at the median value of 326 IU/ml and overall survival (OS), indicating a higher risk of mortality (HR=1.9; 95%CI=1.03-3.36; p=0.038). No significant differences were observed in the survival analyses of malignant ovarian tumors when the levels of dsDNA and H3Cit-DNA were examined. In conclusion, our findings indicated a lack of correlation between markers of neutrophil extracellular traps (NETs) and ovarian tumors.

Study II: Preoperative peripheral blood samples were collected from 116 patients diagnosed with epithelial ovarian cancer (EOC) and scheduled to undergo primary debulking surgery. Among these patients, 28 had early-stage EOC (International Federation of Gynecology and Obstetrics (FIGO) stages I–II) and 88 had advanced-stage EOC (FIGO stages III–IV). Protein measurements were performed using

Olink Oncology II and Inflammation panels. In total, 177 protein biomarkers were analyzed. The combined use of cross-validation and least absolute shrinkage and selection operator (LASSO) regression was used to determine the optimal prediction models for OS. Results: The model, which incorporated age and a combination of three biomarkers (neurotrophin-3, transmembrane glycoprotein NMB, and mesothelin), demonstrated a significant association with worse overall survival. The area under the curve (AUC) for this model was 0.79, with a p-value of 0.004. The inclusion of CA125 and HE4 in the model significantly enhanced the performance (AUC=0.83; p=0.003). In a postoperative model that incorporated age and stage (III + IV vs. I + II), the inclusion of a three-biomarker panel consisting of chemokine (C-C motif) ligand 28 (CCL28), T-cell leukemia/lymphoma protein 1A (TCL1A), and glycoprotein NMB (GPNMB) demonstrated enhanced predictive capability for OS. Specifically, the addition of this biomarker panel increased the AUC from 0.83 to 0.90, which was statistically significant (p=0.05). In the model, the inclusion of age and dichotomized stage (III vs. I + II) demonstrated an enhanced predictive capability for OS when incorporating the biomarkers CCL28 and GPNMB1 (AUC=0.86; p<0.001). Elevated CA125 and HE4 levels were significantly associated with poor survival outcomes (p=0.05).

Study III: DNA was extracted from the tumor tissues of 41 patients with ovarian tumors. Targeted sequencing was performed using a panel consisting of 127 genes that are frequently mutated in cancer to identify potential somatic mutations in the tumor DNA. This study used SAGAsafe digital PCR (dPCR) to measure the levels of circulating tumor DNA (ctDNA) in plasma samples obtained from patients before surgery.

In this study, somatic mutations were detected in 24 tumors. Of these tumors, seven were obtained from patients who had been diagnosed with borderline tumors and 17 were obtained from patients with invasive cancer. The TP53 gene exhibited the highest frequency of mutations. Of the total cohort of 24 patients, 15 showed detectable levels of ctDNA in the preoperative plasma. The concentration of mutant ctDNA in the plasma demonstrated a significant positive correlation with the advanced stage, as indicated by a statistically significant linear trend (ptrend <0.001). Patients diagnosed with cancer who showed a plasma concentration of > 10 copies/mL of ctDNA before surgery experienced notably reduced OS (p=0.008).

Study IV: A retrospective cohort study was conducted between 1995 and 2018, in which samples obtained from cases with borderline ovarian tumors (BOTs) and subsequent or simultaneous cancer diagnoses were collected from the Swedish Cancer Register and linked to the Total Population Register. Observation and tracking of each female participant were carried out until the point of non-ovarian cancer occurrence, death, or relocation. To ensure the integrity of the outcome analysis, each participant was strictly included only once. The analysis focused on the examination of standardized incidence ratios (SIRs) and their corresponding 95% CIs for non-ovarian cancers.

This study included 4,998 women diagnosed with serous and mucinous BOTs. The average age at diagnosis was found to be 55.7 years, with a standard deviation of 16.0. Women diagnosed with BOTs exhibited significantly higher risks of various non-ovarian cancers, including colon (standardized incidence ratio [SIR]=2.5), rectum (SIR=1.7), small intestine (SIR=5.0), cervix (SIR=2.5), endometrium (SIR=2.4), pancreas (SIR=2.3), upper aerodigestive tract (SIR=2.2), lung (SIR=1.8), kidney (SIR=2.3), and bladder (SIR=1.8). The incidence of thyroid cancer was elevated in women diagnosed with serous borderline ovarian tumors (SIR=3.1). The incidence of lung and pancreatic cancers increased beyond the first year following a diagnosis of BOTs.

In conclusion, our first study found no association between NETs markers and ovarian tumors. However, a second study that explored the performance of plasma protein biomarkers in predicting overall survival (OS), showed promising results. Specifically, the addition of biomarkers, especially NT-3, to the panel improved OS prediction. The third study indicated that ctDNA in preoperative plasma could serve as a valuable predictive biomarker for tumor staging and prognosis in patients with ovarian cancer. Lastly, a Swedish population-based study revealed an elevated risk of multiple malignancies, including lung and pancreatic cancers, beyond the first year of diagnosis in patients with borderline ovarian tumors (BOTs). This finding suggests a potentially shared etiology of these cancers.

Populärvetenskaplig sammanfattning

Äggstockscancer är den mest dödliga gynekologiska cancern. På grund av vaga symtom som kan leda till fördröjd diagnos, är 5-årsöverlevnaden för en patient med avancerad äggstockscancer ungefär 30%. För att undersöka och bedöma tumörer på äggstockarna och oklara cancrar i lilla bäckenet, används oftast biomarkören CA125 och vaginalt ultraljud, men tidig upptäckt är fortfarande svår och specificiteten av dessa undersökningar är låg. Vår förståelse av ursprunget och mångfalden av äggstockscancer har förändrats på grund av ny information om förändringar i arvsmassan (mutationer) som är specifika för äggstockscancer. Mutationer i äggstockstumörer kan hittas i tex blodprover och biopsier från tumörvävnad.

Syftet med min avhandling var att undersöka befintliga och nya biomarkörer och algoritmer för diagnostik av äggstockscancer hos kvinnor med äggstockstumörer. I det första arbetet utvärderades om nivån av så kallade NET-markörer ökar i blodplasman hos patienter med äggstockscancer. NET-markörer kan beskrivas som ett nät av DNA strängar som bildas utanför cellkärnan av en särskild grupp av vita blodkroppar (neutrofiler) och dessa nät fångar sjukdomsalstrande mikrober av olika slag. I det andra arbetet undersöktes hur väl tillägg av vissa biomarkörer i plasma och kombinationer av dessa biomarkörer kan förutsäga total överlevnad vid äggstockscancer. I arbete III diskuteras specifikt analysen av mutationsspecifika markörer i blodprover (ctDNA) erhållna från plasma och hur detta kan bidra till korrekt stadieindelning och prognos för patienter med äggstockscancer. Huvudsyftet med den fjärde studien var att undersöka den potentiella risken för efterföljande cancerformer bland kvinnor med tidigare borderline ovarialtumörer i förhållande till den allmänna kvinnliga befolkningen i Sverige (artikel IV).

Sammanfattningsvis fann den första studien inget samband mellan NET-markörer och äggstockstumörer. Den andra studien, som undersökte prestandan hos plasmaproteinbiomarkörer för att förutsäga total överlevnad, visade dock lovande resultat. Specifikt förbättrade tillägget av biomarkörer, särskilt NT-3, till panelen av biomarkörer förutsägelsen av den totala överlevnaden. Fortsatt, den tredje studien indikerade att mätning av ctDNA i plasma innan operation kan fungera som en värdefull prediktiv biomarkör för tumörstadieindelning och prognos hos äggstockscancerpatienter. Slutligen avslöjade den svenska befolkningsbaserade studien en förhöjd risk för flera andra cancertyper såsom lung- och bukspottkörtelcancer, bortom det första diagnosåret hos patienter med borderline ovarialtumörer. Detta kan tyda på ett möjligt delat ursprung för dessa cancerformer.

Santrauka lietuvių kalba

Kiaušidžių vėžys yra dažniausia mirties priežastis tarp moterų, sergančių ginekologiniais piktybiniais navikais. Dėl nespecifinių simptomų ir sudėtingos diagnostikos, penkerių metų išgyvenamumas vėlyvose stadijos yra maždaug 30%. „Auksinis diagnostikos standartas“ kiaušidžių piktybinių navikų diagnozavime yra biomarkeris CA125 ir ultragarsinis tyrimas, tačiau kiaušidžių vėžio ankstyvas diagnozavimas vis dar sunkus, o specifiškumas žemas. Mūsų supratimas apie kiaušidžių vėžio etiologiją ir nevienalytiškumą pasikeitė dėl naujos informacijos apie genų mutacijas, būdingas kiaušidžių vėžiui. Kiaušidžių tumorų DNR ląstelių mutacijas galima rasti tumoro audinio biopsijose arba kraujo plazmoje bei ascite.

Doktorantūros darbo tikslas buvo ištirti esamus ir naujus biomarkerius bei galimus algoritmus, skirtus kiaušidžių vėžio diagnostikai. Tai buvo pasiekta pirmojoje ir antrojoje mokslinio darbo dalyje. Siekiant išnagrinėti naujausius kiaušidžių vėžio diagnostikos metodus, specifinis mano darbo tikslas buvo skirtas naviko genų mutacijų ir DNR grandinių (ctDNR), cirkuliuojančiu kraujo plazmoje, nustatymui. Pagrindinis ketvirtojo tyrimo tikslas buvo ištirti galimą kitos kilmės vežio riziką moterims, kurioms anksčiau buvo nustatytas ribinio piktybiškumo kiaušidžių navikas ir palyginti su bendrąja Švedijos moterų populiacija.

Apibendrinant galima pasakyti, kad pirmasis tyrimas neįrodė ryšio tarp NETs (Neutrophil extracellular traps) biomarkerio ir kiaušidžių navikų. Tačiau antrasis tyrimas, kurio metu buvo tiriamas plazmos biomarkerių veikimas numatant bendrą išgyvenamumą, parodė daug žadančių rezultatų. Biomarkerių, ypač NT-3, pridėjimas ir panaudojimas algoritmuose pagerino bendrojo išgyvenamumo numatymą. Trečiasis tyrimas įrodė, kad ctDNR matavimas iš kraujo plazmos paimtos prieš chirurginį kiaušidžių vėžio gydymą gali būti vertingas nustatant vėžio stadiją ir prognozuojant pacientų išgyvenamumą. Ketvirtasis tyrimas atskleidė padidėjusią piktybinių navikų, tokių kaip plaučių ir kasos vėžio, riziką po pirmųjų diagnozės metų nuo susirgimo ribiniais kiaušidžių navikais. Ši išvada rodo galimą bendrą šių vėžių etiologiją ir suteikia galimybę tolimesniems tyrimams ateityje.

List of original papers

This thesis is based on the following papers, referred to by Roman numerals:

Paper I

Circulating markers of neutrophil extracellular traps (NETs) in patients with ovarian tumors Arturas Dobilas, Charlotte Thålin, PhD, Håkan Wallén, Christer Borgfeldt *Anticancer Research* DOI: 10.21873/anticanres.15556

Paper II

A Multiplex Biomarker Assay Improves the Prediction of Survival in Epithelial Ovarian Cancer Arturas Dobilas, Anna Åkesson, Pia Leandersson and Christer Borgfeldt. *Cancer Genomics & Proteomics* doi:10.21873/cgp.200xx

Paper III

Preoperative circulating tumor DNA level is associated to poor overall survival in patients with ovarian cancer Arturas Dobilas, Yilun Chen, Miguel Alcaide, Christian Brueffer, Pia Leandersson, Lao H Saal and Christer Borgfeldt. *Submitted.*

Paper IV

Risks of non-ovarian cancers in women with borderline ovarian tumor: a national cohort study in Sweden Arturas Dobilas, Filip Jansåker, Xinjun Li, Kristina Sundquist and Christer Borgfeldt. *Submitted.*

The following publication is not included in the thesis but is of relevance to the field: Areas to Improve Quality of Life After Ovarian Tumor Surgery and Adjuvant Treatment Arturas Dobilas, Louise Moberg and Christer Borgfeldt. DOI: 10.21873/invivo.12517

Abbreviations

ASA	American Society of Anaesthesiologists' classification
AUC	Area under the curve
BMI	Body mass index
BOT	Borderline type tumors
BRAF	V-raf murine sarcoma viral oncogene homolog B1
BRCA1/2	Breast cancer type 1/2 susceptibility protein
CA125	Cancer antigen 125
CCL28	chemokine (C-C motif) ligand 28
CCC	clear-cell carcinoma
cfDNA	Cell-free DNA
ctDNA	Circulating tumor DNA
CI	Confidence interval
CT	Computed tomography
CTC	Circulating tumor cells
EOC	Epithelial ovarian cancer
DNA	Deoxyribonucleic acid
EC	endometrioid carcinoma
ERBB2 (HER2)	Avian erythroblastic leukemia viral homolog 2
FDA	US Food and drug administration
FIGO	International Federation of Gynecology and Obstetrics
GPNMB	transmembrane glycoprotein NMB
HE4	Human epididymis protein 4
HGSC	High-grade serous carcinoma
IOTA	International Ovarian Tumor Analysis
IL-8	Interleucin-8
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LGSC	Low-grade serous carcinoma
MAF	Mutant Allele Fraction

MSLN	mesothelin
NGS	Next Generation Sequencing
NPV	Negative predictive value
NT-3	neurotrophin-3
OC	Ovarian cancer
OVA-1	Multivariate index assay
p53	Tumor protein p53
PAD	Pathologic anatomic diagnosis
PEA	Proximity extension assay
PID	Pelvic inflammatory disease
PIKC3CA	Phosphatidylinositol 3-kinase
PLCO	The prostate, lung, colorectal, and ovarian trial
PPV	Positive predictive value
RCC	Regional cancer center
RMI	Risk of Malignancy Index
ROMA	Risk of Malignancy Algorithm
STIC	Serous tubal intraepithelial carcinoma
TP53	Tumor protein p53 gene
TCGA	The Cancer Genome Atlas
TCL1A	T-cell leukemia/lymphoma protein 1A
TVU	Transvaginal ultrasound
UKTOCS	The UK collaborative Trial of ovarian cancer screening

Introduction

Epidemiology

Ovarian cancer is the 8th most common cancer in women worldwide, with more than 300,000 new cases per year (1, 2). The incidence varies geographically and the highest incidence is in the central and eastern parts of Europe (6.8–15.1 per 100,000) and North America (6.8–8.4 per 100,000) (2). In Sweden, approximately 700 new cases of ovarian cancer were diagnosed in 2021, with an incidence of 11.2 per 100,000, mostly in advanced stages. Ovarian cancer can be diagnosed in women of all age groups but is rare in younger patients (3), mainly affecting postmenopausal women (>50 years). According to data from the Scandinavian Cancer Registry (NORDic CANcer database (NORDCAN)), the incidence of ovarian cancer has declined (3).

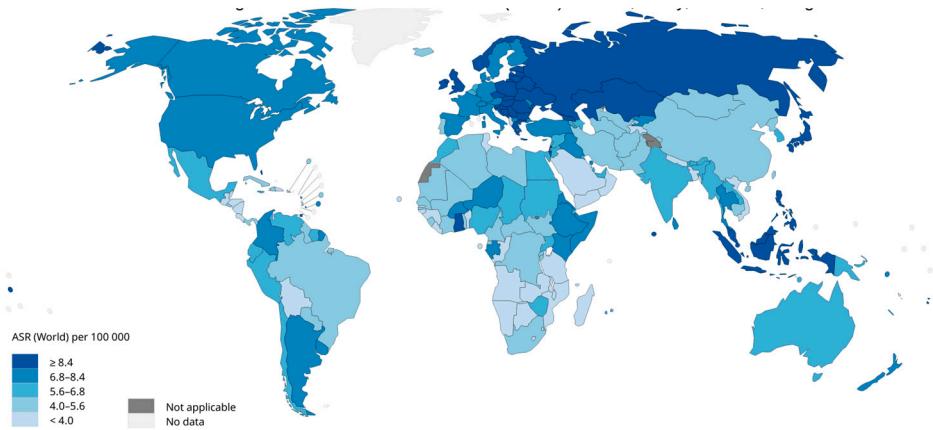


Figure 1. Worldwide variations in ovarian cancer incidence. Source: Global Cancer Observatory (GLOBOCAN). Estimated global ovarian cancer incidence for the year 2020, considering age-standardized incidence rates.

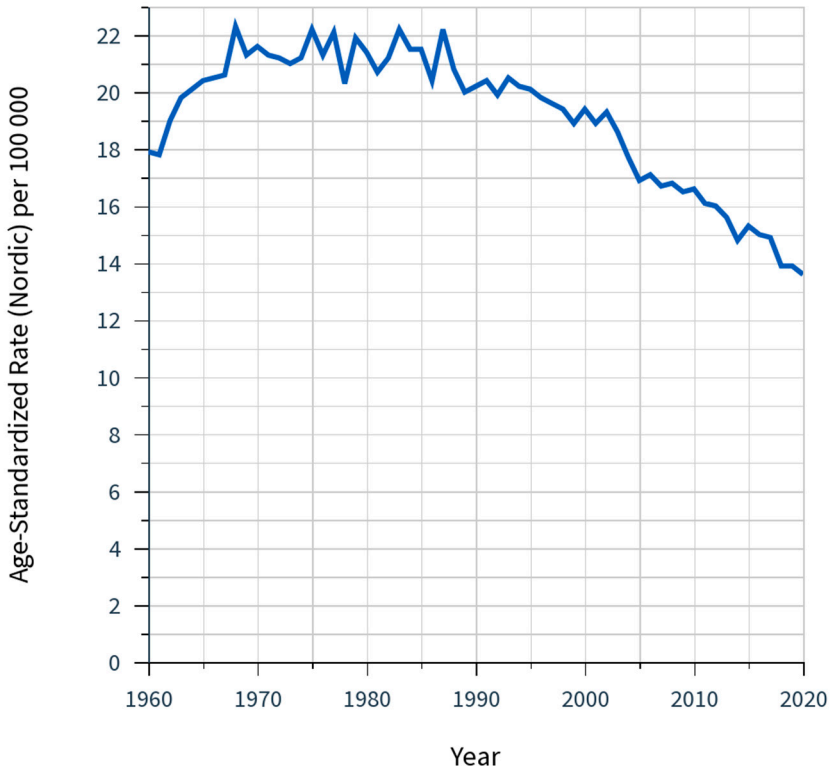


Figure 2. A decline in the incidence of ovarian cancer in Nordic countries since 1960. Source: NORDCAN. Incidence of ovarian cancer in Nordic countries from 1960 to 2020, age-standardized incidence rates.

