COULD UNNECESSARY SURGERIES FOR SUSPECTED OVARIAN CANCER BE AVOIDED IN WOMEN WITH A PATHOGENIC MUTATION IN THE BRCA1/2 GENES?

FINAL DEGREE PROJECT

FEBRUARY 2023

UNIVERSITAT DE GIRONA

Author: Maria Ràfols Pérez

Clinical tutor: Laura Cárdenas Puiggrós

Methodological tutor: Xavier Castells Cervelló

Acknowledgments

I would like to express my gratitude to my clinical tutor Dra Laura Cárdenas, for guiding me throughout the completion of the study and internship. Her corrections and feedback have been very profitable.

Thanks to my methodological tutor Xavier Castells, for advising me in the methodological part and for making me reflect on the section's contents. I also appreciate Professor Marc Sáez for his help with the statistical analysis. And Anna Roca, her comments on the bibliography were truly helpful.

Lastly, thanks to my friends and family, especially to my mother Núria for her unconditional support, and to Alex, for always being there.

INDEX

LIST OF FIGURES

LIST OF TABLES

1. ABSTRACT

BACKGROUND. Ovarian cancer is the most lethal gynaecological cancer, particularly due to the non-specificity of symptoms and the lack of a screening strategy. The general population has a 1-1.5% lifetime risk of developing ovarian cancer. Women with a pathogenic mutation in the BRCA1 or BRCA2 genes have a higher lifetime risk of developing ovarian cancer, being at the age of 70, 39-44% for BRCA1 and 11-18% for BRCA2. Currently, the recommendation with the most evidence in terms of risk reduction is risk-reducing prophylactic bilateral salpingo-oophorectomy. Nevertheless, some women refuse or postpone this surgery due to the subsequent effects. Thus, they are offered a six-monthly screening strategy based on transvaginal ultrasound (TVUS) and CA125 marker analysis. This strategy presents a false-positive rate of 15%, with the consequence of performing unnecessary surgeries, since when these tests are altered the woman is considered candidate for individualized surgery.

OBJECTIVES. The main objective of this study is to determine whether the application of the combined follow-up strategy based on transvaginal ultrasound, serum tumour marker CA125 and PapSEEK is more specific than the current follow-up strategy based on transvaginal ultrasound and serum tumour marker CA125 , in the context of the six-monthly follow-up for the early detection of ovarian cancer in women who carry a pathogenic mutation in the BRCA1 or BRCA2 genes and who are candidates for surgery after presenting a transvaginal ultrasound and/or an altered CA125. Secondary objectives aim to determine the sensibility of the study strategy and assess patient acceptability.

DESIGN AND METHODOLOGY. This study was designed as a cross-sectional study. It will be a multicentre study performed in 9 Catalan hospitals.

PARTICIPANTS. 62 women with a pathogenic mutation in the BRCA1/2 genes who undergo the current six-monthly follow-up strategy and have these tests altered (CA125 > 35 U/mL and/or TVUS O-RADS ≥ 2).

KEYWORDS. *Ovarian cancer; BRCA; risk-reducing prophylactic bilateral salpingo-oophorectomy; CA125; TVUS; PapSEEK; specificity; false-positive rate; patient acceptability*.

2. ABBREVIATIONS

BOC – Breast and Ovarian Cancer

BRCA – Breast Cancer Gene

CPG – Clinical Practice Guideline

EOC – Epithelial Ovarian Cancer

HBOCS – Hereditary Breast and Ovarian Cancer Syndrome

HDI – Human Development Index

HHR – Homologous Recombination Repair

HPV – Human Papilloma Virus

IOTA – International Ovarian Tumour Analysis

OC – Ovarian Cancer

O-RADS – Ovarian-Adnexal Reporting and Data System

OSE – Ovarian Surface Epithelium

ROMA – Risk of Ovarian Malignancy Algorithm

RR-PBSO – Risk Reducing Prophylactic Bilateral Salpingo-Oophorectomy

SEE-FIM – Sectioning and Extensively Examining the Fimbriated end

SEGO – "Sociedad Española de Ginecología y Obstetricia"

STIC – Serous Tubal Intraepithelial Carcinoma

TFA – Theoretical Framework of Acceptability

TVUS – TransVaginal UltraSound

US – Ultrasound

3. INTRODUCTION

3.1. BASIC CONCEPTS

Ovarian cancer is defined as the uncontrolled division and proliferation of abnormal cells of the ovaries. Recent evidence on histology, molecular biology and genetics in relation to ovarian cancer has demonstrated the frequent fimbriae origin in the fallopian tubes (1). Therefore, it has been proposed to redefine the nomenclature and consider serous ovarian, fallopian tube and peritoneal cancers collectively, thus constituting an heterogeneous group of malignant tumours differentiated by cell/site of origin, pathological grade, risk factors, prognosis and treatment (1,2). Currently, the most accepted term is "pelvic serous carcinoma" (1). However, it should be noted that the term "ovarian cancer" continues to be used to refer interchangeably to the set of fallopian tube, ovarian and peritoneal cancers. Thus, when throughout our study we use the term "ovarian cancer", it includes fallopian tube and peritoneum cancers too.