Mortality

According to the World Health Organization, 207,252 patients with ovarian cancer died in 2020. Ovarian cancer is the most lethal gynecological malignancy worldwide and the fifth leading cause of cancer-related deaths (4). The mortality rate is highest in Northern Europe. A majority of ovarian cancer cases are diagnosed at an advanced stage primarily due to the presence of diffuse symptoms. Considering these diagnostic challenges, the current scenario entails approximately 600 annual deaths in Sweden. The projected age-standardized mortality rate for 2020 was reported at 4.3 per 100,000 individuals. The one-year and five-year relative survival (RS) rates were estimated to be 88 and 52%, respectively, in the year 2020 (3). A woman's lifetime risk for ovarian cancer is approximately 2%, and the estimated risk of death from OC is 1 out of 100 (5, 6).

Etiology

Ovarian cancer is a heterogeneous disease with a complex and poorly understood etiology. While we possess knowledge about certain factors that elevate a woman's susceptibility to developing epithelial ovarian cancer (EOC), our understanding is considerably less comprehensive concerning the risk factors associated with ovarian germ cells and stromal tumors. EOC accounts for the majority of ovarian cancers, constituting approximately 90–95% of the cases, whereas the remaining subset, including germ cells and stromal tumors, accounts only for 5–10%. The recent breakthrough on the origin of ovarian cancer indicates its origin form cells located in the fallopian tube fimbriae, rather than solely originating within the ovary. This revelation bears significant implications for early detection, development of preventive strategies, and specialized therapeutic interventions tailored to combat this fatal illness.

Different theories have been proposed regarding the etiology of EOC. Pregnancy, breastfeeding, and use of birth control pills reduce the risk of ovarian cancer. These events reduce the number of ovulations (7, 8). These data suggest that the development of EOC may be influenced by the impairment of the ovarian surface. Repeated trauma to the ovarian epithelium and exposure of the epithelium to estrogen-rich follicular fluid lead to epithelial neoplasia. In 2020, Trabert et al. reported the occurrence of a linear relationship between lifetime ovulatory cycles and ovarian cancer risk (9).

Another theory suggests that androgens cause ovarian cancer. Modugno et al. reviewed epidemiological evidence that androgens might play a role in the development of EOC (10); however, other studies do not support this assumption. Pooled data from 12 prospective cohort studies identified 2,000 epithelial ovarian cancers in approximately half a million women and reported an association between a height of 01.7 m and EOC risk, especially in premenopausal women (11). The association between height and susceptibility to EOC may align with hormone-related mechanisms, given that gonadal hormones promote growth during puberty (11).

Dualistic model -Type I and Type II

Over the past decades, major advances have been made in our understanding of the pathogenesis of EOC. Kurman and Shih proposed a dualistic model of epithelial ovarian carcinogenesis, categorizing epithelial ovarian cancer into two main groups based on its origin and pathogenesis (12, 13). Type I tumors develop over time from benign precursors (cystadenomas or endometriosis) to borderline tumors (BOT), and then to invasive carcinomas. They grow slowly and are genetically stable. Type I tumors include low-grade serous carcinoma (LGSC), endometrioid carcinoma (EC), clear cell carcinoma (CCC), mucinous carcinoma, and Brenner tumors. Type

I tumors are low-grade tumors, except for clear-cell carcinomas. Mutations can be observed in Kirsten rat sarcoma viral oncogene homolog (KRAS), v-raf murine sarcoma viral oncogene homolog B (BRAF), receptor tyrosine-protein kinase ErbB-2 (ERBB2), phosphatase and tensin homolog (PTEN), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), AT-rich interactive domain 1A (ARID1A), catenin beta-1 (CTNNB1), and DNA mismatch repair (MMR) genes. Type I tumors have a more favorable prognosis, especially if restricted to the ovary when diagnosed (stage I) (13).

Most type II tumors develop from serous tubal intraepithelial carcinoma (STIC) (Figure 3), account for 70% of all EOC cases, and are responsible for 90% of all deaths from OC, as they are typically diagnosed at an advanced stage with secondary spread to the ovaries and peritoneum (stages III–IV). Type II tumors are characterized by TP53 mutations and common homologous recombination defects (HRD) including breast cancer gene (BRCA)1/2 mutations. They are more aggressive, fast-growing, and chromosomally unstable compared to type I tumors. Type II tumors include high-grade serous carcinoma (HGSC), carcinosarcoma, and undifferentiated carcinomas (13).

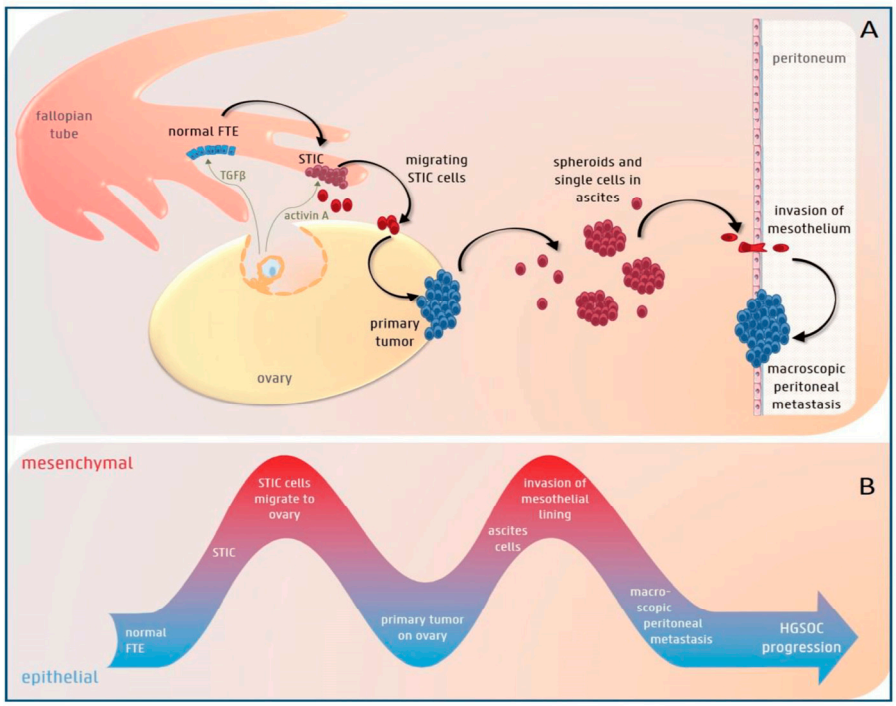


Figure 3. (A) The potential role of epithelial-to-mesenchymal (EMT) plasticity during high-grade serous (HGS) ovarian cancer progression. (B) EMT plasticity with EMT and mesenchymal-epithelial transition (MET) occurring alternately during HGS ovarian cancer progression. Nele Loret et al., The Role of Epithelial-to-Mesenchymal Plasticity in Ovarian Cancer Progression and Therapy Resistance. Cancers. 2019.

Tumor characteristics

Ovarian carcinoma

Because of the complex origin of epithelial ovarian cancer, we grouped it into subtypes, each of which exhibited different morphological features and biological behaviours. Histological subtypes differ in their pathogenesis, gene expression, origin, cell subtypes, and molecular characteristics (Table 1) (14, 15). Five main types:

- High-grade serous carcinoma (70%),
- Endometrioid carcinoma (10%),
- Clear cell carcinoma (10%),
- Mucinous carcinoma (3%),
- Low-grade serous carcinoma (<5%).

Furthermore, smaller histological variants, including Brenner, seromucinous, undifferentiated tumors, and carcinosarcomas, are also present.

Table 1. Features of the five major subtypes of epithelial ovarian carcinoma (EOC).

	High-grade serous carcinoma	Low-grade serous carcinoma	Endometroid	Clear-cell	Mucinous
Frequency	70%	<5%	10%	5–10%	3%
Low- or high- grade	High-grade	Low-grade	Low-grade	High-grade	Low-grade
Dualistic model	Type II	Type I	Type I	Type I	Type I
Precursor lesions	Serous tubal intraepithelial carcinoma (STIC)	Low-grade malignant lesions, serous BOT	Endometriosis, retrograde menstruation	Endometriosis, retrograde menstruation	Low-grade malignant lesions, mucinous BOT
Genetic rise	BRCAI/2		HNPCC/Lynch		
Molecular abnormalities and common mutations	TP 53, BRCAI/2	KRAS, BRAF	PTEN, ARID IA, CTNNBI, PIK3CA, KRAS, CDKN2A, BRAF	ARIDIA, PIK3CA, CTNNB I, PTEN, KRAS	KRAS, CDKN2A, PTEN
The typical stage of diagnosis	Advanced	Early or advanced	Early	Early	Early
Prognosis	Poor	Favorable	Favorable	Intermediate	Favorable

As mentioned earlier, HGSC comprises the majority of EOCs that form according to the dualistic model type II (12, 13). Although all these categories belong to EOC, their characteristics vary significantly.

Borderline ovarian tumors

Another important subgroup of epithelial ovarian tumors is borderline tumors. In contrast to epithelial carcinomas, they do not invade the stroma and have low malignant potential. As described by Kurman and Shih, it is formed by the dualistic model I when the primary tumor is formed in the ovarian epithelium. In comparison to benign epithelial tumors (adenomas), borderline tumors can be considered atypical proliferating epithelia with nuclear atypia and are called tumors with low malignant potential. The most common borderline tumors are serous (55%) and mucinous (40%) (12, 16). Due to the clinical similarity of BOT to the early stages of EOC, we face challenges in making a correct diagnosis. In such instances, transvaginal ultrasound (TVU) and computed tomography (CT) can aid in diagnosing ovarian carcinoma. However, due to the prevalence of borderline ovarian tumors (BOTs) being diagnosed in the premenopausal age, when preserving fertility is crucial, supplementary diagnostic approaches should be considered. (16-18).

Ovarian cancer staging

The recommendations for staging EOC were made according to FIGO and tumor-node-metastasis (TNM) (19). The FIGO staging criteria and equivalent TNM classifications are listed below (20). The first stage is limited to the ovaries, in which the disease has not spread to the pelvic peritoneum. In advanced EOC stages, cancer spreads to distant organs, most commonly the omentum. The EOC growth path and metastatic status have prognostic significance and bear critical importance when determining the treatment choice (19, 21, 22).

Stage I: Tumor confined to ovaries or fallopian tube(s)	**T1-NO-MO**
IA: Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	T1a-NO-MO
1B: Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	T1b-N0-M0
IC: Tumor limited to 1 or both ovaries or fallopian tubes, with any of the following:	
– IC1: Surgical spill	T1c1-N0·M0
– IC2: Capsule ruptured before surgery or tumor on ovarian or fallopian tube surface	T1c2-N0-M0
– IC3: Malignant cells in the ascites or peritoneal washings	T1c3-N0-M0
Stage II: Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or peritoneal cancer	**T2-N0-M0**
IIA: Extension and/or implants on uterus and/or fallopian tubes and/or ovaries	T2a-N0-M0
IIB: Extension to other pelvic intraperitoneal tissues	T2b-N0-M0
Stage III: Tumor involves 1 or both ovaries or fallopian tubes, or peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes	**T1-3/N01/M0**
IIIA1: Positive retroperitoneal lymph nodes only (cytologically or histologically proven):	T1/T2-N1-M0
– IIIA1(i) Metastasis up to 10 mm in greatest dimension	
– IIIA1(ii) Metastasis more than 10 mm in greatest dimension	
IIIA2: Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes	T3a2-N0/N1-M0
IIIB: Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes	T3b-N0/N1·M0
IIIC: Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)	T3c-N0/N1-M0
Stage IV: Distant metastasis excluding peritoneal metastases	**Any T, any N, M1**
Stage IVA: Pleural effusion with positive cytology	
Stage IVB: Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)	

Figure 4. Staging of ovarian-, tubal-, and primary- peritoneal cancer according to FIGO and TNM.

Risk factors for epithelial ovarian cancer

Several risk factors are associated with EOC. Some of them are determined by lifestyle factors such as smoking, obesity (7), nulliparity (23) and potentially the use of hormone replacement therapy (HRT) (24). Other factors include early menarche (before the age of 12 years), late natural menopause (1), familial history of OC, endometriosis, and genetic mutations (25).

Endometriosis

The term endometriosis and the relationship between endometriosis and EOC were first described at the beginning of the 20th century by Sampson et al. (26). Globally, endometriosis affects approximately 10% of women and girls within the reproductive age (27) and this condition is associated with an elevated risk of developing clear cell and endometrioid cancers (28). In a study conducted in the Netherlands, the relationship between endometriosis and EOC was shown by the age-adjusted incidence rate ratios (IRR) of 3.92 (95% CI 2.19–7.01) for clear cell ovarian cancer and 2.39 (95% CI 1.28–4.45) for endometrioid ovarian cancer (29). Endometriosis is considered a primary risk factor associated with the development of CCC of the ovary, as it is identified in more than 50% of patients with clear cell carcinoma (30) and 30–40% of all endometrioid OC (31). Associations between endometriosis and EOC have been reported previously; however, the underlying molecular and cellular mechanisms remain unclear. Recent studies have suggested a possible genetic link between EC, CCC, and endometriosis (22, 26, 30). Driver mutations in PIK3CA, KRAS, ARID1A, and other genes have been found in pelvic endometriotic tissues and EOC (32).

BRCA

An inherited susceptibility to EOC is present in at least 15% of patients, most of whom have germline mutations in BRCA1/2. Up to 50% of patients with HGSC may exhibit homologous recombination deficiency (HRD) mediated by mechanisms, such as germline BRCA mutations, somatic BRCA mutations, or BRCA promoter methylation (33). Carriers of BRCA1/2 mutations have an increased lifetime risk of ovarian cancer (BRCA1 40–60% (34) and BRCA2 11–30%) (35). Patients diagnosed with EOC with BRCA1/2 mutations exhibit several clinical characteristics, including an increased likelihood of platinum sensitivity and enhanced survival rates compared to those with non-BRCA-related ovarian cancer (33).

EOC formation from serous tubal intraepithelial carcinoma (STIC) in dualistic model II is characterized by TP53 mutations. Mutations in the TP53 gene are extremely common and occur early in the progression of serous ovarian cancer (36). TP53 mutations, which result in p53 dysfunction, are associated with genomic instability in cancer cells. This instability can manifest as high levels of copy

number alterations, that is, changes in the copy numbers of certain genes in the genome. These alterations can occur in extended chromosomal regions and small focal aberrations, resulting in gene amplification or loss (37, 38).

The tumor suppressor protein, p53, plays a crucial role in maintaining genomic stability by regulating cell cycle, DNA repair, and apoptosis. Mutations in the TP53 gene, which encodes p53, can impair its function and lead to the loss of genomic stability. This can result in the accumulation of DNA damage, errors in DNA replication, and an increased risk of chromosomal abnormalities (39).

One consequence of genomic instability in cancer cells is copy number alterations. These alterations include amplifications or losses, which result in an increase or decrease in the number of copies of a gene, respectively. Copy number alterations can affect key oncogenes or tumor suppressor genes and contribute to the development and progression of cancer (40).

Studies have shown that TP53 mutations are associated with high copy number alterations in several types of cancer, including ovarian cancer. This genomic instability can lead to drug resistance, making TP53 mutations an important factor to consider when developing targeted cancer therapies (37, 41).

Overall, TP53 mutations and p53 dysfunction can contribute to genomic instability and copy number alterations in cancer cells, which carry profound implications for both the development and progression of cancer. Understanding these mechanisms may provide new insights into cancer biology and lead to the development of novel therapeutic strategies for cancer treatment (42, 43). Furthermore, there are additional gene mutations that have not undergone as thorough scrutiny as the aforementioned ones, underscoring the need for an expanded research effort.

Protective factors

In the context of risk factors, it is pertinent to acknowledge that certain factors could potentially be subject to modification by patients, whereas others could remain unalterable. It has already been proved that the number of births, breastfeeding, and use of oral contraceptives reduce the number of ovulations and risk of EOC (7). Given the etiological considerations of EOC originating from fallopian tube fimbriae, the practice of prophylactic salpingectomy becomes significantly relevant for patients undergoing benign hysterectomy (44-46).

Prophylactic salpingo-oophorectomy has been recommended as a risk-reducing strategy for women with BRCA gene mutations who are at a high risk of developing ovarian cancer. The fallopian tubes have been identified as the site of origin in many cases of high-grade serous ovarian carcinoma.

Several studies have suggested that prophylactic salpingo-oophorectomy can significantly reduce the risk of HGSC in women with BRCA gene mutations. One study reported that prophylactic salpingo-oophorectomy was associated with an 80% reduction in the risk of developing ovarian cancer in women with BRCA1 or BRCA2 mutations compared to those who did not undergo the procedure (47, 48).

Prophylactic salpingectomy can be performed either alone or in combination with prophylactic oophorectomy. Some women may choose to undergo both procedures to reduce their risk of developing ovarian cancer, whereas others may choose to undergo salpingectomy alone to preserve their fertility.

Prophylactic salpingo-oophorectomy is generally considered a safe and effective risk-reducing strategy with a low risk of surgical complications. However, it is important to note that the procedure is not 100% effective in preventing ovarian cancer, and women who undergo the procedure need to be closely monitored for signs or symptoms of the disease (48).

In general, prophylactic salpingo-oophorectomy is a crucial risk-reducing strategy for women with BRCA gene mutations who are at a high risk of developing ovarian cancer. It can be suggested as an integral component of a comprehensive risk reduction plan that may also include regular surveillance (49).

Diagnosis

Ovarian cancer is usually diagnosed at advanced stages owing to non-specific symptoms, such as abdominal distension, frequent urination, fatigue, and dyspepsia, among others. Patient survival rates and quality of life vary significantly depending on the EOC stage. As mentioned above, patients diagnosed with EOC in the first stage demonstrate a five-year survival rate exceeding 90%. In light of these survival outcomes contingent on the disease stage and leveraging the successful screening for other gynecological malignancies, such as cervical cancer, and the progress made in diagnostic methodologies, dedicated efforts are being made to develop an effective screening program. High expectations have revolved around the biomarker CA125, both in isolation or in combination with other biomarkers, as well as its integration with ultrasound. A large-scale study called the United Kingdom collaborative trial of ovarian cancer screening (UKCTOCS) (50) examined whether screening could be useful in ovarian cancer. Over 200,000 women participated in this study. At the end of the study, screening did not significantly reduce ovarian and tubal cancer deaths, and screening in the general population could not be recommended (51). However, even before the results of the UKCTOCS study were published, other research and diagnostic methods were attempted (51).

Biomarkers

In the biomedical context, a biomarker, also known as a biological marker, refers to a quantifiable characteristic that serves as an indicator of a biological state or condition (52). In the context being discussed, the CA125 biomarker is specifically relevant to ovarian cancer. Biomarkers play a crucial role in disease diagnosis and are essential for accurate and efficient identification of diseases. However, it is critical that biomarkers are not only accurate and precise but also fast and cost-effective. These qualities are necessary to ensure that biomarkers are widely accessible and can be used in clinical practice. Identifying new biomarkers is a complex and slow process, often requiring several years before they can be used in clinical practice (52).

Several biomarkers have already been used for the diagnosis of ovarian cancer, the most commonly used being Cancer Antigen 125 (CA125) and human epididymis protein 4 (HE4) antigens. Notably, new biomarkers that can potentially be used in practice to further personalize the diagnosis and treatment of EOC are available. The most promising biomarkers are cell-free deoxyribonucleic acid (cfDNA), circulating tumor DNA (ctDNA), exosomes, and microRNAs.

CA125

Robert Bast and Robert Knapp and their research team first isolated this monoclonal antibody in 1981 (53). The protein received the name "cancer antigen 125" because it was recognized by the OC125 antibody, which happened to be the 125th antibody produced during the study of the ovarian cancer cell line. (54). The ovarian cancer-related tumor marker, CA125, is a protein that in humans is encoded by the mucin (MUC)16 gene (55, 56) and is important for the diagnosis of EOC. CA125 is expressed and secreted by the epithelial ovarian tumors and other tissues (fallopian tubes, endometrium, endocervix, peritoneum, pleura, and pericardium). CA125 expression varies between tumor histological subtypes and can be elevated in benign ovarian tumors as well as in many other benign conditions, including endometriosis, pelvic inflammatory disease, early pregnancy, all-cause ascites, and congestive heart failure (57, 58). Owing to these limitations in sensitivity and specificity, CA125 is less useful as a diagnostic tool for EOC. Furthermore, in the advanced stages of EOC, more than 80% of patients exhibit elevated serum levels of CA125 (>35 U/mL), whereas, in the early stages, less than half of the EOC cases demonstrate an increase. (59). To improve diagnostic performance, CA125 has been used in combination with other biomarkers and transvaginal ultrasound (TVS).

HE4

HE4 is also known as the human epididymis protein 4 or WFDC2 (WAP four-disulfide core domain protein 2). HE4 is expressed in normal tissues of the female

reproductive tract, including the ovaries and fallopian tubes. It is also expressed in several types of cancer cells, including ovarian, endometrial, and lung cancer cells.

In ovarian cancer, HE4 is elevated and it has been studied as a potential biomarker for its diagnosis and monitoring of ovarian cancer. HE4 is often used in combination with another biomarker, CA125, in diagnostic tests for ovarian cancer, as exemplified by the Risk of Ovarian Malignancy Algorithm (ROMA).

HE4 has several advantages over CA125 as an OC biomarker. For example, HE4 is less likely to be elevated in women with non-cancerous conditions, such as smoking, endometriosis, or fibroids, which can lead to false positives when using CA125 alone (60, 61). Additionally, HE4 is more sensitive and specific than CA125 for detecting early-stage ovarian cancer (62, 63).

HE4 has also been studied as a potential prognostic biomarker for ovarian cancer, with higher levels of HE4 in the blood associated with poor prognosis and shorter survival times in women with ovarian cancer (64).

Although HE4 is a promising biomarker for ovarian cancer, but can be elevated in pancreatic, lung, and other cancer types (65, 66). Therefore, HE4 levels should be interpreted in conjunction with other diagnostic tests and clinical assessments when evaluating women with ovarian or other cancer types.

Circulating tumor DNA (ctDNA)

Circulating tumor DNA is the genetic material that is released into the bloodstream by cancer cells as they undergo apoptosis or necrosis (67). ctDNAs can be detected and analyzed using a simple blood test, making it a promising tool for the early detection and monitoring of cancer (68).

In ovarian cancer, ctDNAs have been studied as potential biomarkers for the diagnosis and monitoring of the disease (69). Analysis involving ctDNA has shown promise in detecting ovarian cancer at earlier stages than traditional diagnostic methods involving biomarker tests. In addition, ctDNA analysis may detect residual disease or recurrence earlier than imaging or other clinical assessments (70).

One study published in the Journal of Ovarian Research in 2020 evaluated the use of ctDNA to monitor response to treatment in women with ovarian cancer (71). This study found that ctDNA levels decreased in women who responded to treatment and increased in those who did not, suggesting that ctDNA analysis may be a useful tool for monitoring the treatment response and guiding treatment decisions (71).

Overall, ctDNA analysis holds promise as a tool for early detection and monitoring of ovarian cancer. However, further research is required to fully validate its clinical utility and optimize its use in the diagnosis and management of ovarian cancer.

Algorithms

Risk of Ovarian Malignancy Algorithm (ROMA)

The Risk of Ovarian Malignancy Algorithm (ROMA) is widely used to differentiate benign pelvic tumors from ovarian cancer. The ROMA score was calculated using the levels of two proteins, CA125 and HE4, and the patient's menopausal status. This score is used to assess the likelihood of ovarian cancer development.

More than a decade ago, Moore et al. introduced the Risk of Ovarian Malignancy Evaluation (ROME) Study, which aimed to validate a predictive model for assessing the risk of EOC in women with pelvic masses (72). The study found that the ROMA model served as a robust tool for predicting the risk of EOC, thus demonstrating its potential to effectively triage patients, warranting subsequent assessment and management at specialized centers of expertise.

However, the ROMA score is not intended to be used as a screening tool for ovarian cancer. Instead, it is used to help clinicians differentiate benign pelvic masses from ovarian cancer in women who have already been diagnosed with pelvic masses. The ROMA score is the most useful in guiding further diagnostic testing and referral to gynecologic oncologists for women with a high likelihood of ovarian cancer based on their ROMA score (73, 74).

Risk of Malignancy Index (RMI)

The Risk of Malignancy Index (RMI) was used to assess the likelihood of ovarian cancer in women with ovarian tumors. The RMI combines three factors: ultrasound features of the tumor, serum CA125 levels, and menopausal status.

The RMI was developed to help healthcare providers identify women with ovarian tumors who are at higher risk of ovarian cancer and may need further testing or referral to a gynecological oncologist for surgical intervention (75). This index has been validated in multiple studies and is considered a reliable tool for predicting the risk of ovarian cancer (76).

The RMI score is calculated using the following formula:

RMI = U x M x CA125

Where:

U = ultrasound score (1–3)

M = menopausal status score (1–3)

CA125 = serum CA125 level in U/ml

The ultrasound score is based on the appearance of the tumor on ultrasound imaging, with higher scores indicating a greater likelihood of malignancy. The menopausal status score is based on a woman's age and menopausal status, with higher scores indicating a greater likelihood of malignancy in postmenopausal women.

The RMI score ranges from 0 to > 200, with higher scores indicating a greater likelihood of malignancy. A score reaching or exceeding 200 is indicative of a high level of suspicion for ovarian cancer, necessitating an immediate referral to a gynecological oncologist (77).

It is important to note that the RMI is not a definitive diagnostic tool for ovarian cancer and should be used in conjunction with other diagnostic tests and clinical assessments. Women with a high RMI score may still have benign tumors, and those with a low RMI score may still have ovarian cancer. Therefore, it is important to individualize the management of ovarian tumors based on the patient's clinical presentation and risk factors (78).

Ultrasound

Ultrasound is an excellent diagnostic method of considerable significance in the diagnosis of ovarian cancer (79, 80). Although its potential as a screening method has not yet been definitively established, its value remains notably high, particularly when performed by highly skilled specialists (51). Guidelines have been developed to provide a structure and assistance in distinguishing between benign and cancerous tumors. The Simple Rules comprise a preoperative classification system for ovarian tumors. It consists of ten features, five of which are typical for benign tumors (B-features) and the other five for malignant tumors (M-features) (81). Simple Rules can be applied to diagnose ovarian cancer in women who require surgery and have at least one persistent adnexal (ovarian, para-ovarian, or tubal) tumor. Depending on whether B-features, M-features, both, or none are present, tumors are classified as Benign, Malignant, or Inconclusive, respectively. Specifically, if only B-features are applied, the tumor is classified as benign; if only M-features are applied, it is classified as malignant; and if no features are applied or both B- and M-features are applied, the tumor is classified as inconclusive.

The Logistic Regression model 2 and ADNEX model

The Logistic Regression model 2 (LR2) and Assessment of Different NEoplasias in the adneXa model (ADNEX) multivariable models are two ultrasound-based models with greater diagnostic accuracy for the preoperative evaluation of ovarian cancers recommended by the International Ovarian Tumor Analysis (IOTA). The LR2 is a multivariable model that calculates the likelihood (%) of malignancy of adnexal mass (82). Timmerman et al. introduced the LR2-model, which integrates

clinical data (age) and ultrasounds findings (ascites, blood flow within a solid papillary projection, maximum width of the solid component within a mass, irregular cyst walls, and the existence of acoustic shadows) (83). The ADNEX multivariable model was used to calculate the probability of malignancy of an adnexal tumor. The combination of clinical data (age, healthcare setting), ultrasound characteristics (maximum mass diameter, proportion of solid tissue, number of papillary projections, presence of more than 10 cyst locules, acoustic shadows, and presence of ascites), and CA125 levels, holds promise for the preoperative differentiation of benign, borderline, early stage, and advanced malignancies in ovarian masses (84).

Computed tomography (CT), positron emission tomography-CT, and magnetic resonance imaging (MRI)

Computed tomography is an effective diagnostic method with potential diagnostic value for cancer (85). However, for evident reasons (cumulative radiation dose or specificity), this method cannot be used for screening. CT has limitations in accurately identifying and defining the extent of lesions in the small pelvis. This imaging method is generally not specific, except in cases of fatty or calcified dermoid cysts or teratomas, where it can provide more precise information. However, the true strength of CT lies in its ability to detect intraperitoneal spread, such as tumor growth within the abdominal cavity, liver metastases, and metastases outside the abdominal region. Therefore, although CT may have limited value in assessing the characteristics and delineation of lesions in the small pelvis, it is highly valuable for identifying and evaluating the spread of cancer to other areas, aiding in the overall staging and management of the disease.

Advanced imaging methods such as positron emission tomography-CT (PET-CT) and MRI have been useful for identifying and staging a variety of malignancies (86). While PET/CT and MRI can provide valuable insights into ovarian cancer diagnosis and staging, their limitations in terms of specificity, sensitivity, tissue differentiation, and the complexity of ovarian tissue can impact their role in routine diagnosis (87, 88). Nonetheless, they have been proven to be excellent diagnostic tools when disease relapse or distant metastasis is suspected.

Computed tomography (CT) continues to be the initial diagnostic step for ovarian cancer. However, owing to recent advancements, particularly in MRI technology, radiologists can now provide imaging characteristics that can help gynecologists identify patients suitable for achieving complete cytoreduction (86, 89).

Prognostic factors

Several prognostic factors influence ovarian cancer outcomes.

Stage: Ovarian cancer is staged based on the extent of the disease, with higher stages indicating a more advanced disease and worse prognosis (1).

Histology: The histology or subtype of ovarian cancer can also affect the prognosis, with high-grade serous ovarian cancer being the most common and aggressive subtype (90, 91).

Tumor grade: Tumor grade, which reflects the degree of cancer cell differentiation, can also affect prognosis. Higher-grade tumors are generally more aggressive and have a worse prognosis (91, 92).

Age and performance status: Younger women with ovarian cancer tend to have a better prognosis than older women because younger patients are often more responsive to treatment and have a lower risk of developing comorbidities. The performance status of patients, which reflects their ability to perform daily activities and self-care, can also influence their prognosis. Patients with better performance status tend to have a better prognosis (2, 93).

Residual disease after surgery and response to treatment: The amount of residual disease left after surgery, or the presence of residual disease, also affects prognosis. Patients with no or minimal residual disease (less than 0.5 cm) have a better prognosis than those with a larger amount of residual disease (94, 95). The response of the tumor to initial treatments, such as chemotherapy, can also influence prognosis. Patients who respond well to treatment tend to have a better prognosis than those who do not (96, 97).

Genetic mutations: The presence of BRCA1/2 mutations is another factor. Women with these mutations are more responsive to certain types of platinum-based chemotherapy and are also candidates for targeted therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors (98, 99). In terms of prognosis, women with BRCA/2 mutations diagnosed with ovarian cancer exhibit better outcomes than noncarriers lacking BRCA1/2 mutations (99, 100).

Biomarkers: CA125 (101) and HE4 (102, 103) have emerged as significant prognostic indicators for ovarian cancer (104), aiding in the assessment of disease severity and potential outcomes. The EOC stage was associated with the absolute values of CA125. CA125 levels higher than 500 U/mL in high-grade serous carcinoma (105) and the advantageous stage of EOC can predict patient operability and survival rate (101).

Overall, a combination of these factors can be used to determine the prognosis of patients with ovarian cancer and guide treatment decisions.

Aims of the thesis

The primary objective of my doctoral thesis is to evaluate new biomarkers for EOC and analyze the risk of subsequent or simultaneous cancers in women with borderline tumors.

- The first study aims to evaluate whether the levels of NETs (double-stranded DNA (dsDNA) and the NET-specific marker H3Cit) increase in the plasma of patients with malignant ovarian tumors.
- The second study aims to identify new protein biomarkers and biomarker panels to improve the prediction of ovarian cancer survival.
- The third study aims to measure tumor-specific mutations in the plasma of ovarian cancer and borderline ovarian tumors (BOT) following targeted sequencing of primary tumor tissues and to relate ctDNA measurements to clinicopathological features and patient outcomes.
- The fourth study aims to analyze the risk of subsequent or simultaneous cancers in women with BOTs compared to the general female Swedish population.

Material and methods

Paper I

Prior to primary surgery for the removal of adnexal masses, peripheral blood samples were obtained from 199 patients admitted to the Department of Obstetrics and Gynecology in Lund, Sweden, between October 2014 and December 2017. The blood was collected in citrate tubes and the plasma was stored at −80˚C until analyzed. These patients were chosen to represent a range of ovarian tumor patients as well as some other pelvic malignancies from the GUNNEL biobank at Lund University, Medicon Village. Patient data and information on thromboembolic events were obtained from the GynOp Registry. Data collection from patients undergoing gynecological surgery according to the Swedish GynOp Registry began in 1997. Since 2004, GynOp has included major gynecological surgical procedures. The use of the registry is not obligatory, and certain regions have traditionally submitted data to other surgical quality registries (Gyn-Kvalitets-Registret (GKR)).

All data were sorted based on the tumor type and cancer stage, including benign (B), borderline (BOT), Stage I ovarian (EOC Stage I), Stage II–IV ovarian (EOC Stage II-IV), and other cancers. The amount of dsDNA was measured using a Quant-iT PicoGreen dsDNA assay (Invitrogen) following the manufacturer's instructions. Nucleosomal H3Cit (H3Cit-DNA complexes) was measured using an in-house enzyme-linked immunosorbent assay (ELISA) as previously described (106). CA125 levels were analyzed using a commercial electrochemiluminescence immunoassay Elecsys CA125 kit (Roche Diagnostics Scandinavia AB, Bromma, Sweden) following the manufacturer's instructions.

Statistical analyses

The data were analyzed using a variety of statistical tests, including Student's t-test, Pearson's chi-square test, and analysis of variance (ANOVA) with Bonferroni's post hoc test. Linear regression with log-transformed values was used to analyze the trends across groups. Survival probabilities were calculated using the Kaplan-Meier method and log-rank test. The Cox proportional hazards model was used for both univariate and multivariate analyses, with hazard ratios (HR) and 95% confidence intervals (CI) reported as point estimates. Assumptions of proportional hazards were visually verified as required. Power analysis was conducted with a 5% significance level and 90% power, which determined that a minimum of 21 patients were needed

in each group to detect a difference of 7% or more in the mean values of the H3Cit variable between the groups. All statistical analyses were performed using SPSSTM 26.0 2019 (IBM Corp, Armonk, NY, USA), and two-sided comparisons were performed.

Paper II

Peripheral blood samples were collected from 180 women with adnexal masses who were admitted for surgery at the Department of Obstetrics and Gynecology, Skåne University Hospital, Lund, Sweden, between 2005 and 2012. The blood samples were collected before the surgery in citrate tubes, then centrifuged, and the plasma was stored at −20°C until analyzed. All diagnoses were confirmed by histopathological examination and the disease was staged according to the FIGO criteria. Patients who received neoadjuvant chemotherapy were excluded. The patient group consisted of 28 early-stage EOC cases (FIGO stages I–II) and 88 advanced-stage EOC cases (FIGO stages III–IV). Frozen plasma samples were analyzed using Olink Proteomics AB (Uppsala, Sweden).

To measure proteins, the Olink Oncology II and Inflammation Panels from Olink Proteomics AB were used, following the manufacturer's instructions. The specific biomarkers included in each panel are listed in a previous study by Leandersson et al. (107). The assays employed Proximity Extension Assay (PEA) technology, which has been previously described (108) and was carried out at Olink Proteomics AB. The technicians who conducted the analyses were blinded to the patients' disease status. The samples were randomly distributed across the plates and analyzed in duplicate. Internal controls were used for quality assurance and normalization, and an interplate control was used to adjust for intra- or interplate variability. All assay validation data are available on the manufacturer's website. Of the EOC cases, six did not pass the internal quality control of the PEA analyses and were excluded from the statistical analyses because of either significant intracorrelation variance or the inability to read one of the duplicate samples.

Statistical analyses

To determine whether certain combinations of proteins were linked to survival at the 60-month mark, a combination of LASSO regression and cross-validation techniques was used. The selection of the 60-month time point was based on the frequent reporting of five-year survival rates in the existing literature. The data was randomly split into a training set and a test set, with the shrinkage parameter (λ) being determined using k-fold cross-validation on the training set. The λCV was subsequently utilized on the test set to perform variable selection, with the selected variables and their coefficients being saved and the process repeated ten times. The variables were then ranked based on their selection frequency and the sum of their

estimated coefficients, with the lowest ranked variable removed, and the process was repeated until the final model was selected. This method has been previously described by Leandersson et al. (2020). The final models were estimated using logistic regression, and the probabilities predicted by this model were used to evaluate their discriminatory abilities. A nonparametric bootstrap procedure was used to construct the receiver operating characteristic (ROCs) curves, and the AUC was calculated with 95% confidence intervals. All analyses were conducted using R v 4.1.0 (109).