Although most cases of OC are sporadic, 14% of ovarian neoplasms are linked to an inherited variant in a hereditary cancer predisposition gene (3). The most mutated genes are the BRCA1 and BRCA2 genes (60-65%). Nonetheless, mutations have also been described in the hereditary cancer predisposition genes related to Lynch syndrome (10%) and in other OC predisposition genes (11%), such as TP53, among others (3).

Hereditary Breast and Ovarian Cancer Syndrome, according to the National Cancer Institute, is defined as "An inherited disorder in which the risk of breast and ovarian cancer is higher than normal" (4). BRCA1 and BRCA2 are the high penetrance genes associated with higher proportion of hereditary BC and OC cases (5). The genetic predisposition model followed by BRCA genes is autosomal dominant type, in which the inheritance of a single mutation in one of these genes confers a high risk of developing BC or OC throughout life (6). HBOCS is also linked with an increased risk of developing other neoplasms, like melanoma, pancreatic, prostate, colon and other gynaecological cancers (4,6).

OC is the most lethal gynaecological cancer with the worst overall prognosis among all other gynaecological cancers (7,8). The non-specificity of the symptoms, particularly in early-stages, and the absence of a screening strategy, cause that around 75% of patients presented advanced-stage disease at diagnosis assessment (7–9).

3.2. OVARIAN CANCER OVERVIEW

3.2.1. HISTOPATHOLOGIC CLASSIFICATION OUTLINE

There are distinct types of OC according to the main cell of origin: (i) epithelial, (ii) germ cell and (iii) sex cord-stroma ovarian tumours (Figure 1, ANNEX 1). These correspond to different entities, diverging therefore, in their histological, clinical, diagnostic, and therapeutic features.

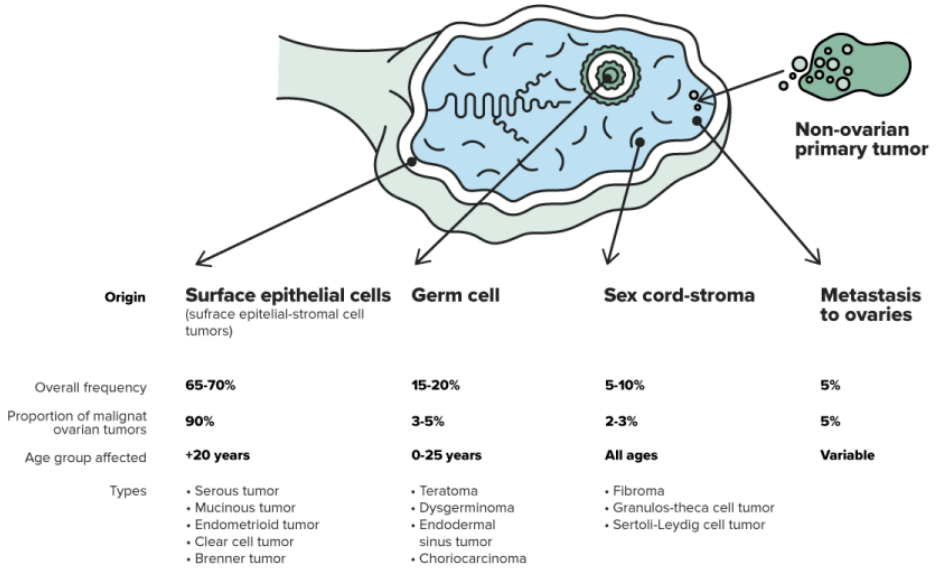

Figure 1. Histological subtypes of ovarian cancer - Taken from(10).

It is meaningful to note that throughout our study we refer to epithelial ovarian tumours and do not deal with the aspects related to non-epithelial ovarian tumours.

EOC is also classified according to cell type, into serous, mucinous, endometrioid, clear cell, transitional, and squamous cell tumours (ANNEX 2). The main characteristics are depicted in Table 1.

3.2.1.1. Pathology of ovarian tumours associated with BRCA mutations

It is estimated that 75-100% of OC in BRCA1 or BRCA2 carrier women are high-grade serous carcinomas. OC cases of endometrioid and clear cell carcinomas have also been described (5,11). Over and above, mucinous and borderline OC and non-epithelial tumours (germ cell and stromal-sex cord tumours) are not associated with BRCA mutations (5).

Table 1. Main types of EOC. (12). HNPCC: Hereditary Non-Polyposis Colorectal Cancer (Lynch syndrome).