Paper III

In this study, peripheral plasma samples were collected from 41 patients admitted to the Department of Obstetrics and Gynecology in Lund, Sweden between October 2004 and December 2012 for primary surgery of adnexal masses. The samples were obtained either the day before surgery or the same day and stored at −80°C until further analysis. Fresh frozen tumor biopsies from the same patients were also obtained and stored at −80°C until further analysis. The patient cohort was selected as a representative sample of patients with ovarian tumors from the biobank and grouped according to tumor type and cancer stage: benign (B), borderline (BOT), and ovarian cancer (OvCa Stage I–IV).

DNA was extracted from tumor samples using the AllPrep DNA/RNA Mini Kit (Qiagen) from samples obtained between 2018 and 2020. The tumor DNA samples were sheared to an average size of 250 bp using a Covaris ultrasonicator before generating Illumina-compatible sequencing libraries using the KAPA HyperPrep Kit (Roche). Total library yields were measured using Qubit and size distributions were checked using a BioAnalyzer (Agilent) before adding equimolar amounts of each library to pools subjected to target enrichment. The targeted regions of the 41 libraries were hybridized according to the manufacturer's protocol with either the xGen Pan-Cancer Panel (Integrated DNA Technologies; IDT) containing 127 cancer-associated genes or a custom xGen Lockdown Probes panel covering exons and hotspots in 14 genes recurrently mutated in cancer (IDT). Finally, the hybridized libraries were sequenced using either an Illumina NextSeq 550 or MiSeq instrument. A more comprehensive explanation of these methods is provided in the published article.

Statistical analysis of the data involved the use of ANOVA with Bonferroni correction as a post hoc test, and trends across ordered groups were analyzed using linear regression with log-transformed values. Overall survival probabilities were calculated using the Kaplan-Meier method and assessed using the log-rank test. Statistical comparisons were conducted on a two-sided basis with a 5% level of significance. SPSS 26.0 (IBM Corp, Armonk, NY, USA) was used for all statistical analyses.

Paper IV

Study design, population, and setting: A nationwide open-cohort study was conducted in Sweden between 1995 and 2018 that included a total population of 6,838,524 women residing in the country at the study's inception. The study had a total follow-up of 116,406,014 person-years.

Ascertainment of the main predictor variable: Serous or mucinous ovarian borderline tumors (BOTs) were obtained from the Swedish Cancer Register between 1995 and 2018, which had achieved full national coverage by that time. Serous BOTs (84423 and 84513) and mucinous BOTs (84723) were identified using SNOMED-10 codes. However, codes for clear cell (84,623, n=384) and endometrioid borderline tumors (83801, 83802, 83811, n=35) were excluded because of the small number of cases. The study included 4,998 women diagnosed with any type of BOTs, except for two cases with missing information.

The study's outcome variables encompassed non-ovarian cancer, identified through meticulous utilization of the comprehensive Swedish Cancer Register, employing the 7th revision of the International Classification of Diseases (ICD-7) (as illustrated in the results (Table 2)). Each participant was included only once for each study outcome. To evaluate the potential differences among women who later developed nonovarian cancers, certain parameters, such as age, period (years since BOT diagnosis), highest educational level (as a proxy for socioeconomic status), and geographical region were included as covariates. The age groups selected were <60, 60–69, and >70 years of age, as most cancers occur in older age groups. The aim of including these covariates was to investigate whether there were any specific period, age, socioeconomic status, or regional differences among women who developed non-ovarian cancers.

The data for the study were collected from two sources: the Swedish Cancer Register (managed by the National Board of Health and Welfare; in Swedish: *Socialstyrelsen)* and the Total Population Register, managed by Statistics Sweden. The Cancer Register provides information on BOTs and outcomes, whereas the Population Register contains data on the population of women in Sweden during the study period, along with covariates and details on emigration and death. The Total Population Register is considered nearly 100% complete for the entire national population. All linkages between registry data were conducted using a unique 10-digit personal identification number assigned to each person for their lifetime upon birth or immigration. However, the research group only had access to a pseudonymized version of this number to maintain the confidentiality of all individuals.

Table 2. Number of cases of cancer (non-ovarian cancer) in women in Sweden, 1995–2018

Tumor	ICD-7 codes	Number of cases	%
Breast	170	152,517	29
Colon	153	41,720	7,9
Lung	162, 163	36,128	6,9
Endometrium	172, 174	31,922	6,1
Skin	191	31,693	6
Melanoma	190	27,427	5,2
Primary unknown	199	18,040	3,4
Leukemia	204, 205, 206, 207, 208, 209	17,987	3,4
Rectum	154	17,450	3,3
Nervous system	193	16,798	3,2
Non-Hodgkin's lymphoma	200, 202	16,123	3,1
Bladder	181	13,633	2,6
Endocrine gland	195	13,228	2,5
Pancreas	157	11,593	2,2
Cervix	171	11,014	2,1
Liver	155, 156	10,183	1,9
Kidney	180	9,538	1,8
Stomach	151	8,103	1,5
Upper aerodigestive tract	140, 141, 143, 144, 145, 146, 147, 148, 161	7,295	1,4
Thyroid gland	194	6,534	1,2
Myeloma	203	6,190	1,2
Vulvovaginal cancer	176	4,823	0,9
Connective tissue	197	3,013	0,6
Esophagus	150	2,505	0,5
Small intestine	152	2,272	0,4
Anus	1541	2,127	0,4
Hodgkin's disease	201	1,961	0,4
Others		1,552	0,3
Eye	192	1,387	0,3
Salivary gland	142	1,033	0,2
Bone	196	735	0,1
All		**526,524**	**100**

Statistical analyses

Person-year calculations were initiated from the initial diagnosis of BOTs in 1995 onwards, extending until the diagnosis of any non-ovarian cancer, death, emigration, or the conclusion of the follow-up period in 2018. Standardized incidence ratios (SIRs) were calculated to compare the relative risk of non-ovarian cancers in BOTs and in individuals who had never been registered for BOTs. The SIR was calculated as the ratio of the observed (O) to expected (E) number of non-ovarian cancers by indirect standardization methods using the following formula:

$$SIR = \frac{\sum_{j=1}^{J} O_j}{\sum_{i=1}^{J} n_j \lambda_j^*} = \frac{O}{E^*}$$

where: O = ∑Oj denotes the total observed number of non-ovary cancer cases in the study group (registered for BOTs); E* is calculated by applying stratum-specific standard incidence rates (λ_j^*) obtained from the reference group (no registration for BOTs) to the stratum-specific person-year (n_j) experience of the study group; O_j represents the observed number of cases that the cohort subjects contribute to the jth stratum; and j represents the strata defined by the cross-classification of various adjustment variables, including age, educational level, and region (110, 111). All calculations were standardized by age (five-year-age-groups), period (five-year-period-groups), highest educational level (as a proxy for socioeconomic status), and geographical region. The 95% confidence intervals (CIs) of the SIRs were calculated assuming a Poisson distribution. Statistical significance was defined as $p < 0.05$. For multiple comparisons, 99% of CIs for significant sites are shown in footnotes. All analyses were performed using the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA).

Ethical considerations

Conducting medical research on human subjects is crucial for gaining a comprehensive understanding of various human diseases and improving prevention, diagnosis, and treatment methods. However, this research must adhere to the ethical standards outlined by the Declaration of Helsinki. These standards ensure the protection of human health and rights and promote respect for all individuals. Only studies in which the benefits outweigh the potential risks and burdens to the research subjects can be conducted. Additionally, research participation must be voluntary and subjects must provide informed consent while being informed of their right to withdraw consent at any time. (World Medical Association, 2013).

The outcomes of the four studies in this thesis did not affect the treatment or prognosis of women who participated in the studies. Nonetheless, improving diagnostic methods for future patients with ovarian cancer is crucial, and the potential benefits outweigh the minimal harm caused by blood and tissue samples. Registry research provides valuable population-level information; however, researchers must prioritize protecting the privacy and integrity of subjects to avoid harm from personal information dissemination. It is the researcher's responsibility to ensure the safety and respect of all research participants according to ethical standards (World Medical Association, 2013).

At the time of admission to the Department of Obstetrics and Gynecology, Skåne University Hospital, Lund, the participating women provided written and oral informed consent for the biomarker studies (Papers I, II, and III). Paper IV was a registry study in which no informed consent was necessary for ethical approval.

Study I: Ethical approval was granted by the Ethical Review Board at the Faculty of Medicine, Lund University, Sweden, Dnr 495 2016 (amendment to Dnr 558–2004 and 94–2006) and the Regional Ethics Board, University of Umeå Dnr 2013-155-32M (Supplement to 08-120M).

Study II-III: Ethical approval was granted by the Ethical Review Board of the Faculty of Medicine at Lund University, Sweden. Dnr 495 2016 (amendment to Dnr 558–2004 and 94–2006).

Study IV: This was a non-intervention nationwide register study of pseudonymized secondary data obtained from Swedish authorities after approval from the Ethical Review Board in Lund, Sweden (2013/736 and later amendments).

Results

Paper I

The patients were categorized into different groups based on the type of tumor: benign (n=39), BOT (n=32), stage I (n=23), stages II–IV (n=99), and other cancers (n=6) (Table 1). The BOT group had the youngest patients with a mean age of 53 years ±19.59, while the EOC stages II–IV group had the oldest patients with a mean age of 66 years ±9.38. The highest body mass index (BMI) of all patient groups was not greater than 30 kg/m^2. The longest surgeries were observed in the EOC stages II–IV group ($p < 0.01$), and blood loss (ml) was significantly higher in this group than in the benign and borderline groups ($p < 0.01$). Most analyzed patients belonged to the American Society of Anesthesiologists (ASA) I–II group. Patients with cancer required a longer time to return to normal daily living ($p = 0.05$). Among the 98 patients who responded to the eight-week follow-up questionnaire, a significant difference in thromboembolic events was not evident between the EOC stages II–IV group (three events out of 60 (5%)) and the remaining patients with ovarian tumors (five events out of 33 (15%)).

The plasma levels of dsDNA and H3Cit-DNA did not differ between patients with benign, borderline, or malignant ovarian tumors, or other cancer types (Figures 5 and 6). However, plasma CA125 levels were higher in BOT, EOC stage I, and EOC stages II–IV in comparison to benign ovarian tumors and other pelvic cancer types ($p_{trend} < 0.001$).

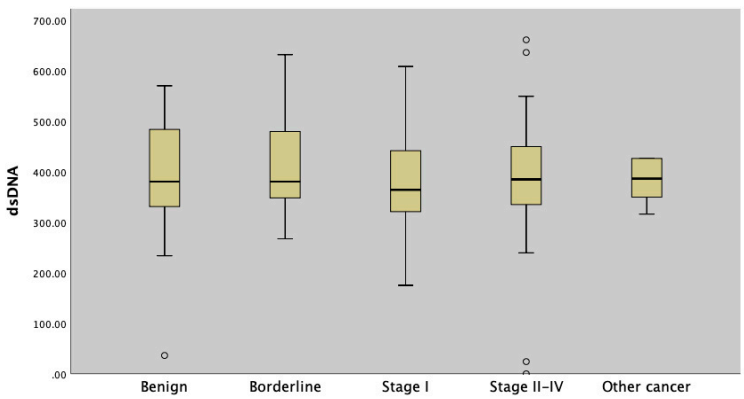


Figure 5. Plasma levels of dsDNA did not differ between patients with benign, borderline, or malignant ovarian tumors.

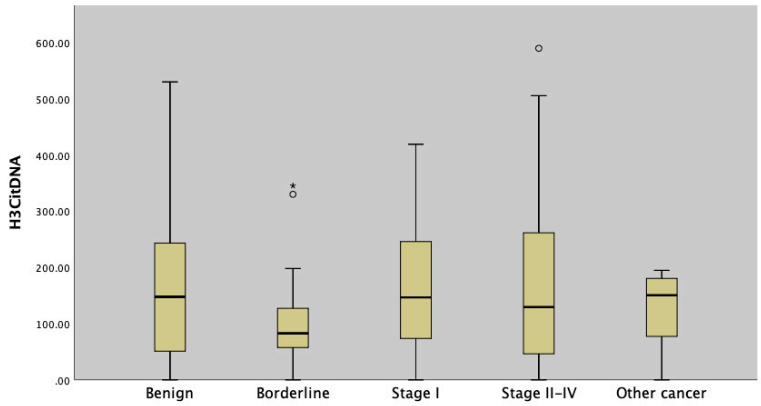


Figure 6. Plasma levels of H3CitDNA did not differ between patients with benign, borderline, or malignant ovarian tumors.

Survival analyses were conducted, excluding benign and borderline tumors. The biomarkers CA125, dsDNA, and H3Cit-DNA were analyzed as continuous variables, dichotomized, and divided into quartiles. Uni- and multivariable Cox regression analyses with continuous variables showed that increased levels of CA125 were associated with worse overall survival (HR 2.0, 95% CI 1.2–3.1, p=0.004). No significant differences were observed in the survival of malignant ovarian tumors when the dsDNA and H3Cit-DNA levels were analyzed. However, in the univariable Cox regression analysis, high levels of CA125 dichotomized at 326 IU/ml (median) showed worse overall survival, with a hazard ratio of 1.9 (95% C.I. 1.03–3.36; p=0.038) and a log-rank test (p=0.034) (median follow-up time 37 months).

Paper II

This study analyzed the diagnostic performance of various biomarkers in predicting survival outcomes in patients with EOC. The analysis included plasma samples from 116 patients with EOC with different histopathological morphologies.

The diagnostic performance of the reference model, which included age alone in predicting survival, was poor. However, adding NT-3 alone and in combination with GPNMB and MSLN improved the performance of the model. The best results were obtained for the model that included age and the three-biomarker panel combination of NT-3 + GPNMB + MSLN [AUC=0.792 (0.707–0.878); p=0.004] (Table 3, Figure 7).

Table 3. Biomarker model based on LASSO regression when the reference model included age.

Reference model	AUC (95%)	*p*-value*	Sensitivity at 95% specificity	Specificity at 95% sensitivity	Specificity at the best point	Sensitivity at the best point
Age	0.624 (0.516-0.732)	-	0.253 (0.024-0.410)	0.091 (0.000-0.242)	0.879 (0.394-1.000)	0.422 (0.217-0.855)
Additional marker combinations						
NT-3 + GPNMB + MSLN	0.792 (0.707-0.878)	0.004	0.410 (0.265-0.602)	0.333 (0.121-0.546)	0.818 (0.514-1.000)	0.699 (0.410-0.964)
NT-3 + GPNMB	0.768 (0.705-0.870)	0.008	0.410 (0.253-0.578)	0.333 (0.152-0.525)	0.879 (0.455-1.000)	0.615 (0.374-0.976)
NT-3	0.730 (0.632-0.828)	0.054	0.325 (0.205-0.446)	0.242 (0.091-0.424)	0.758 (0.515-1.000)	0.699 (0.301-0.868)

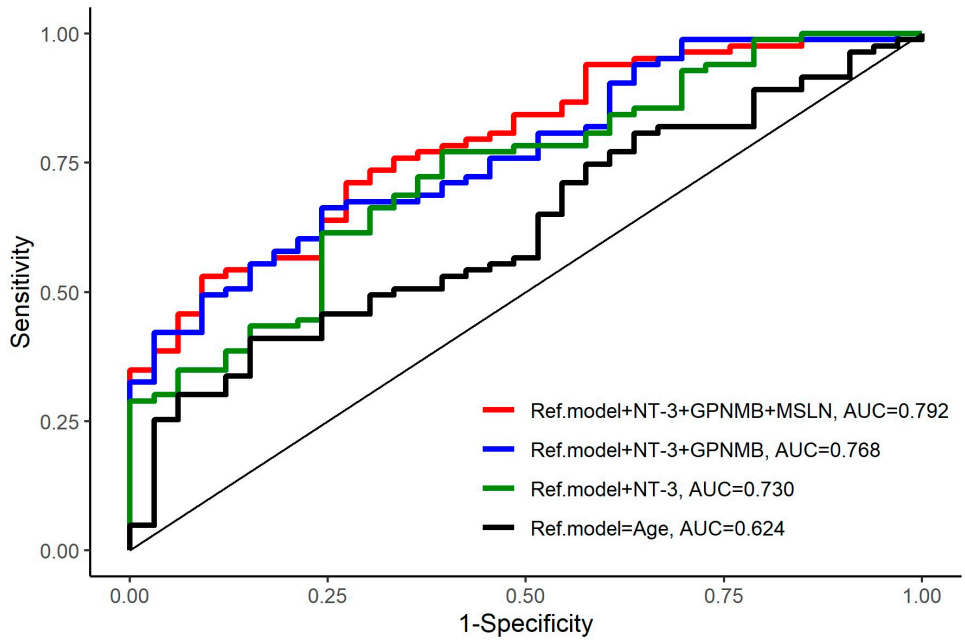


Figure 7. Comparison of the reference model and models with added biomarkers. A three-biomarker model including NT-3, GPNMB, and MSLN with age as the reference model demonstrated the highest prediction of survival (AUC=0.792, p=0.004)

Incorporating the biomarkers CA125 and HE4 into the reference model further improved the diagnostic performance. The addition of NT-3 + GPNMB + MSLN to the reference model that included age, CA125, and HE4 (0.748–0.902); p= 0.003] provided the best results for predicting survival outcomes.

Another reference model that included age and stage (early vs. late) was tested. The addition of the biomarker CCL28 + TCL1A + GPNMB to this reference model

improved the prediction of overall survival (AUC=0.899 (0.831–0.966; p=0.048]. However, the exclusion of GPNMB and TCL1A from the model resulted in outcomes with no statistically significant differences.

Finally, a model was created starting with a reference model that included age alone. Adding stage (early vs. late) and CCL28 + GPNMB biomarkers to this model significantly improved the prediction of OS (AUC=0.864 (0.783–0.946); p<0.00) (Table 4, Figure 8).

Table 4. Biomarker models based on LASSO regression when the reference model exclusively includes age (and stage IV is excluded).

Reference model	AUC (95%)	*p*-value*	Sensitivity at 95% specificity	Specificity at 95% sensitivity	Specificity at the best point	Sensitivity at the best point
Age	0.633 (0.519-0.746)	-	0.258 (0.015-0.424)	0.063 (0.000-0.250)	0.889 (0.394-1.000)	0.422 (0.217-0.855)
Additional marker combinations						
Stage I+II vs. III + CCL28 + GPNMB1	0.864 (0.783-0.946)	<0.001	0.364 (0.030-0.788)	0.406 (0.188-0.750)	0.844 (0.656-1.000)	0.818 (0.621-0.955)
Stage I+II vs. III + CCL28	0.856 (0.770-0.942)	0.001	0.318 (0.000-0.803)	0.281 (0.031-0.781)	0.875 (0.688-0.969)	0.803 (0.667-0.955)
Stage I+II vs. III	0.834 (0.750-0.918)	<0.001	0.439 (0.258-0.621)	0.375 (0.031-0.750)	0.781 (0.563-0.969)	0.849 (0.606-0.970)

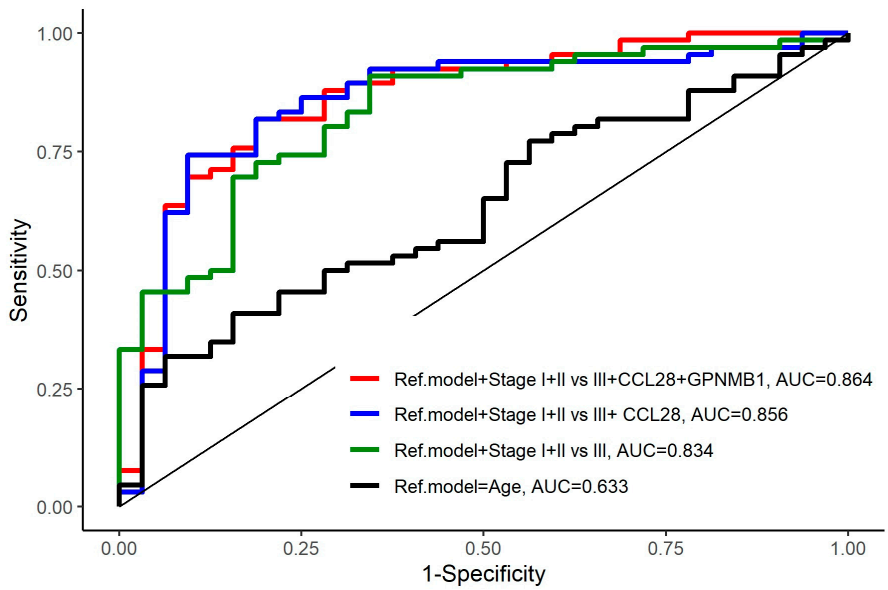


Figure 8. Comparison of the reference model and models with added biomarkers. The model including stage I+II vs. III+CCL28+GPNMB1 with age as the reference model showed the highest prediction of survival (AUC=0.864, p<0.001).

Additionally, in this study, we observed that a combination of high levels of both CA125 and HE4 efficiently predicted unfavorable survival outcomes (p=0.05) (Figure 9).

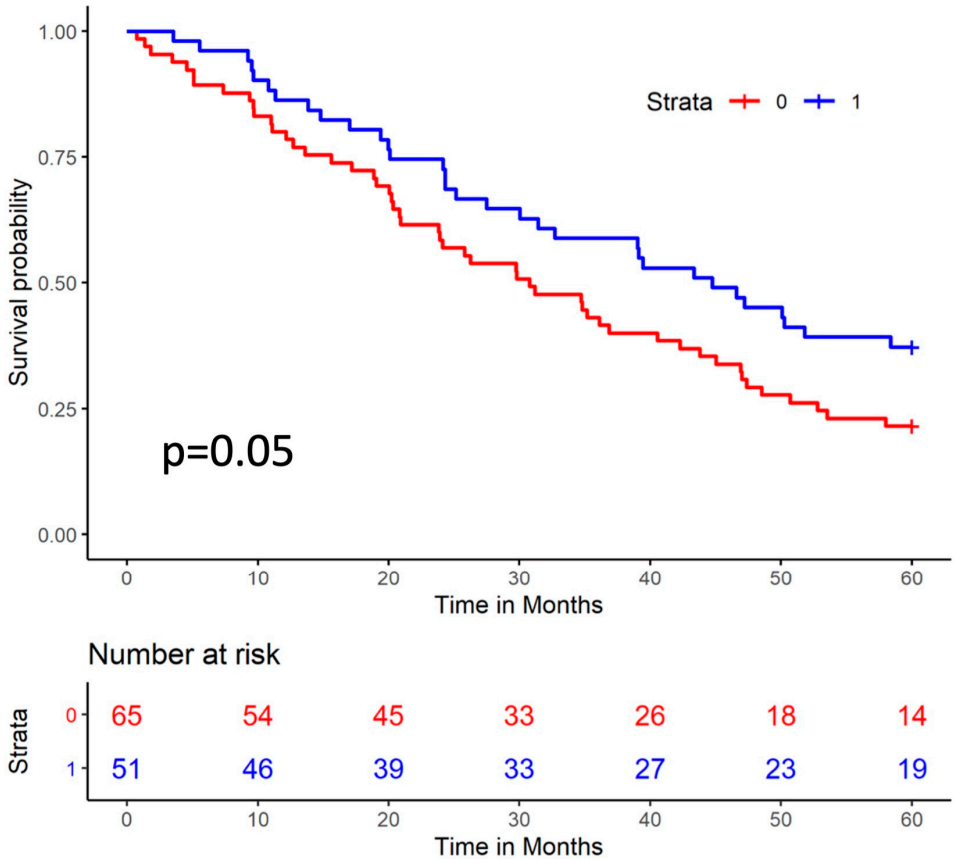


Figure 9. Kaplan-Meier analysis of overall survival in patients in terms of CA125 and HE4 levels. Patients with both values above the median are shown in red, and patients with one or both biomarkers below the median are shown in blue.

Paper III

Patient data were stratified into three groups based on the tumor type: benign (n=6), borderline ovarian (n=9), and EOC (n=26). The benign group mainly comprised younger patients (mean age: 48 years ±13.3) in comparison to the cancer group (mean age: 64 years ±11.9). The preoperative levels of CA125 were 293.7 ±627.3 units/mL in the BOT group and 1,264.3±1,147.1 units/mL in the cancer group. Somatic mutations were identified in 24 tumor samples, of which seven were detected in BOT patients and 17 in those with ovarian cancer. The most commonly mutated genes were TP53, KRAS, PIK3CA, PIK3R1, and BRAF (Figure 10). In stage III ovarian cancer, TP53 mutations were present in eight tumors, and one tumor exhibited PIK3R1 mutations (Figure 10). KRAS mutations were observed in four mucinous borderline tumors and two serous borderline tumors.

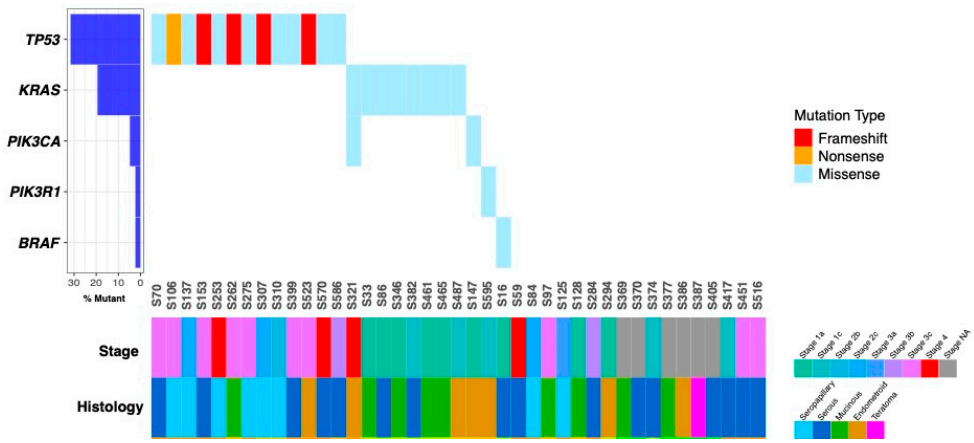


Figure 10. Waterfall plot of validated somatic mutations in the patient tumors. Genes are indicated in rows, and samples in columns. Mutated samples are shown according to mutation type. Patient and tumor clinicopathological variables are shown below the patient IDs.

Mutations in TP53, KRAS, PIK3CA, and PIK3R1 have been detected in the plasma. Of these patients, seven with stage III, three with stage IV, and four with stage I and II ovarian cancer exhibited detectable ctDNA in their plasma samples. All serous cancer patients with detectable ctDNA exhibited TP53 mutations, and one serous borderline patient exhibited a plasma KRAS mutation. The concentration of ctDNA increased with increasing stages ($p_{trend}<0.001$). ctDNA concentrations in stages III and IV were significantly higher than those in stage I ($p=0.025$ and $p=0.007$, respectively) (Figure 11). Ten mutant copies/mL were chosen for survival analysis after evaluating the results (Figure 11), in which the number of mutant copies was correlated with the stage. Patients with more than 10 mutant copies/mL of plasma demonstrated significantly unfavorable overall survival ($p=0.008$ by log-rank test) (Figure 12).

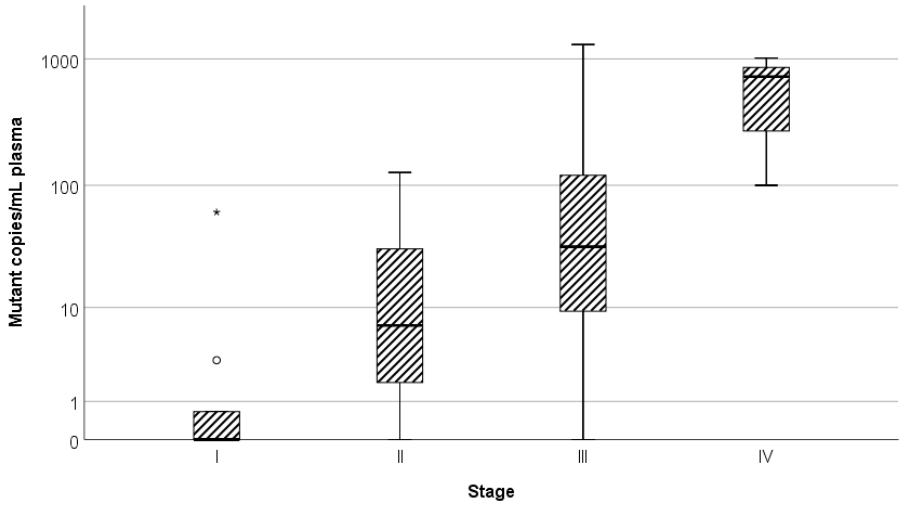


Figure 11. Plasma circulating tumor DNA (ctDNA) concentration increased with higher stage (p_{trend}<0.001). Bars include highest and lowest values, except outliers (○), which are 1.5 to 3 box lengths from the end of the box, and extremes (*) which are more than 3 box lengths from the end of the box.

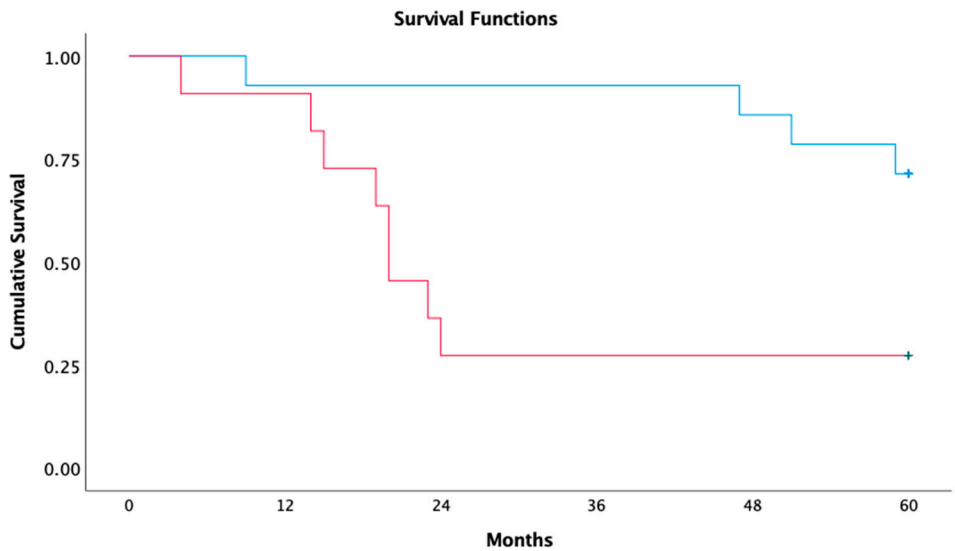


Figure 12. Kaplan Meier analysis of overall survival in patients related to a genetic mutation found in plasma (OS, p=0.008 by log-rank test). Blue line <10 copies/mL, red line >10 copies/mL. The mortality rate was two out of three patients in the group with ctDNA <10 copies/mL and 10 out of 11 patients in the group with ctDNA >10 copies/mL.

Paper IV

This study included 4,998 women diagnosed with BOTs between 1995 and 2018, with a mean age of 55.7 years at diagnosis. Of these, 2,971 (59%) were diagnosed with serous BOTs, and 2,027 (41%) were diagnosed with mucinous BOTs. Additionally, 526,524 women in the general female population were diagnosed with non-ovarian cancer during the same period.

Data analysis showed that women with BOTs demonstrated an increased risk of developing various non-ovarian cancers compared to the general female population. Specifically, women with BOTs exhibited a higher risk of small intestinal, colon, endometrial, pancreatic, cervical, lung, kidney, upper aerodigestive tract, bladder, and rectal cancers, as well as primary unknown cancers (Table 5). Women with serous BOTs also exhibited an increased risk of developing thyroid and vulvovaginal cancers.

The increased risk of non-ovarian cancer was further analyzed based on the age at the time of BOT diagnosis. Women diagnosed with BOTs at the age of 60 years or younger demonstrated a higher risk of colon, pancreatic, endometrial, vulvovaginal, lung, and rectal cancers. Women aged 60–69 years at the time of BOT diagnosis demonstrated a higher risk of cervical, breast, pancreatic, kidney, and upper aerodigestive tract cancers, and melanoma. In contrast, women aged 70 years at the time of BOT diagnosis demonstrated a higher risk of colon, endometrial, lung, bladder, small intestine, pancreatic, and upper aerodigestive tract cancer.

Follow-up duration was also examined concerning the risk of non-ovarian cancer. Women with BOTs exhibited an increased risk of various cancers within the first year of diagnosis, including colon, rectum, small intestine, breast, pancreas, nervous system, bladder, kidney, liver, endometrial, cervical, vulvovaginal, and non-Hodgkin lymphoma. More than ten years after the diagnosis, women with BOTs exhibited a higher risk of developing lung cancer.

Table 5. Subsequent risk of cancers in women with earlier diagnosis of borderline ovarian tumor (BOT), 1995-2018, sorted by sorted by coding system.

			Serous					Mucinous					All				
Cancer	**IDC-7 codes**	**Total number of events**	**O**	**E**	**SIR**	**95% CI**		**O**	**E**	**SIR**	**95% CI**		**O**	**E**	**SIR**	**95% CI**	
Upper aerodigestive tract	a)	7 295	7	3.47	2.02	0.80	4.18	6	2.35	2.55	0.92	5.59	13	5.82	**2.23**	**1.18**	**3.83**
Stomach	151	8 103	6	3.04	1.97	0.71	4.32	2	2.23	0.90	0.08	3.30	8	5.26	1.52	0.65	3.01
Small intestine	152	2 272	5	1.07	**4.67**	**1.47**	**10.99**	**4**	0.74	**5.41**	**1.41**	**13.98**	9	1.81	**4.97**	**2.25**	**9.48**
Colon	153	41 720	52	18.92	**2.75**	**2.05**	**3.61**	29	13.24	**2.19**	**1.47**	**3.15**	81	32.2	**2.52**	**2.00**	**3.13**
Rectum	154	17 450	13	7.71	1.69	0.89	2.89	9	5.42	1.66	0.75	3.17	22	13.1	**1.68**	**1.05**	**2.54**
Liver	155,156	10 183	7	4.41	1.59	0.63	3.29	6	3.13	1.92	0.69	4.20	13	7.54	1.72	0.91	2.96
Pancreas	157	11 593	11	5.67	1.94	0.96	3.48	11	3.9	**2.82**	**1.40**	**5.06**	22	9.58	**2.30**	**1.44**	**3.48**
Lung	162,163	36 128	28	19.44	1.44	0.96	2.08	31	13.18	**2.35**	**1.60**	**3.34**	59	32.6	**1.81**	**1.38**	**2.33**
Breast	170	152 517	75	68.87	1.09	0.86	1.37	59	46.24	1.28	0.97	1.65	134	115	1.16	0.98	1.38
Cervix	171	11 014	8	3.42	**2.34**	**1.00**	**4.63**	7	2.56	**2.73**	**1.08**	**5.67**	15	5.98	**2.51**	**1.40**	**4.15**
Endometrium	172,174	31 922	43	14.76	**2.91**	**2.11**	**3.93**	18	10.21	**1.76**	**1.04**	**2.79**	61	25	**2.44**	**1.87**	**3.14**
Vulvovaginal cancer	176	4 823	6	2.11	**2.84**	**1.02**	**6.23**	1	1.48	0.68	0.00	3.87	7	3.59	1.95	0.77	4.04
Kidney	180	9 538	9	4.32	2.08	0.94	3.97	8	3.01	**2.66**	**1.14**	**5.26**	17	7.33	**2.32**	**1.35**	**3.72**
Bladder	181	13 633	10	6.35	1.57	0.75	2.91	9	4.41	2.04	0.93	3.89	19	10.8	**1.77**	**1.06**	**2.76**
Melanoma	190	27 427	14	12.31	1.14	0.62	1.91	10	8.27	1.21	0.58	2.23	24	20.6	1.17	0.75	1.74
Skin	191	31 693	23	15.27	1.51	0.95	2.26	6	10.38	0.58	0.21	1.27	29	25.7	1.13	0.76	1.62
Nervous system	193	16 798	10	6.54	1.53	0.73	2.82	6	4.46	1.35	0.48	2.95	16	11	1.45	0.83	2.37
Thyroid gland	194	6 534	7	2.27	**3.08**	**1.22**	**6.39**	1	1.57	0.64	0.00	3.65	8	3.84	2.08	0.89	4.13
Endocrine gland	195	13 228	8	5.11	1.57	0.67	3.10	4	3.5	1.14	0.30	2.96	12	8.61	1.39	0.72	2.44
Connective tissue	197	3 013	3	1.1	2.73	0.51	8.07	2	0.79	2.53	0.24	9.31	5	1.89	2.65	0.83	6.22
Primary unknown	199	18 040	16	6.89	**2.32**	**1.32**	**3.78**	13	5.09	**2.55**	**1.35**	**4.38**	29	12	**2.42**	**1.62**	**3.48**
Non-Hodgkins lymphoma	200,202	16 123	8	7.07	1.13	0.48	2.24	8	4.92	1.63	0.69	3.22	16	12	1.33	0.76	2.17
Leukemia	b)	17 987	11	7.69	1.43	0.71	2.57	5	5.3	0.94	0.30	2.22	16	13	1.23	0.70	2.00
All		526 524	392	235.24	**1.67**	**1.51**	**1.84**	259	161.53	**1.60**	**1.41**	**1.81**	651	397	**1.64**	**1.52**	**1.77**

O=Observed; E=Expected; SIR=Standardized incidence ratio; CI=Confidence intervals. Bold types: 95% CI does not include 1.00.