	High-grade serous	Low-grade serous	Mucinous	Endometrioid	Clear cell
Usual stage at diagnosis	Advanced	Early or advanced	Early	Early	Early
Presumed tissue of origin/precursor lesion	Fallopian tube or tubal neometaplasia in inclusions of OSE	Serous borderline tumour	Adenoma-borderline-carcinoma sequence; teratoma	Endometriosis, adenofibroma	Endometriosis, adenofibroma
Genetic risk	BRCA1/2	?	?	HNPCC	?
Significant molecular abnormalities	TP53 and BRCA	B-RAF or K-RAS	K-RAS and ERBB2	PTEN, CTNNB1, ARID1A, PIK3CA, K-RAS, MI	HNF-1β, ARID1A, PTEN, PIK3CA
Proliferation	High	Low	Intermediate	Low	Low
Response to primary chemotherapy	80%	26-28%	15%	?	15%
Prognosis	Poor	Favourable	Favourable	Favourable	Intermediate

3.2.2. CARCINOGENESIS AND THE LINK WITH THE BRCA MUTATION

Two types of OC have been described depending on the primary site, clinical and pathological features (13), with distinct genetic patterns and different biological behaviour (14). Principal distinctive features are shown in Table 2.

- **Type I tumours**. These tumours are thought to evolve slowly from lower-grade precursor conditions, such as endometriotic cysts, cystadenomas, etc. Low-grade endometrioid carcinomas, clear cell carcinomas, borderline and low-grade serous carcinomas, and mucinous carcinomas are included (1,14,15). They are often diagnosed in early stages (I/II) and have low mortality rates (13,15).
- **Type II tumours**. These tumours are thought to evolve rapidly from more obscure precursors. High-grade endometrioid carcinomas, HGSC, carcinosarcomas and undifferentiated carcinomas are included. The association with mutations in the TP53

gene is highly usual (1,14,15). It is more common to diagnosed them in advanced stages (III/IV) and, therefore, the mortality rate is higher (13,15).

Table 2. Principal distinctive features of the two types of ovarian tumours. - Adapted from (16).

	TYPE I	**TYPE II**
Subtypes	Endometrioid, clear cell, LGSC, mucinous carcinomas, seromucous carcinomas, malignant Brenner tumours	HGSC, carcinosarcoma, undifferentiated carcinoma
Genetic stability	Stable	Unstable
Diagnosis	Early-stage	Advanced stage
Early detection	Frequent	Infrequent
Progression	Slow	Rapid
TP53 mutations	Infrequent	Frequent
Germline BRCA mutations	Infrequent	Frequent
Ki67 proliferative index	10-15%	50-75%
Median CA125 levels	53-413 U/mL	395-1340 U/mL

Until recently, the ovary was considered the primary site of carcinogenesis for high-grade serous ovarian carcinoma and the ovarian surface epithelium, the cell of origin (17). This consideration is mostly based on the "incessant ovulation" hypothesis, whereby tumour development is due to repetitive lesions of the OSE with each ovulatory cycle and exposure to oestrogen-rich follicular fluid (15,17,18). This would cause a pro-inflammatory state in the ovary and genetic damage at cell level due to oxidative stress which, in turn, would eventually lead to the development of a Mullerian "neometaplasia" in the OSE (mesothelium), which derives from the coelomic epithelium. (12). The latter gives rise to the Müllerian ducts, and accordingly, it was proposed that as the OSE became malignant, it would develop the morphological attributes of the Müllerian duct epithelium; being serous (fallopian tube-like), endometrioid (endometrium-like), and mucinous (endocervical-like) (12). This aberrant cell differentiation process provides

the fundament of the current classification of epithelial ovarian tumours (12). Plus, the specified changes would be the main substrate for neoplastic transformation because of the accumulation of unrepaired DNA damage (17,18). Although this evidence may explain the carcinogenesis of type 1 ovarian tumours, it does not clear up the pathogenic development of type 2 tumours (17).

The discovery of the BRCA1 and BRCA2 genes in relation to the increased risk for the development of OC led to the realization of the RRS and the development of a pathology protocol, called the SEE-FIM (17). This fact allowed the anatomopathological analysis of the tissues and the tubal surface epithelium was proposed as a possible origin of ovarian tumours (15,17,18). The proposed mechanism is based on the same evidence as the "incessant ovulation" hypothesis in the OSE and is supported by the high incidence of preneoplastic lesions, chronic inflammation and serous tubal intraepithelial carcinomas, that were discovered in the fallopian tubes of high-risk women (12,17,18). STIC is a developed carcinoma confined to the epithelium that can produce metastases (12). Nevertheless, according to published studies, only 50-60% of ovarian tumours would have a tubal origin. Thus, evidence regarding the presence of precursor lesions and their contribution to tumour development is lacking. It is also not known whether all high-grade serous ovarian carcinomas originate in the fallopian tubes or in other locations (17).

Overall, tumour suppressor genes contribute to cell growth control by inhibiting cell proliferation and tumour development (19). Accordingly, when these genes are inactivated contribute to the abnormal proliferation of carcinogenic cells due to the loss of control of cell cycle regulation (19). BRCA1 and BRCA2 are tumour suppressor genes that repair DNA double-strand breaks through HHR in order to preserve genomic stability (4,5). Genomic instability in conjunction with hormone-dependent carcinogenic environment, among other pathogenic processes mentioned above, lead to cellular neoplastic transformation (4,5). Further functions of the BRCA genes include control of centrosome dynamics, chromosome segregation and cytokinesis, and distinctively BRCA1 partakes in health embryonic development and onset of BOC, centrosome replication and splicing, among others (4,5).