99% CI for significant SIRs: serous, colon (1.77-4.00); endometrium (1.79-4.39); and all (1.44–1.92); Mucinous, colon (1.19-3.59); lung (1.31-3.80); and all (1.33–1.91); All, small intestine (1.38-11.60); pancreas (1.12-4.03); colon (1.79-3.41); lung (1.20-2.58); cervix (1.01-4.91); endometrium (1.64-3.46); kidney (1.00-4.37); primary unknown (1.31-3.97), and all (1.46-1.83).

a) = 140, 141, 143, 144,145,146, 147, 148, 161 b) = 204, 205, 207, 208, 209

Discussion

The identification of cancer biomarkers plays a crucial role in predicting survival outcomes and guiding treatment decisions in patients with ovarian cancer.

Over the past decade, interest in the role of NETs in cancer progression has increased (112, 113). While neutrophils are recognized as an important element in tumor progression, their response varies depending on the stimulus (114), which can result in the activation of neutrophils with various anti- and pro-tumor phenotypes (115). Studies have shown that Markers of NETs formation, such as H3Cit, are found in the plasma of patients with acute microangiopathies (116, 117). Animal models have also demonstrated NETs formation in the omentum of ovarian tumor-bearing mice before the onset of metastasis, and a neutrophil-specific deficiency of PAD4, an enzyme essential for NETs formation, reduced omental metastasis in these mice (118).

In Paper I, the plasma levels of dsDNA and H3Cit-DNA did not differ significantly among the different tumor types. However, plasma CA125 levels were found to be a significant predictor of worse overall survival in patients with malignant ovarian tumors. CA125 levels were significantly higher in borderline and malignant ovarian tumors than in benign tumors and other pelvic cancer types.

This study did not verify the hypothesis. Circulating H3Cit-DNA and dsDNA levels in patients with ovarian cancer were not elevated in patients with ovarian cancer compared to other groups. The study also found that CA125, a commonly used ovarian cancer biomarker, was higher in advanced-stage III and IV cancer patients than in borderline and early-stage tumors. However, this biomarker may also be elevated in other benign gynecological diseases and medical conditions, reducing its sensitivity and specificity (119).

Several studies have suggested that biomarkers of NETs formation are associated with the occurrence of VTE in patients with cancer (120), and patients with ovarian cancer are known to be at high risk for VTE (121, 122). Surgical intervention is a crucial factor for improving the survival of patients with ovarian cancer, and maximal cytoreductive tumor debulking surgery is recommended to achieve no residual tumors (123). However, thorough tumor debulking can increase the risk of venous thromboembolism (VTE), which is why patients with ovarian cancer are classified as high-risk. This study found a lack of increase in the incidence of self-reported VTE events in the ovarian cancer group compared to patients who

underwent surgery without a cancer diagnosis, which may be due to the recommendations of prolonged anticoagulation prophylaxis in ovarian cancer patients (124). Further studies are needed to evaluate the role of NETs formation in various cancer cohorts, especially those with a known increased risk of thromboembolic events.

In the second study, we suggested that combining different factors, such as known biomarkers, age, and disease stage, may provide satisfactory results. We analyzed the diagnostic performance of various biomarkers, including NT-3, GPNMB, MSLN, CA125, and HE4, for predicting survival outcomes in patients with epithelial ovarian cancer.

In this study, we found that biomarker panels could improve the prediction of survival, and the model including age and a combination of neurotrophin-3 (NT-3), transmembrane glycoprotein NMB (GPNMB), mesothelin (MSLN), CA125, and HE4 was the best model for predicting OS.

Neurotrophins such as NT-3 have been shown to regulate angiogenesis and contribute to tumor progression and angiogenesis in several types of cancers (125). Neurotrophins are a family of growth factors that play crucial roles in nervous system development and maintenance. Recent studies have shown that they have important functions in cancer biology (126). For example, NT-3 promotes the survival and proliferation of various types of cancer cells, including breast cancer cells and gliomas, they act by activating the TrkC receptor (125, 127). Moreover, elevated NT-3 levels have been associated with increased tumor aggressiveness and poor patient outcomes in several types of cancer (128). As previously stated in the introduction, CA125 and HE4 are specific and commonly used in the diagnosis of late-stage EOC. But CA125 has a low sensitivity and limited specificity for the detection of early-stage EOC. Our study demonstrated that, in combination with other data, biomarkers can have enhanced specificity for determining OS.

Combining age with CA125 and HE4 levels and the addition of NT-3 significantly improved the OS prediction model. GPNMB and MSLN have also been identified as biomarkers for the prediction of poor survival. Higher levels of GPNMB promote angiogenesis, migration, invasion, and metastasis of cancer cells (129)(130), whereas abnormal expression of MSLN plays an important role in tumor cell growth, invasion, and metastasis (131, 132). CCL28, which regulates cell chemotaxis, has also been identified as a biomarker for the prediction of poor survival in combination with TCL1A, GPNMB, EOC stage, and age.

The study sample had limitations due to the heterogeneity of the epithelial histopathological subtypes, as only patients scheduled for primary upfront surgery were included in the study. Despite these limitations, we obtained positive results, which could provide an impetus for further research.

In the third study, we analyzed the preoperative levels of CA125 and somatic mutations in patients with EOC. This study found that TP53 was the most commonly mutated gene in both borderline and EOC patients. Additionally, CA125 levels were significantly higher in patients with ovarian cancer than in those with borderline ovarian tumors.

The main finding of this study was that ctDNA analyzed in the plasma can be a useful diagnostic and prognostic biomarker for ovarian cancer. This study measured ctDNA levels in plasma samples from patients with ovarian cancer and borderline ovarian tumors using SAGAsafe dPCR technology. The results showed that the plasma levels of ctDNA mutations increased in patients at a higher disease stage. In addition, in patients with EOC, higher plasma ctDNA levels were associated with worse overall survival.

These findings have significant clinical implications. First, the use of ctDNA as a diagnostic biomarker can aid in the early detection and monitoring of ovarian cancer. Early detection of ovarian cancer is critical for improving patient outcomes, as the disease is often diagnosed at an advanced stage when treatment options are limited (94, 95). Secondly, the use of ctDNA as a prognostic biomarker can help identify patients who are at a higher risk of disease progression and may require more aggressive treatment (133).

Identification of specific genetic mutations in circulating tumor DNA has exhibited promise in influencing choices related to cancer treatment interventions (134). In patients with ovarian cancer, the most commonly mutated gene is TP53, particularly in those with advanced-stage disease, which substantiates the second dualistic theory II (13). TP53 mutations have been found in over 90% of high-grade serous ovarian carcinomas, and loss of p53 function can promote the transformation of cells into malignant cells by preventing apoptosis. Targeting pharmacological interventions such as PARP inhibitors have shown efficacy in treating BRCA-mutated ovarian cancers and those with homologous recombination deficiencies. However, few studies have evaluated the efficacy of PARP inhibitors on TP53 expression (135, 136). Targeting mutant p53 may destabilize PARP repair and impede the distant spread of cancer cells (135).

Another gene mutation that has shown potential in guiding ovarian cancer treatment is KRAS, which was found in seven tissue specimens and one plasma ctDNA sample. KRAS is a member of the Ras family of oncogenes that plays important roles in cell division, differentiation, and apoptosis (137). In borderline tumors, frequent mutations in KRAS, BRAF, or ERBB2 and a lack of TP53 mutations may lead to the progression of low-grade serous carcinomas. In two trials, MEK inhibitors have shown activity against LGSOC, particularly in KRAS-mutated diseases (138). Additionally, KRAS inhibition has shown positive results in patients with advanced malignant melanoma with BRAF mutations (139), and mutation status may guide anti-PD-1/PD-L1 immunotherapy in patients with lung

adenocarcinoma with co-occurring TP53 mutations (140). Thus, the identification of specific genetic mutations in ctDNAs has the potential to guide personalized treatment decisions in patients with ovarian cancer.

Our study found that 15 of 24 patients with tumor DNA mutations also exhibited mutations in their plasma ctDNA, indicating a sensitivity of 62.5%. However, the ability to detect ctDNA mutations in plasma is influenced by various factors such as tumor morphology, disease stage, tumor burden, and DNA degradation (70, 141, 142).

In this study, the plasma ctDNA mutant concentration increased with higher cancer stage, which is consistent with previous findings (70). Late-stage cancers in abdominal organs, such as the colon, pancreas, or ovaries, commonly contain ctDNA, which is detected in over 60% of patients (142). Interestingly, this study also detected ctDNA mutations in the plasma of one patient with a borderline tumor, which is a novel observation. Other studies have shown that ctDNA levels correlate with tumor volume, as assessed using CT imaging in patients with relapsed high-grade serous ovarian cancer (143, 144). Median concentrations of 100–1,000 mutated gene copies per 5 mL of plasma have been reported in patients with advanced ovarian cancer (144). In the current study, the analyses detected less than 10 copies/mL of ctDNA in the plasma in the early stage of the disease, suggesting high sensitivity. Taken together, these results indicate that the amount of ctDNA in plasma is related to tumor volume and disease stage and has the potential to be a valuable marker for cancer detection and monitoring.

Our study aimed to evaluate the feasibility of detecting ctDNA mutations in patients with ovarian cancer, as well as the potential of ctDNA as a prognostic factor. Our results showed that ctDNA mutations could be detected in the plasma of EOC patients and that patients with advanced-stage disease had a higher concentration of mutated gene copies than those in the early stages of the disease.

The importance of prognostic markers in ovarian cancer cannot be overstated because the prognosis of ovarian cancer patients is closely linked to the disease stage (4). Previous studies have shown that higher percentages of ctDNAs in gynecological cancers correlate with worse survival outcomes (145). Similarly, ctDNA has been identified as a prognostic factor for disease recurrence in colorectal cancers (146).

Our study found that patients with more than 10 ctDNA mutations per mL of plasma demonstrated significantly worse overall survival compared to those with fewer mutations. It is important to note that the optimal cutoff level of mutant copies per milliliter needs to be evaluated in larger studies. Nonetheless, our study suggests that the amount of ctDNA in plasma may be a valuable prognostic marker for patients with ovarian cancer.

The results of this study suggest that ctDNA analysis is a useful tool for detecting ovarian cancer and predicting patient survival. However, this study was conducted on a relatively small cohort of patients, and the results need to be validated. Additionally, only genetic variants in the coding regions were investigated, and further studies are needed to evaluate the potential of ctDNA analysis for the detection of genetic variants in non-coding regions. The single allele genotyping by amplicon sequencing - safe digital polymerase chain reaction (SAGAsafe dPCR) technology has several advantages over other methods, including high sensitivity and specificity and the ability to detect low levels of ctDNA mutations. However, this technology has not been widely used. The presence of ctDNA in the bloodstream is directly proportional to tumor burden, and the sensitivity of ctDNA detection can be influenced by the technology employed for genetic material analysis. Thus, the quality and quantity of ctDNA can also be influenced by other factors such as age and overall health status.

During the course of the first three studies aimed at identifying new biomarkers to aid in determining the survival of patients with ovarian cancer, a question arose regarding the future outcomes of patients who had undergone treatment for borderline tumors. To address this question, a subsequent study was conducted to determine whether patients with borderline tumors were at risk of developing other cancer types. This study sought to investigate the potential link between BOT and other cancers and to identify any potential risk factors that may be associated with the development of non-ovarian cancers in patients with BOTs. The results of this study provide important insights into the management and care of patients with borderline tumors.

In the fourth nationwide study, we analyzed data from 4,998 women with BOTs diagnosed between 1995 and 2018. Our study revealed that women with BOTs face increased risks of certain types of cancer, including lung and pancreatic cancers, more than one year after their diagnosis. Furthermore, BOTs were associated with an increased risk of several other types of cancer, which were diagnosed within the same year or later.

Notably, we observed a double risk of lung cancer in women with mucinous BOTs, which was consistent across all age groups and follow-up periods. This finding is consistent with earlier studies linking smoking to an increased risk of mucinous ovarian tumors (147) (148). Similarly, we found an increased risk of pancreatic cancer in women older than 60 years with BOTs, which is in line with a known association between smoking and pancreatic cancer (149). Without access to in-depth data on lifestyle habits, we can merely conjecture the extent of the influence of these risk factors on pancreatic carcinogenesis, lung cancer, and BOTs.

Our study also revealed a higher risk of colon and small intestinal cancers in women with both serous and mucinous BOTs, although this association was significant only within the first year of BOT diagnosis. Previous studies reported a higher risk of

colorectal cancer in women with BOTs (150), which has been attributed to shared genetics such as Lynch syndrome (151, 152). Our large register-based study with a longer follow-up period allowed the identification of colorectal cancer diagnoses following BOTs.

Interestingly, we found an association between serous BOTs and increased thyroid cancer risk, likely due to hormonal risk factors and obesity (150). Serous BOTs can be associated with KRAS and BRAF mutations (16), which are also associated with a subset of thyroid cancers.

It is important to note that our study did not observe significantly increased rates of subsequent breast cancer, nor did we find any association between BOTs and myeloid leukemia or malignant melanoma (150).

One of the strengths of our study was the large number of patients included and the use of the complete Swedish Cancer Registry. Our findings are consistent with those of a similar Danish cohort study (150), further supporting the validity of our results. However, our study also has limitations, including the lack of information on potential confounding factors such as lifestyle factors and family history. Nonetheless, our study sheds light on the increased risk of certain cancer types in women with BOTs, thereby potentially influencing the design of upcoming strategies for screening and surveillance.

Conclusions

The first study did not reveal a notable increase in H3Cit-DNA levels in patients with EOC. However, CA125 levels were significantly elevated in EOC cases, thereby confirming its potential as a biomarker for diagnosing and predicting EOC outcomes.

The second study found that the prediction of overall survival in patients with EOC was enhanced by a combination of biomarkers, including NT-3, GPNMB, MSLN, cancer CA125, and HE4.

Age was identified as a significant factor in the predictive model affecting the prognosis of patients with EOC. These findings demonstrate the potential of biomarker panels for enhancing the prognosis of patients with EOC.

The third study revealed that the level of mutant copies of ctDNA in plasma was associated with the EOC stage, being higher in more advanced stages.

Patients with higher plasma ctDNA concentrations had worse overall survival. Plasma was identified as a widely accessible source of ctDNA, making it a promising candidate for EOC prognostic prediction.

The fourth population-based study found that women with BOTs had a significantly increased risk of pancreatic and lung cancers more than a year after BOTs diagnosis compared with women in the general population.

The study also revealed a link between BOTs and an increased risk of malignancies with primary unknown etiology, renal, cervical, intestinal, endometrial, and other cancers.

Future perspectives

Studies on NETs formation and its potential role in cancer progression have gained increasing interest in recent years. The response of neutrophils to tumors varies, leading to their activation with various antitumor and pro-tumor phenotypes. There are several potential paths for future research based on the findings and limitations of this study. First, larger sample sizes may improve the generalizability of the results and offer a deeper understanding of the biomarkers linked to ovarian cancer. The discovery of new biomarkers for diagnosis and prognosis may also result from investigating other biomarkers and analyzing changes in biomarker levels over time. It is also necessary to conduct further research on how NET formation affects the development of cancer. Finally, investigating additional potential variables that can affect the risk of VTE in patients with ovarian cancer may aid in the development of new preventive and therapeutic approaches. The shortcomings of this study underscore the need for further research, although it offers extensive information on the potential diagnostic and prognostic utility of biomarkers, such as H3Cit-DNA, dsDNA, and CA125 in ovarian cancer.

The second study demonstrated the potential of biomarker panels for enhancing the prognosis of patients with EOC. Future analyses should consider the fact that this study only included patients scheduled for primary upfront surgery and excluded those receiving neoadjuvant chemotherapy, which may restrict the generalizability of the results to all patients with EOC. Further limiting the interpretation of the study findings may be the heterogeneity of the histological epithelial subtypes. The absence of statistically significant results for some biomarkers may have been due to the small sample size. Therefore, the findings of this study should be interpreted with caution. Further studies are required to validate these results and develop novel biomarkers that can be included in panels to predict the prognosis of patients with EOC.

The findings and results of the third study indicated that ctDNA is a promising candidate for ovarian cancer diagnosis and prognostic prediction. Future studies should focus on the clinical value of ctDNA analysis for ovarian cancer detection and survival prediction. It is crucial to remember that before being used in clinical settings, the findings of studies, such as those conducted on small patient cohorts, would need to be proven in larger patient cohorts. The potential of ctDNA analysis for the discovery of genetic variations in non-coding regions requires further research. According to earlier studies, such polymorphisms may help identify those

who are genetically at risk of developing ovarian cancer. Future sequencing studies may encounter difficulties in identifying genetic variations linked to EOC in non-coding areas. In the future, patients with a greater ovarian tumor burden will be more likely to have detectable ctDNA. The technique used for genetic material analysis, as well as other elements such as age and general health status, may continue to have an impact on the sensitivity of ctDNA detection. However, this can only be assessed during studies to accurately assess their impact.

In circumstances where tumor material is not available, larger genomic panels may be developed for ctDNA analysis in the plasma for one day. Utilizing such panels could simplify ctDNA analysis and potentially improve the accuracy of ovarian cancer detection while retaining the high sensitivity of ctDNA analysis.

The findings of the fourth population-based study conducted nationally may have had a significant impact on cancer prevention and follow-up of women with BOTs. Future studies can continue to benefit from the strengths of this population cohort study. The study's large patient population and the use of the entire Swedish Cancer Registry, which allowed for a precise diagnosis and minimal loss of follow-up, are two of its key advantages. Furthermore, this study goes beyond prior research attempts by considering confounding variables such as socioeconomic status. Future studies should address several limitations. For instance, there was an absence of certain potential confounders, such as obesity and smoking. The lack of information regarding the stage of BOTs and the 2014 modification to the classification of borderline ovarian cancer and tumors may also have had an impact, and it will be crucial for future research to consider these characteristics whenever possible and incorporate them into the study design.

Acknowledgments

I would like to express my sincere gratitude to all the women who participated in this study. I also extend my heartfelt appreciation to those who have experienced, are currently battling, or facing oncological challenges. My unwavering belief in the potential to offer healing to patients with cancer was the driving force behind my chosen profession.

Moreover, I would like to express my gratitude to the individuals acknowledged in the following paragraphs.

I extend my heartfelt appreciation to Professor Christer Borgfeldt, my primary supervisor, for introducing me to the vast, captivating, and challenging domain of ovarian cancer research. Your patient guidance throughout my years as a Ph.D. student, coupled with your unwavering support and availability for discussions at all hours, has been invaluable. Thank you for your contribution and dedication.

My co-supervisor Louise Moberg for all the advice, dedicated mentorship, and wise answers. Your help with academic-related questions was essential.

I would like to thank my friend Dr. Eimantas Švedas. You brought me to this path of oncogynecology from the very first steps, helped and continued to help me improve in clinical practice. Your support and faith are beyond words.

Charlotte Hellsten, head of the gynecology team, I deeply appreciate your understanding and professionalism. Since my earliest days in Sweden, you have been acquainted with me, guiding my growth, and affording me the chance to evolve into the person I am today.

I would like to thank my colleagues in the Gynecology and Obstetrics Department for their help. You have taught me much about both gynecology and life, and I am grateful for the opportunity to work with you.

I would like to thank all the co-authors who participated in the projects.

My Family. I extend my heartfelt thanks to my wife Vaida for her constant support and unwavering belief in me. This dissertation would not have been possible without your support. To our fortunate children, Goda, Adas, and Domas, your presence brings boundless happiness. As several professors have wisely noted, life's most important moments often occur outside the clinic.

This work was supported by grants from the Southern Health Care Region of Sweden, the Swedish Cancer Society, and Cancerfonden.

References

1. International. WCRF. Ovarian cancer statistics: WCRF; 2022 [Available from: https://www.wcrf.org/cancer-trends/ovarian-cancer-statistics/.
2. Cancer. IAfRo. Estimated age-standardized incidence rates (World) in 2020, ovary, females, all ages: World Health Organization; 2022 [Available from: https://gco.iarc.fr/today/online-analysis-map?v=2020&mode=population&mode_population=continents&population=900&populations=900&key=asr&sex=2&cancer=25&type=0&statistic=5&prevalence=0&population_group=0&ages_group%5B%5D=0&ages_group%5B%5D=17&nb_items=10&group_cancer=1&include_nmsc=0&include_nmsc_other=0&projection=natural-earth&color_palette=default&map_scale=quantile&map_nb_colors=5&continent=0&show_ranking=0&rotate=%255B10%252C0%255D.
3. Registries. AotNC. Crude rate per 100 000, Incidence, Females Sweden Ovary and tubes: NORDCAN; 2022 [Available from: https://nordcan.iarc.fr/en/dataviz/trends?mode=age&group_populations=0&multiple_cancers=0&sexes=2&cancers=220&populations=752&multiple_populations=0.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.
5. Gupta KK, Gupta VK, Naumann RW. Ovarian cancer: screening and future directions. Int J Gynecol Cancer. 2019;29(1):195-200.
6. Leandersson P, Hogberg T, Dickman PW, Malander S, Borgfeldt C. Incidence and survival of epithelial ovarian, fallopian tube, peritoneal, and undesignated abdominal/pelvic cancers in Sweden 1960-2014: A population-based cohort study. BMC Cancer. 2021;21(1):465.
7. La Vecchia C. Ovarian cancer: epidemiology and risk factors. Eur J Cancer Prev. 2017;26(1):55-62.
8. Riman T, Dickman PW, Nilsson S, Correia N, Nordlinder H, Magnusson CM, et al. Risk factors for epithelial borderline ovarian tumors: results of a Swedish case-control study. Gynecol Oncol. 2001;83(3):575-85.
9. Trabert B, Tworoger SS, O'Brien KM, Townsend MK, Fortner RT, Iversen ES, et al. The Risk of Ovarian Cancer Increases with an Increase in the Lifetime Number of Ovulatory Cycles: An Analysis from the Ovarian Cancer Cohort Consortium (OC3). Cancer Res. 2020;80(5):1210-8.
10. Modugno F, Laskey R, Smith A, Andersen C, Haluska P, Oesterreich S. Hormone response in ovarian cancer: time to reconsider as a clinical target? Endocrine-Related Cancer. 2012;19:R255-R79.

11. Schouten L, Rivera C, Hunter D, Spiegelman D, Adami H, Arslan A, et al. Height, body mass index, and ovarian cancer: a pooled analysis of 12 cohort studies. Cancer Epidemiology, Biomarkers & Prevention. 2008;17:902-12.
12. Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. Am J Pathol. 2004;164(5):1511-8.
13. Kurman RJ, Shih Ie M. The Dualistic Model of Ovarian Carcinogenesis: Revisited, Revised, and Expanded. Am J Pathol. 2016;186(4):733-47.
14. Ramalingam P. Morphologic, Immunophenotypic, and Molecular Features of Epithelial Ovarian Cancer. Oncology (Williston Park). 2016;30(2):166-76.
15. Prat J, D'Angelo E, Espinosa I. Ovarian carcinomas: at least five different diseases with distinct histological features and molecular genetics. Hum Pathol. 2018;80:11-27.
16. Hauptmann S, Friedrich K, Redline R, Avril S. Ovarian borderline tumors in the 2014 WHO classification: evolving concepts and diagnostic criteria. Virchows Arch. 2017;470(2):125-42.
17. Poulain M, Vandame J, Tran C, Koutchinsky S, Pirtea P, Ayoubi JM. Fertility preservation in borderline ovarian tumor patients and survivors. Horm Mol Biol Clin Investig. 2020;43(2):179-86.
18. Gershenson DM. Management of borderline ovarian tumours. Best Pract Res Clin Obstet Gynaecol. 2017;41:49-59.
19. Medscape. Ovarian Cancer Staging.: Medscape; 2022 [Available from: https://emedicine.medscape.com/article/2007140-overview.
20. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. Int J Gynaecol Obstet. 2021;155 Suppl 1(Suppl 1):61-85.
21. Armstrong DK, Alvarez RD, Bakkum-Gamez JN, Barroilhet L, Behbakht K, Berchuck A, et al. Ovarian Cancer, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 2021;19(2):191-226.
22. Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. Ann Oncol. 2019;30(5):672-705.
23. Spaan M, van den Belt-Dusebout AW, Lambalk CB, van Boven HH, Schats R, Kortman M, et al. Long-Term Risk of Ovarian Cancer and Borderline Tumors After Assisted Reproductive Technology. J Natl Cancer Inst. 2021;113(6):699-709.
24. Beral V, Gaitskell K, Hermon C, Moser K, Reeves G, Peto R. Menopausal hormone use and ovarian cancer risk: individual participant meta-analysis of 52 epidemiological studies. Lancet. 2015;385(9980):1835-42.
25. Tanha K, Mottaghi A, Nojomi M, Moradi M, Rajabzadeh R, Lotfi S, et al. Investigation on factors associated with ovarian cancer: an umbrella review of systematic review and meta-analyses. J Ovarian Res. 2021;14(1):153.
26. Sampson JA. Endometrial carcinoma of the ovary, arising in endometrial tissue in that organ. Archives of Surgery. 1925;10(1):1-72.

27. Zondervan KT, Becker CM, Missmer SA. Endometriosis. New England Journal of Medicine. 2020;382(13):1244-56.
28. Kalaitzopoulos DR, Mitsopoulou A, Iliopoulou SM, Daniilidis A, Samartzis EP, Economopoulos KP. Association between endometriosis and gynecological cancers: a critical review of the literature. Arch Gynecol Obstet. 2020;301(2):355-67.
29. Hermens M, van Altena AM, Bulten J, van Vliet H, Siebers AG, Bekkers RLM. Increased incidence of ovarian cancer in both endometriosis and adenomyosis. Gynecol Oncol. 2021;162(3):735-40.
30. Gadducci A, Multinu F, Cosio S, Carinelli S, Ghioni M, Aletti GD. Clear cell carcinoma of the ovary: Epidemiology, pathological and biological features, treatment options and clinical outcomes. Gynecol Oncol. 2021;162(3):741-50.
31. Yu HC, Lin CY, Chang WC, Shen BJ, Chang WP, Chuang CM. Increased association between endometriosis and endometrial cancer: a nationwide population-based retrospective cohort study. Int J Gynecol Cancer. 2015;25(3):447-52.
32. Bulun SE, Wan Y, Matei D. Epithelial Mutations in Endometriosis: Link to Ovarian Cancer. Endocrinology. 2019;160(3):626-38.
33. Moschetta M, George A, Kaye SB, Banerjee S. BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. Ann Oncol. 2016;27(8):1449-55.
34. Easton DF, Ford D, Bishop DT. Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet. 1995;56(1):265-71.
35. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The Breast Cancer Linkage Consortium. Am J Hum Genet. 1998;62(3):676-89.
36. Bernardini MQ, Baba T, Lee PS, Barnett JC, Sfakianos GP, Secord AA, et al. Expression signatures of TP53 mutations in serous ovarian cancers. BMC Cancer. 2010;10:237.
37. Vaddavalli PL, Schumacher B. The p53 network: cellular and systemic DNA damage responses in cancer and aging. Trends Genet. 2022;38(6):598-612.
38. Nakamura M, Obata T, Daikoku T, Fujiwara H. The Association and Significance of p53 in Gynecologic Cancers: The Potential of Targeted Therapy. Int J Mol Sci. 2019;20(21).
39. Olivier M, Hollstein M, Hainaut P. TP53 mutations in human cancers: origins, consequences, and clinical use. Cold Spring Harb Perspect Biol. 2010;2(1):a001008.
40. Levine AJ. p53: 800 million years of evolution and 40 years of discovery. Nat Rev Cancer. 2020;20(8):471-80.
41. Aubrey BJ, Strasser A, Kelly GL. Tumor-Suppressor Functions of the TP53 Pathway. Cold Spring Harb Perspect Med. 2016;6(5).
42. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474(7353):609-15.

43. Felicio PS, Grasel RS, Campacci N, de Paula AE, Galvão HCR, Torrezan GT, et al. Whole-exome sequencing of non-BRCA1/BRCA2 mutation carrier cases at high-risk for hereditary breast/ovarian cancer. Hum Mutat. 2021;42(3):290-9.
44. Gockley AA, Elias KM. Fallopian tube tumorigenesis and clinical implications for ovarian cancer risk-reduction. Cancer Treat Rev. 2018;69:66-71.
45. Salvador S, Scott S, Francis JA, Agrawal A, Giede C. No. 344-Opportunistic Salpingectomy and Other Methods of Risk Reduction for Ovarian/Fallopian Tube/Peritoneal Cancer in the General Population. J Obstet Gynaecol Can. 2017;39(6):480-93.
46. Falconer H, Yin L, Grönberg H, Altman D. Ovarian cancer risk after salpingectomy: a nationwide population-based study. J Natl Cancer Inst. 2015;107(2).
47. Finch AP, Lubinski J, Møller P, Singer CF, Karlan B, Senter L, et al. Impact of oophorectomy on cancer incidence and mortality in women with a BRCA1 or BRCA2 mutation. J Clin Oncol. 2014;32(15):1547-53.
48. Finch A, Beiner M, Lubinski J, Lynch HT, Moller P, Rosen B, et al. Salpingo-oophorectomy and the risk of ovarian, fallopian tube, and peritoneal cancers in women with a BRCA1 or BRCA2 Mutation. Jama. 2006;296(2):185-92.
49. Park JY, Lee JY, Lee YY, Shim SH, Suh DH, Kim JW. Major clinical research advances in gynecologic cancer in 2021. J Gynecol Oncol. 2022;33(2):e43.
50. Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, et al. Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. The Lancet. 2016;387(10022):945-56.
51. Menon U, Gentry-Maharaj A, Burnell M, Singh N, Ryan A, Karpinskyj C, et al. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet. 2021;397(10290):2182-93.
52. Califf RM. Biomarker definitions and their applications. Exp Biol Med (Maywood). 2018;243(3):213-21.
53. Bast RC, Jr., Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981;68(5):1331-7.
54. Schmidt C. CA-125: a biomarker put to the test. J Natl Cancer Inst. 2011;103(17):1290-1.
55. Yin BW, Dnistrian A, Lloyd KO. Ovarian cancer antigen CA125 is encoded by the MUC16 mucin gene. Int J Cancer. 2002;98(5):737-40.
56. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: identification as a new mucin, MUC16. J Biol Chem. 2001;276(29):27371-5.
57. Muyldermans M, Cornillie FJ, Koninckx PR. CA125 and endometriosis. Hum Reprod Update. 1995;1(2):173-87.
58. Sikaris KA. CA125--a test with a change of heart. Heart Lung Circ. 2011;20(10):634-40.

59. Benjapibal M, Neungton C. Pre-operative prediction of serum CA125 level in women with ovarian masses. J Med Assoc Thai. 2007;90(10):1986-91.
60. Gasiorowska E, Kluz T, Lipski D, Warchoł W, Tykarski A, Nowak-Markwitz E. Human Epididymis Protein 4 (HE4) Reference Limits in Polish Population of Healthy Women, Pregnant Women, and Women with Benign Ovarian Tumors. Dis Markers. 2019;2019:3890906.
61. McKinnon B, Mueller MD, Nirgianakis K, Bersinger NA. Comparison of ovarian cancer markers in endometriosis favours HE4 over CA125. Mol Med Rep. 2015;12(4):5179-84.
62. Hellström I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003;63(13):3695-700.
63. Scaletta G, Plotti F, Luvero D, Capriglione S, Montera R, Miranda A, et al. The role of novel biomarker HE4 in the diagnosis, prognosis and follow-up of ovarian cancer: a systematic review. Expert Rev Anticancer Ther. 2017;17(9):827-39.
64. Piovano E, Attamante L, Macchi C, Cavallero C, Romagnolo C, Maggino T, et al. The role of HE4 in ovarian cancer follow-up: a review. Int J Gynecol Cancer. 2014;24(8):1359-65.
65. He YP, Li LX, Tang JX, Yi L, Zhao Y, Zhang HW, et al. HE4 as a biomarker for diagnosis of lung cancer: A meta-analysis. Medicine (Baltimore). 2019;98(39):e17198.
66. Ohkuma R, Yada E, Ishikawa S, Komura D, Kubota Y, Hamada K, et al. High levels of human epididymis protein 4 mRNA and protein expression are associated with chemoresistance and a poor prognosis in pancreatic cancer. Int J Oncol. 2021;58(1):57-69.
67. Pessoa LS, Heringer M, Ferrer VP. ctDNA as a cancer biomarker: A broad overview. Crit Rev Oncol Hematol. 2020;155:103109.
68. Papakonstantinou A, Gonzalez NS, Pimentel I, Suñol A, Zamora E, Ortiz C, et al. Prognostic value of ctDNA detection in patients with early breast cancer undergoing neoadjuvant therapy: A systematic review and meta-analysis. Cancer Treat Rev. 2022;104:102362.
69. Asante DB, Calapre L, Ziman M, Meniawy TM, Gray ES. Liquid biopsy in ovarian cancer using circulating tumor DNA and cells: Ready for prime time? Cancer Lett. 2020;468:59-71.
70. Thusgaard CF, Korsholm M, Koldby KM, Kruse TA, Thomassen M, Jochumsen KM. Epithelial ovarian cancer and the use of circulating tumor DNA: A systematic review. Gynecol Oncol. 2021;161(3):884-95.
71. Sharbatoghli M, Vafaei S, Aboulkheyr Es H, Asadi-Lari M, Totonchi M, Madjd Z. Prediction of the treatment response in ovarian cancer: a ctDNA approach. Journal of Ovarian Research. 2020;13(1):124.
72. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2009;112(1):40-6.

73. Salim E, Zubairi AM, Danish SH, Ali U. Diagnostic Accuracy of Risk of Ovarian Malignancy Algorithm (ROMA) in Post-Menopausal Patients with Ovarian Mass. J Coll Physicians Surg Pak. 2018;28(6):440-4.
74. Montagnana M, Danese E, Ruzzenente O, Bresciani V, Nuzzo T, Gelati M, et al. The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in women presenting with pelvic mass: is it really useful? Clin Chem Lab Med. 2011;49(3):521-5.
75. Aktürk E, Karaca RE, Alanbay I, Dede M, Karaşahin E, Yenen MC, et al. Comparison of four malignancy risk indices in the detection of malignant ovarian masses. J Gynecol Oncol. 2011;22(3):177-82.
76. Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas JG. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. Br J Obstet Gynaecol. 1990;97(10):922-9.
77. Tingulstad S, Hagen B, Skjeldestad FE, Onsrud M, Kiserud T, Halvorsen T, et al. Evaluation of a risk of malignancy index based on serum CA125, ultrasound findings and menopausal status in the pre-operative diagnosis of pelvic masses. Br J Obstet Gynaecol. 1996;103(8):826-31.
78. Hada A, Han L-p, Chen Y, Hu Q-h, Yuan Y, Liu L. Comparison of the predictive performance of risk of malignancy indexes 1–4, HE4 and risk of malignancy algorithm in the triage of adnexal masses. Journal of Ovarian Research. 2020;13(1):46.
79. Froyman W, Timmerman D. Methods of Assessing Ovarian Masses: International Ovarian Tumor Analysis Approach. Obstet Gynecol Clin North Am. 2019;46(4):625-41.
80. Timmerman D, Planchamp F, Bourne T, Landolfo C, du Bois A, Chiva L, et al. ESGO/ISUOG/IOTA/ESGE Consensus Statement on pre-operative diagnosis of ovarian tumors. Int J Gynecol Cancer. 2021;31(7):961-82.
81. Timmerman D, Testa AC, Bourne T, Ameye L, Jurkovic D, Van Holsbeke C, et al. Simple ultrasound-based rules for the diagnosis of ovarian cancer. Ultrasound Obstet Gynecol. 2008;31(6):681-90.
82. Testa A, Kaijser J, Wynants L, Fischerova D, Van Holsbeke C, Franchi D, et al. Strategies to diagnose ovarian cancer: new evidence from phase 3 of the multicentre international IOTA study. Br J Cancer. 2014;111(4):680-8.
83. Timmerman D, Van Calster B, Testa AC, Guerriero S, Fischerova D, Lissoni AA, et al. Ovarian cancer prediction in adnexal masses using ultrasound-based logistic regression models: a temporal and external validation study by the IOTA group. Ultrasound Obstet Gynecol. 2010;36(2):226-34.
84. Van Calster B, Van Hoorde K, Valentin L, Testa AC, Fischerova D, Van Holsbeke C, et al. Evaluating the risk of ovarian cancer before surgery using the ADNEX model to differentiate between benign, borderline, early and advanced stage invasive, and secondary metastatic tumours: prospective multicentre diagnostic study. Bmj. 2014;349:g5920.