3.2.3. *EPIDEMIOLOGY*

3.2.3.1. OC statistics worldwide

As of 2018, ovarian cancer is considered the seventh most common cancer in women worldwide (7). Furthermore, OC is the eighth leading cause of cancer-related mortality in women worldwide, with more than 310.000 new cases and about 200.000 deaths in 2020 (20,21). The worldwide incidence and mortality is 3.4% and 4.7%, respectively (20). Differences in incidence and mortality in high/very high HDI versus low/medium HDI are shown in Figure 3.

Incidence rates of ovarian cancer varies across the world. The overall incidence of epithelial tumours varies from 9-17 per 100,000 (1) and is greater in countries undergoing transition (22) and high-income countries (1,23). Around 30% of OC cases occur in Europe (22). In 2012, the highest rates of OC occurred in China (14.60% of all cases), India (11.33% of all cases), the United States (8.81% of all cases) (22), Russian federation (5.64% of all cases) and Indonesia (4.32% of all cases) (22,23).

Even so, the incidence rate increases proportionally with age, being more frequent in the 60-64 age group (1). Moreover, it is important to emphasize that the frequency of the histological subtypes varies according to the age group. Germ cell tumours predominate in women younger than 20 years of age, borderline tumours are typically found in women between 30 and 40 years, and invasive epithelial OC mostly occur after 50s (1).

In the United States, the mean annual OC incidence rate was 11.5 per 100,000 women from 2010 to 2014 (2). Differences in incidence rates of OC based on race or ethnicity have been described. Non-Hispanic white women have the highest incidence rates (12 per 100,000), in comparison with Asian/Pacific Islander, who have the lowest incidence rates (9.2 per 100,000). The incidence rate for Hispanic women is 10.3 per 100,000 and 9,4 per 100,000 for non-Hispanic black women (2,22).

The epidemiological diversity of OC is closely linked to the risk factors that are related to the development of OC (22). Although the main causes of the epidemiological differences remain unknown, the prevalence of risk factors partially explains the differences that have been objectified in relation to the risk of developing OC according to race or ethnicity (2).

In contrast to the incidence, the mortality of ovarian cancer shows a distinct pattern (22). In recent years, the mortality rate has decreased in Europe and North America. Nevertheless, the African population has the highest mortality-to-incidence ratio. As for Asia, India is the Asian country with the highest mortality rate from OC (22).

Likely, the low incidence of ovarian cancer in African countries, compared to other countries, is mostly explained by the absence of cancer registries (24). The high mortality-to-incidence ratio can be explained by the lack of access to optimal treatments and more comorbidities compared with other women (2,22,24).

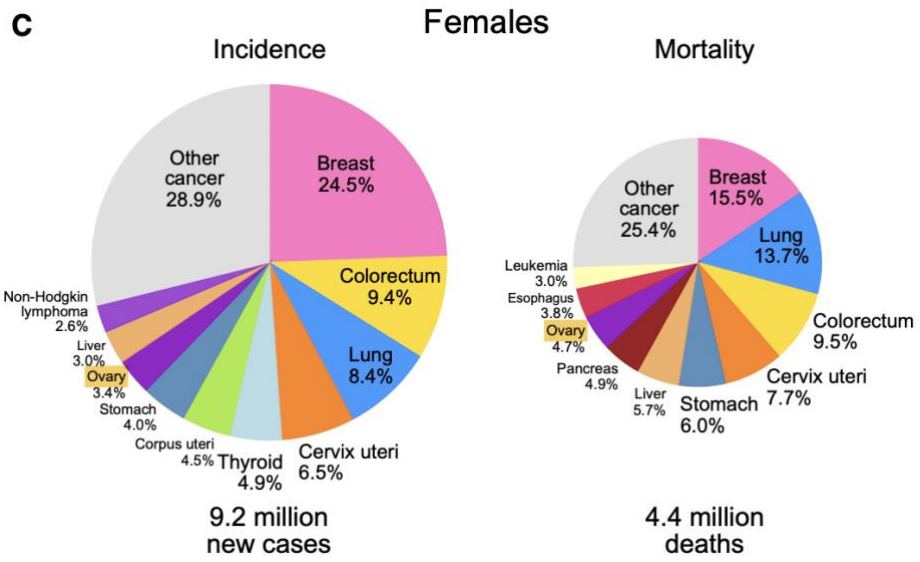

Figure 2. Distribution of Cases and Deaths for the top 10 most common cancers in 2020 among women. (20)

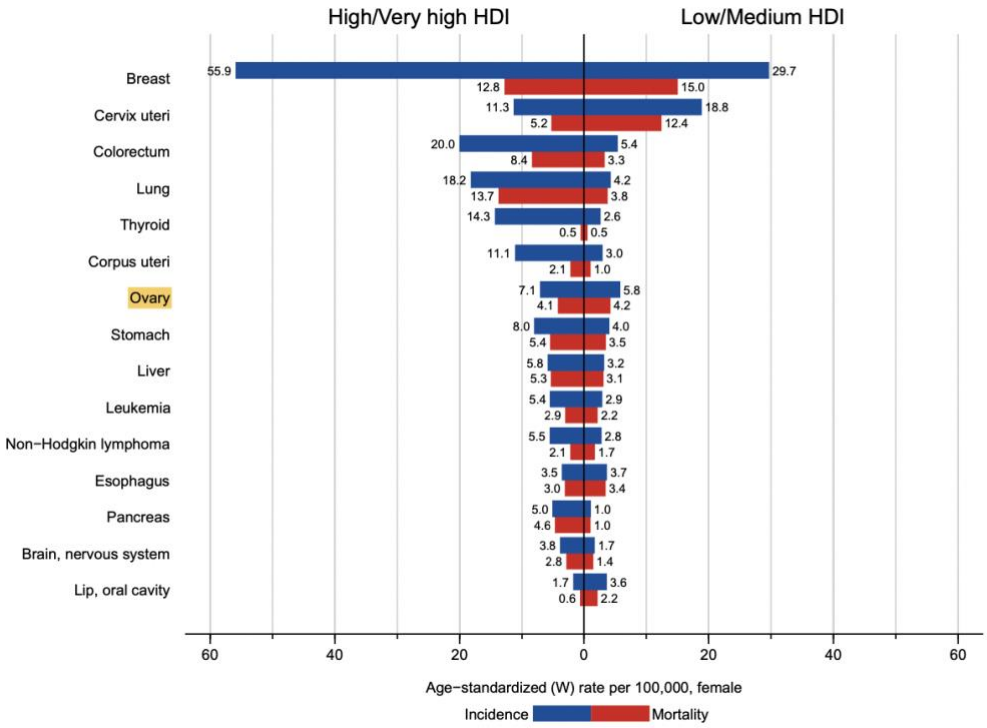

Figure 3. Incidence and Mortality Age-Standardized Rates in High/Very High Human Development Index (HDI) Countries Versus Low/Medium HDI Countries Among Women in 2020. The 15 most common cancers in the world are shown in descending order. (20).

3.2.3.2. Trends and evolution of OC

Broadly, OC incidence has been declining since the mid-1980s, with a 29% decrease in the incidence rate, from 16.6 per 100,000 in 1985 to 11.8 per 100,000 in 2014 (2,25). Nonetheless, trends are different according to age. The female population over the age of 65 showed an increase in the incidence rate from 1975 until it began to decrease in 1990 (2,25). Given that the risk of invasive epithelial OC is reduced by 20% with the first birth and by 10% by each subsequent birth, the increased incidence rate can be explained by the decrease in parity during the beginning and middle of the 20th century (2).

Hormone replacement therapy, particularly estrogenic methods, used during menopause has been linked to an increased risk of ovarian cancer (2). Women who have used oestrogen-HRT have a 20% higher risk of developing the neoplasia than women who have never used (2). The risk is greater, with approximately a 40% excess risk, in those women who are currently taking HRT and those who have stopped it in the last 5 years (2). In this sense, the publication of a study during the 2000s that associates HRT to an increased risk of breast cancer and that led to the reduction in the prescription of this treatment, could partially explain the decrease in the incidence rate of OC in white women in the mentioned age range (2,25).

The opposite happens with the consumption of oral contraceptives, since it has been seen that there is an approximate 35% reduction in risk in those women who consume them for 5 to 9 years, prolonging the protective effect they confer (2). This fact, therefore, could explain the tendency towards a decrease in the incidence among women under 65 years of age since 1975 (2).

Mortality trends resemble those of incidence as a consequence of the low survival rate of OC (25). Due to the decrease of incidence and improvement of treatment, the mortality rate from OC dropped off around 33% between 1976 (10.0 per 100,000) and 2015 (6.7 per 100,000) (2).

3.2.3.3. BRCA mutation epidemiology

The strongest risk factor for developing OC is a family history of breast and/or ovarian cancer (2,22,26). About 18% of EOC, particularly, high-grade serous carcinomas, are caused by inherited pathogenic mutations, mostly in BRCA1 and BRCA2 genes (2,8). Manifold studies reported variation in the worldwide prevalence of BRCA1 and BRCA2 pathogenic sequence variants, probably due to ethnic diverseness, barriers to access, histopathological variation, among others (8). The frequency of BRCA1 and BRCA2 pathogenic variants in the general population has been estimated to be 1 in 400-500, excluding the Ashkenazi Jews, in whom the frequency is higher

(4). Carriers of inherited pathogenic mutations in BRCA1 or BRCA2 are at an increased lifetime risk of developing ovarian cancers; recent reports pointed out that the cumulative risk of ovarian cancer to age 70 years was **39-44% for BRCA1 carriers** and **11-18% for BRCA2 carriers** (3,8,27), compared to 1-1,5% risk in the general population (3,13). Typically, the age of cancer presentation in these patients occurs earlier compared to sporadic cases of OC (1).