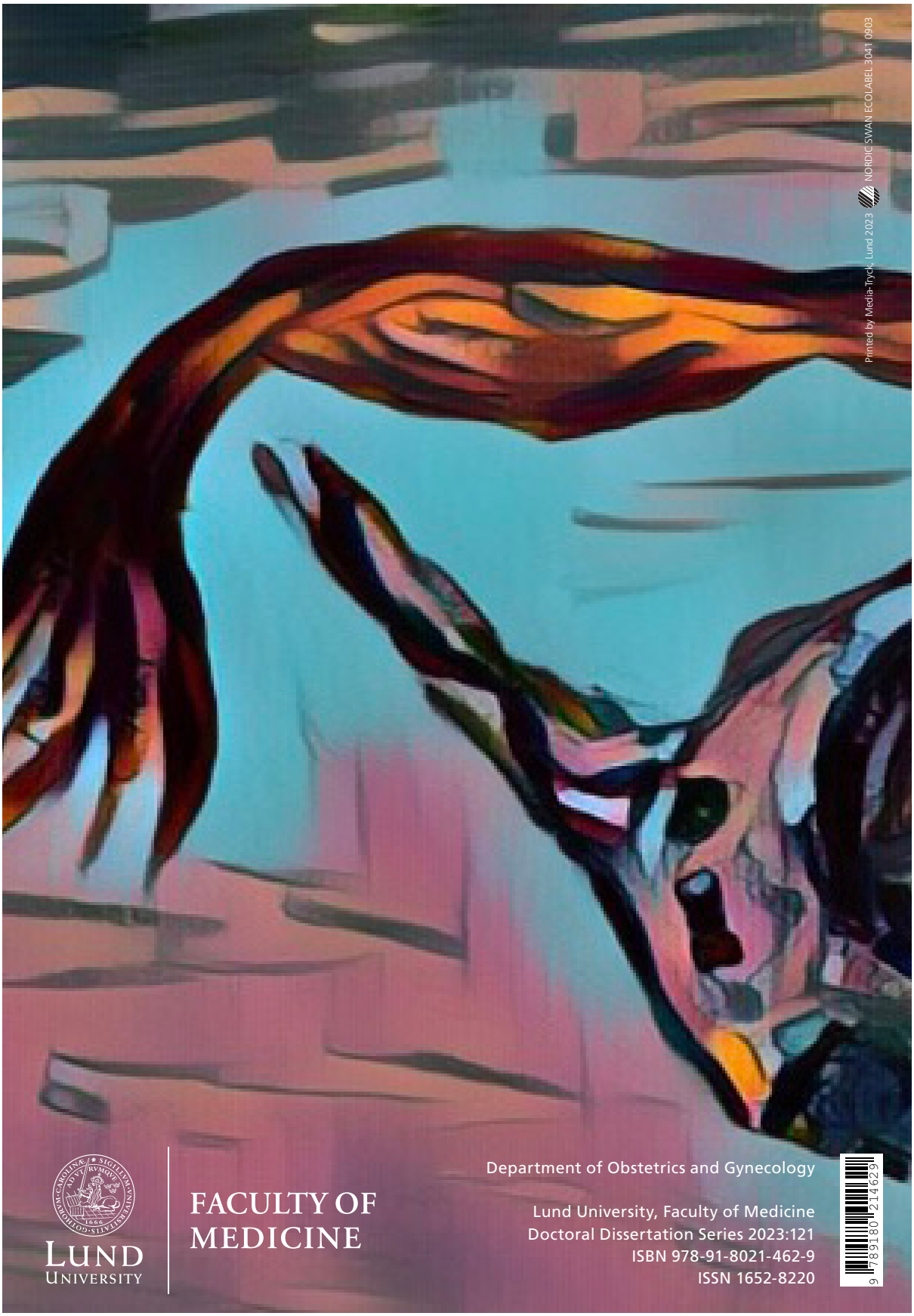
85. An H, Wang Y, Wong EMF, Lyu S, Han L, Perucho JAU, et al. CT texture analysis in histological classification of epithelial ovarian carcinoma. Eur Radiol. 2021;31(7):5050-8.
86. Engbersen MP, Van Driel W, Lambregts D, Lahaye M. The role of CT, PET-CT, and MRI in ovarian cancer. Br J Radiol. 2021;94(1125):20210117.
87. Cengiz A, Koç ZP, Özcan Kara P, Yürekli Y. The Role of (18)F-FDG PET/CT in Detecting Ovarian Cancer Recurrence in Patients with Elevated CA-125 Levels. Mol Imaging Radionucl Ther. 2019;28(1):8-14.
88. Sohaib SA, Reznek RH. MR imaging in ovarian cancer. Cancer Imaging. 2007;7 Spec No A(Special issue A):S119-29.
89. Asp M, Malander S, Wallengren NO, Pudaric S, Bengtsson J, Sartor H, et al. The role of computed tomography in the assessment of tumour extent and the risk of residual disease after upfront surgery in advanced ovarian cancer (AOC). Arch Gynecol Obstet. 2022;306(4):1235-43.
90. Vang R, Shih Ie M, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. Adv Anat Pathol. 2009;16(5):267-82.
91. Chen M, Jin Y, Bi Y, Yin J, Wang Y, Pan L. A survival analysis comparing women with ovarian low-grade serous carcinoma to those with high-grade histology. Onco Targets Ther. 2014;7:1891-9.
92. Yang SP, Su HL, Chen XB, Hua L, Chen JX, Hu M, et al. Long-Term Survival Among Histological Subtypes in Advanced Epithelial Ovarian Cancer: Population-Based Study Using the Surveillance, Epidemiology, and End Results Database. JMIR Public Health Surveill. 2021;7(11):e25976.
93. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68(4):284-96.
94. Bryant A, Hiu S, Kunonga PT, Gajjar K, Craig D, Vale L, et al. Impact of residual disease as a prognostic factor for survival in women with advanced epithelial ovarian cancer after primary surgery. Cochrane Database Syst Rev. 2022;9(9):Cd015048.
95. Cummins C, Kumar S, Long J, Balega J, Broadhead T, Duncan T, et al. Investigating the Impact of Ultra-Radical Surgery on Survival in Advanced Ovarian Cancer Using Population-Based Data in a Multicentre UK Study. Cancers. 2022;14(18):4362.
96. Kim A, Ueda Y, Naka T, Enomoto T. Therapeutic strategies in epithelial ovarian cancer. Journal of Experimental & Clinical Cancer Research. 2012;31(1):14.
97. Agarwal R, Kaye SB. Ovarian cancer: strategies for overcoming resistance to chemotherapy. Nature Reviews Cancer. 2003;3(7):502-16.
98. Tangutoori S, Baldwin P, Sridhar S. PARP inhibitors: A new era of targeted therapy. Maturitas. 2015;81(1):5-9.
99. Moore K, Colombo N, Scambia G, Kim BG, Oaknin A, Friedlander M, et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. N Engl J Med. 2018;379(26):2495-505.

100. Banerjee S, Moore KN, Colombo N, Scambia G, Kim BG, Oaknin A, et al. Maintenance olaparib for patients with newly diagnosed advanced ovarian cancer and a BRCA mutation (SOLO1/GOG 3004): 5-year follow-up of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2021;22(12):1721-31.
101. Bachmann R, Brucker S, Stäbler A, Krämer B, Ladurner R, Königsrainer A, et al. Prognostic relevance of high pretreatment CA125 levels in primary serous ovarian cancer. Mol Clin Oncol. 2021;14(1):8.
102. Kong S-Y, Han MH, Yoo H-J, Hwang JH, Lim MC, Seo S-S, et al. Serum HE4 Level is an Independent Prognostic Factor in Epithelial Ovarian Cancer. Annals of Surgical Oncology. 2012;19(5):1707-12.
103. Yuan C, Li R, Yan S, Kong B. Prognostic value of HE4 in patients with ovarian cancer. Clin Chem Lab Med. 2018;56(7):1026-34.
104. Steffensen KD, Waldstrøm M, Brandslund I, Petzold M, Jakobsen A. The prognostic and predictive value of combined HE4 and CA-125 in ovarian cancer patients. Int J Gynecol Cancer. 2012;22(9):1474-82.
105. Morales-Vásquez F, Pedernera E, Reynaga-Obregón J, López-Basave HN, Gómora MJ, Carlón E, et al. High levels of pretreatment CA125 are associated to improved survival in high grade serous ovarian carcinoma. J Ovarian Res. 2016;9(1):41.
106. Thålin C, Aguilera K, Hall NW, Marunde MR, Burg JM, Rosell A, et al. Quantification of citrullinated histones: Development of an improved assay to reliably quantify nucleosomal H3Cit in human plasma. J Thromb Haemost. 2020;18(10):2732-43.
107. 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.
108. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One. 2014;9(4):e95192.
109. Team RC. R: A language and environment for statistical computing. 2013.
110. Rothman KJ, Greenland S, Lash TL. Types of epidemiologic studies. Modern epidemiology. 1998;3:95-7.
111. Estève J, Benhamou E, Raymond L. Statistical methods in cancer research. Volume IV. Descriptive epidemiology. IARC Sci Publ. 1994(128):1-302.
112. Rivera-Franco MM, Leon-Rodriguez E, Torres-Ruiz JJ, Gómez-Martín D, Angles-Cano E, de la Luz Sevilla-González M. Neutrophil Extracellular Traps Associate with Clinical Stages in Breast Cancer. Pathology and Oncology Research. 2020;26(3):1781-5.
113. Castaño M, Tomás-Pérez S, González-Cantó E, Aghababyan C, Mascarós-Martínez A, Santonja N, et al. Neutrophil Extracellular Traps and Cancer: Trapping Our Attention with Their Involvement in Ovarian Cancer. Int J Mol Sci. 2023;24(6).
114. Scapini P, Lapinet-Vera JA, Gasperini S, Calzetti F, Bazzoni F, Cassatella MA. The neutrophil as a cellular source of chemokines. Immunological Reviews. 2000;177:195-203.

115. Wu L, Saxena S, Awaji M, Singh RK. Tumor-Associated Neutrophils in Cancer: Going Pro. Cancers. 2019;11(4):564.
116. Demers M, Krause DS, Schatzberg D, Martinod K, Voorhees JR, Fuchs TA, et al. Cancers predispose neutrophils to release extracellular DNA traps that contribute to cancer-associated thrombosis. Proc Natl Acad Sci U S A. 2012;109(32):13076-81.
117. Demers M, Wagner DD. NETosis: a new factor in tumor progression and cancer-associated thrombosis. Semin Thromb Hemost. 2014;40(3):277-83.
118. Lee W, Ko SY, Mohamed MS, Kenny HA, Lengyel E, Naora H. Neutrophils facilitate ovarian cancer premetastatic niche formation in the omentum. Journal of Experimental Medicine. 2019;216(1):176-94.
119. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecologic Oncology. 2009;112(1):40-6.
120. Mauracher LM, Posch F, Martinod K, Grilz E, Däullary T, Hell L, et al. Citrullinated histone H3, a biomarker of neutrophil extracellular trap formation, predicts the risk of venous thromboembolism in cancer patients. J Thromb Haemost. 2018;16(3):508-18.
121. Rodriguez AO, Wun T, Chew H, Zhou H, Harvey D, White RH. Venous thromboembolism in ovarian cancer. Gynecologic Oncology. 2007;105(3):784-90.
122. Leandersson P, Granåsen G, Borgfeldt C. Ovarian Cancer Surgery - A Population-based Registry Study. Anticancer Res. 2017;37(4):1837-45.
123. Wimberger P, Lehmann N, Kimmig R, Burges A, Meier W, Du Bois A. Prognostic factors for complete debulking in advanced ovarian cancer and its impact on survival. An exploratory analysis of a prospectively randomized phase III study of the Arbeitsgemeinschaft Gynaekologische Onkologie Ovarian Cancer Study Group (AGO-OVAR). Gynecologic Oncology. 2007;106(1):69-74.
124. Kim Y-N, Kim JC, Chung YS, Park J, Lee YJ, Lee J-Y, et al. Incidence of postoperative thrombotic events in ovarian cancer patients with a de-escalated prophylactic strategy: A retrospective cohort study. Gynecologic Oncology. 2022;165(1):75-81.
125. Garrido MP, Torres I, Vega M, Romero C. Angiogenesis in Gynecological Cancers: Role of Neurotrophins. Front Oncol. 2019;9:913.
126. Griffin N, Faulkner S, Jobling P, Hondermarck H. Targeting neurotrophin signaling in cancer: The renaissance. Pharmacol Res. 2018;135:12-7.
127. Louie E, Chen XF, Coomes A, Ji K, Tsirka S, Chen EI. Neurotrophin-3 modulates breast cancer cells and the microenvironment to promote the growth of breast cancer brain metastasis. Oncogene. 2013;32(35):4064-77.
128. Blondy S, Christou N, David V, Verdier M, Jauberteau M-O, Mathonnet M, et al. Neurotrophins and their involvement in digestive cancers. Cell Death & Disease. 2019;10(2):123.
129. Rose AA, Annis MG, Dong Z, Pepin F, Hallett M, Park M, et al. ADAM10 releases a soluble form of the GPNMB/Osteoactivin extracellular domain with angiogenic properties. PLoS One. 2010;5(8):e12093.

130. Rich JN, Shi Q, Hjelmeland M, Cummings TJ, Kuan CT, Bigner DD, et al. Bone-related genes expressed in advanced malignancies induce invasion and metastasis in a genetically defined human cancer model. J Biol Chem. 2003;278(18):15951-7.
131. Avula LR, Rudloff M, El-Behaedi S, Arons D, Albalawy R, Chen X, et al. Mesothelin Enhances Tumor Vascularity in Newly Forming Pancreatic Peritoneal Metastases. Mol Cancer Res. 2020;18(2):229-39.
132. Inoue S, Tsunoda T, Riku M, Ito H, Inoko A, Murakami H, et al. Diffuse mesothelin expression leads to worse prognosis through enhanced cellular proliferation in colorectal cancer. Oncol Lett. 2020;19(3):1741-50.
133. Zhu JW, Charkhchi P, Akbari MR. Potential clinical utility of liquid biopsies in ovarian cancer. Mol Cancer. 2022;21(1):114.
134. Kamat AA, Baldwin M, Urbauer D, Dang D, Han LY, Godwin A, et al. Plasma cell-free DNA in ovarian cancer: an independent prognostic biomarker. Cancer. 2010;116(8):1918-25.
135. Smeby J, Kryeziu K, Berg KCG, Eilertsen IA, Eide PW, Johannessen B, et al. Molecular correlates of sensitivity to PARP inhibition beyond homologous recombination deficiency in pre-clinical models of colorectal cancer point to wild-type TP53 activity. EBioMedicine. 2020;59.
136. Corcoran NM, Clarkson MJ, Stuchbery R, Hovens CM. Molecular Pathways: Targeting DNA Repair Pathway Defects Enriched in Metastasis. Clin Cancer Res. 2016;22(13):3132-7.
137. Timar J, Kashofer K. Molecular epidemiology and diagnostics of KRAS mutations in human cancer. Cancer Metastasis Rev. 2020;39(4):1029-38.
138. Pauly N, Ehmann S, Ricciardi E, Ataseven B, Bommert M, Heitz F, et al. Low-grade Serous Tumors: Are We Making Progress? Curr Oncol Rep. 2020;22(1):8.
139. Dietrich P, Kuphal S, Spruss T, Hellerbrand C, Bosserhoff AK. Wild-type KRAS is a novel therapeutic target for melanoma contributing to primary and acquired resistance to BRAF inhibition. Oncogene. 2018;37(7):897-911.
140. Dong ZY, Zhong WZ, Zhang XC, Su J, Xie Z, Liu SY, et al. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. Clinical Cancer Research. 2017;23(12):3012-24.
141. Phallen J, Sausen M, Adleff V, Leal A, Hruban C, White J, et al. Direct detection of early-stage cancers using circulating tumor DNA. Science translational medicine. 2017;9(403):eaan2415.
142. Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science Translational Medicine. 2014;6(224).
143. Parkinson CA, Gale D, Piskorz AM, Biggs H, Hodgkin C, Addley H, et al. Exploratory Analysis of TP53 Mutations in Circulating Tumour DNA as Biomarkers of Treatment Response for Patients with Relapsed High-Grade Serous Ovarian Carcinoma: A Retrospective Study. PLoS Med. 2016;13(12):e1002198.

144. Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, et al. Liquid biopsies come of age: towards implementation of circulating tumour DNA. Nature Reviews Cancer. 2017;17(4):223-38.
145. Charo LM, Eskander RN, Okamura R, Patel SP, Nikanjam M, Lanman RB, et al. Clinical implications of plasma circulating tumor DNA in gynecologic cancer patients. Mol Oncol. 2021;15(1):67-79.
146. Benhaim L, Bouché O, Normand C, Didelot A, Mulot C, Le Corre D, et al. Circulating tumor DNA is a prognostic marker of tumor recurrence in stage II and III colorectal cancer: multicentric, prospective cohort study (ALGECOLS). European Journal of Cancer. 2021;159:24-33.
147. Gram IT, Braaten T, Adami HO, Lund E, Weiderpass E. Cigarette smoking and risk of borderline and invasive epithelial ovarian cancer. Int J Cancer. 2008;122(3):647-52.
148. Schneider IJC, Schmidt TP, Correa VP, Santos A, Rocha BVD, Garcia LP, et al. Tobacco-related neoplasms: survival analysis and risk of death of population data from Florianópolis, SC. Rev Saude Publica. 2022;56:16.
149. Yadav D, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. Gastroenterology. 2013;144(6):1252-61.
150. Hannibal CG, Frederiksen K, Vang R, Kurman RJ, Kjaer SK. Risk of specific types of ovarian cancer after borderline ovarian tumors in Denmark: A nationwide study. Int J Cancer. 2020;147(4):990-5.
151. Dominguez-Valentin M, Sampson JR, Seppälä TT, Ten Broeke SW, Plazzer JP, Nakken S, et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: findings from the Prospective Lynch Syndrome Database. Genet Med. 2020;22(1):15-25.
152. Haraldsdottir S, Rafnar T, Frankel WL, Einarsdottir S, Sigurdsson A, Hampel H, et al. Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. Nat Commun. 2017;8:14755.



Printed by Media-Tryck, Lund 2023 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Obstetrics and Gynecology

Lund University, Faculty of Medicine

Doctoral Dissertation Series 2023:121
ISBN 978-91-8021-462-9
ISSN 1652-8220