3.2.4. CLINICAL FEATURES

The difficulty regarding the diagnosis of ovarian cancer lies in the **non-specificity** of the symptoms, especially in early stages (28–30). The clinical presentation can include persistent abdominal or pelvic pain, bloating or lower abdominal distension, pressure, early satiety and urinary frequency or urgency, due to tumour growth (9,28,29); vaginal and rectal bleeding and change in bowel habit or nonspecific gastrointestinal symptoms (14,30). OC may also present like a surgical emergency secondary to torsion or rupture of the mass (29). In advanced stages, patients may present with ascites and clinical symptoms due to intestinal and omental invasion ("omental cake") (29), as well as constitutional symptoms (28).

Regarding the clinical signs, the finding of a **pelvic mass** in the physical examination is a key sign. Characteristics such as fixation, solid composition, nodular and irregular morphology of the mass point to malignant character (29).

3.2.5. DIAGNOSIS

Adnexal masses are frequent findings in the gynaecological consultation, with a prevalence of 7,8% in premenopausal women and 2,5-18% in postmenopausal women. Most of these will be benign, 10% malignant and 25% unclassifiable (11).

The finding of a suspicious adnexal mass must lead to the application of a diagnostic strategy in order to target the possible malignant character of the mass and refer the patient to the gynaecologic oncology team for study and treatment (11).

Diagnostic management (Figure 4) includes the following tools:

- **Anamnesis** including personal and family medical history, emphasizing the possibility of high-risk mutations and history of other neoplasms (11,14).

- **Physical examination** to evaluate the overall condition, focusing in abdominal and pelvic examination to assess the size of the mass, signs of infiltration and the presence of ascites (11,30). Rectovaginal examination should be performed (30).
- **Complementary tests**: there is no test that has demonstrated 100% sensitivity, specificity and predictive value to establish the malignant character of adnexal masses (11).
    - Transvaginal and abdominal ultrasound is a first-line test. It must be performed by an expert sonographer or analysed according to the O-RADS score system or IOTA criteria (11,14,31) (ANNEX 3).
    - Magnetic resonance imaging should be considered if indeterminate ovarian masses are detected in US (11).
    - Extension study with computed tomography scan (including abdomen and pelvis +/- thorax) to rule out metastases and synchronous tumours (11).
    - Biomarkers can contribute to guide diagnosis in conjunction with the other tests (11).
        - Quantitative analysis of **CA125 +/- HE4** markers is recommended.
        - The determination of **Ca19.9** and **CEA** may be useful in suspected adnexal masses of mucinous or metastatic origin from the digestive tract (11).
        - Quantitative analysis of **AFP** and **hCG** in addition to CA125 +/- HE4 is recommended in women under 40 years of age and/or with an adnexal mass suspicious of a germinal tumour (11).
- Predictive and probabilistic indices based on test combinations, such as biomarkers and US, are used in order to improve diagnostic accuracy to set the malignant character of an adnexal mass (11,14). The SEGO clinical practice guideline recommends the application of the **ROMA algorithm** if a suspicious or indeterminate adnexal mass is objectified according to the O-RADS US criteria (11). The ROMA algorithm combines the determination of HE4 and CA125 with menopausal status to estimate the probability of malignant character in an adnexal mass, with its diagnostic performance being superior in postmenopausal women (11,14).

Despite all these diagnostic tools, the definitive diagnosis is currently provided by the **pathological anatomy of the surgical specimen** (11). There are two valid strategies:

- The intraoperative histological analysis with complete surgical staging after the diagnosis of malignancy, all in a single surgical procedure (11).

- The deferred analysis of the mass with complete surgical staging in a second surgical procedure, according to the histological diagnosis (11).

The SEGO CPG recommend the deferred histological analysis in those cases in which the findings may modify the surgical strategy: mucinous, metastatic tumours and patients who want to preserve fertility (11).

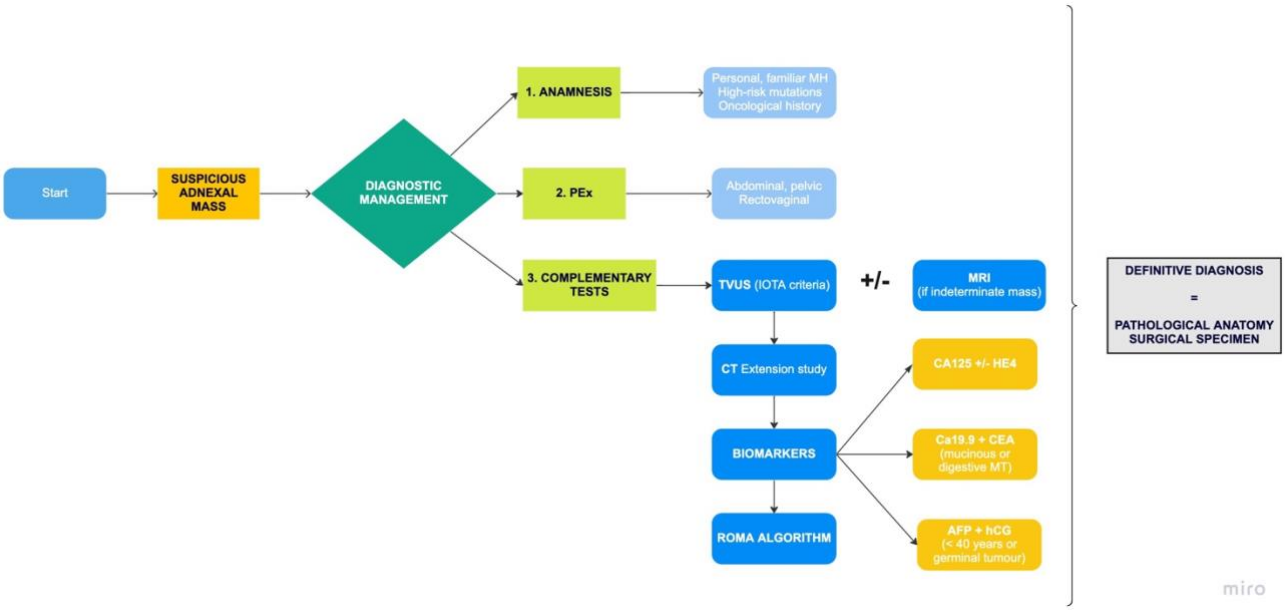

Figure 4. Diagram of the diagnostic management of a suspicious adnexal mass

Cancer staging is conducted at the end of the described procedure (ANNEX 4).

3.2.5.1. Genetic risk assessment

Criteria for genetic counselling in cases of suspected HBOCS are shown in Table 3.

Table 3. Criteria for genetic counselling in cases of suspected HBOCS – Adapted from (11).

An individual from a family with a known germline or somatic BRCA1/2 mutation in a patient.
Personal or family history of breast cancer in one of the following criteria: - Diagnosed at 45 years of age or earlier. - Diagnosed at 50 years of age or earlier with a 1st, 2nd or 3rd degree relative with BC before 50 years of age and/or at least one 1st, 2nd or 3rd degree relative with OC at any age. - Two BC when the first occurred before 50 years of age. - OC at any age, with at least two relatives in 1st, 2nd or 3rd degree with BC and/or OC at any age.

- Male with BC in 1st, 2nd or 3rd generation. - In those people of ethnic groups associated with a high frequency of mutations, even if they have no additional history (Ashkenazi Jew).
Personal history of ovarian cancer.
Personal history of breast cancer in male.
Healthy, with the following family history: - Relatives in 1st or 2nd degree with any of the above criteria. - 3rd degree relatives with BC and/or OC with at least two relatives in 1st, 2nd or 3rd generation with BC (one before 50 years of age) and/or OC.

3.2.5.2. Study of mutations in the BRCA genes

The most common pathogenic mutations of the BRCA genes include small deletions, insertions or nucleotide changes that affect coding regions, exons and intron-exon junction regions (6). These alterations usually cause the premature termination of the synthesis of the BRCA proteins resulting in non-functional proteins (5,6). About 1.700 distinct pathogenic point mutations detected worldwide have been compiled (5,6).

Massive sequencing techniques are used for mutation identification (5,6). Currently, manifold laboratories offer the cancer predisposition analysis through various multigenic panels, which allow the detection of mutations in multiple genes, including the BRCA1 and BRCA2 genes (5).

3.2.6. TREATMENT OUTLINE

The treatment of EOC is based on surgery and adjuvant or neoadjuvant treatments (32) (Figure 5), at all times subject to stage and histological features. It is worth noting the definition of the various surgeries that can be performed.

- **Staging surgery** is the surgery performed in the early-stage disease to know the real spread of it (11).
- **Debulking surgery** is based on the resection of the disease in advanced stages (11).
    - Primary: before the start of any other treatment (11).
    - Interval: after having administered three or four cycles of chemotherapy (11).

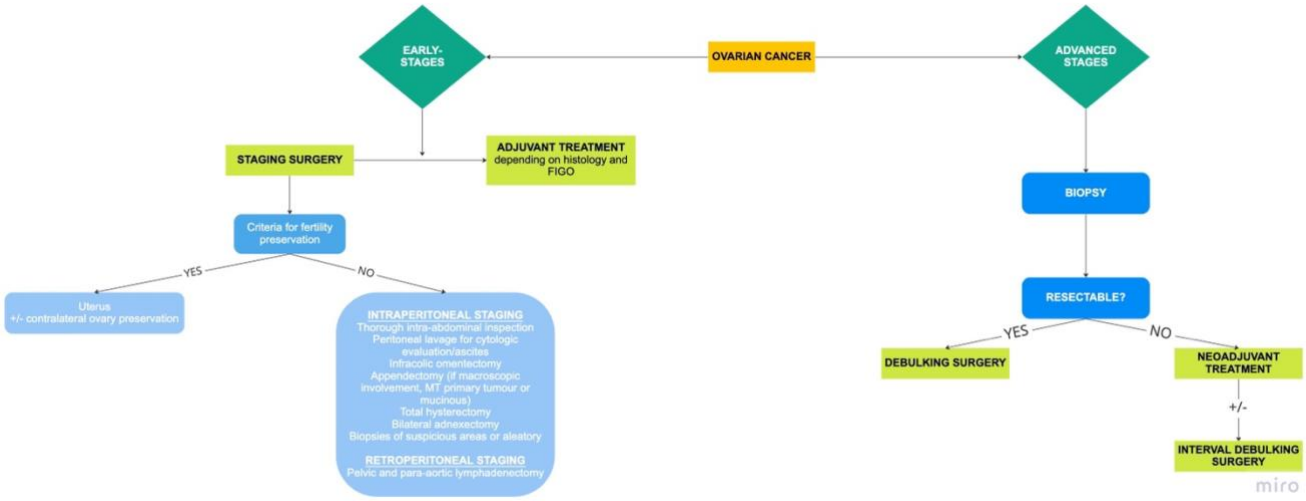

Figure 5. Ovarian cancer treatment diagram. – Adapted from (11,32).

3.2.7. OVARIAN CANCER RISK MANAGEMENT: CURRENT STRATEGY

The most effective strategy for OC risk reduction in women with a pathogenic mutation in BRCA1 or BRCA2 genes is **risk-reducing prophylactic bilateral salpingo-oophorectomy** (3,33). Nevertheless, two situations that contraindicate this recommendation have been described:

- Surgical risk greater than benefit (3).
- Active cancer and poor short-term prognosis (3).

RRS has shown about 80-90% reduction in the risk of gynaecological cancers, including ovarian, fallopian tube and peritoneal cancer (3,33), and a 77% reduction in all-cause mortality (33). Current protocols recommend performing RRS between the ages of 35-40 in carriers of pathogenic or probably pathogenic germline mutations in BRCA1 and between the ages of 40-45 in BRCA2 (3). However, when deciding the timing of the surgical intervention, pathogenic variant type, patient's preference, and family history should be taken into consideration (3,33). Accordingly, this recommendation can be advanced 5-10 years depending on gestational desire and family history (3).

The surgical approach of choice for performing RRS is minimally invasive laparoscopic surgery to reduce morbidity, hospitalization time, and achieve a better cosmetic outcome (33).

Once the surgery has been performed, the patient will have a consultation at the Gynaecology Service every six months for:

- Report on the pathological anatomy of the surgical specimens (3).

- Continue breast monitoring (3).
- Cervical cancer screening according to the population program, except those patients who have a previous hysterectomy (3).
- Establish the indication for HRT and, if starting it, assess the evolution, therapeutic adherence, and adverse effects (3).

Despite RRS being the most effective strategy in terms of reducing mortality, some patients wish to **postpone or refuse** this intervention. The most common situations are the following:

- Women **over 30** until they reach the recommended age for RRS (3).
- Women until their **reproductive desire is fulfilled** (3).
- Women who **refuse RRS** for various reasons (3).

Several follow-up strategies have been proposed for the early diagnosis of ovarian cancer in this group of patients. The screening strategy practiced in our setting is based on a follow-up plan with pelvic examination, **transvaginal ultrasound and serum tumour marker CA125 every 6 months** (3,33) and the performance of **individualized surgery if these tests present alterations**. Nevertheless, there is no clear evidence of its effectiveness (26). Some studies reported that this screening strategy is ineffective in detecting tumours in a sufficiently early stage to influence prognosis (34). Plus, it is also unclear whether screening improves survival in women who carry a pathogenic mutation in the BRCA genes (35), nor what is the optimal time interval to conduct the follow-up plan (33,36). Thus, in this context, there is a lack of evidence concerning new approaches with the aim of improving the follow-up management in this group of patients (34).

Besides, the **false-positive rate** is **significant** for both TVUS and CA125, being higher in premenopausal women (34). On the one hand, while TVUS can examine the ovary, it is only able to detect large tumours and cannot discriminate benign from malignant tumours in a definite way (37). On the other hand, CA125 is elevated in a variety of benign conditions; being elevated in around 1% of the healthy population in previous studies (16,37–39). These facts lead the performance of unnecessary surgeries, with all the risks derived from surgical procedures (infection, bleeding, bladder or bowel damage, blood clots and lower extremities lymphedema, among others) (40). Another relevant drawback derived from this issue is the psychological effect that the suspected diagnosis of a neoplasia has on the patient, causing anxiety and discomfort (16,34).

The available options in OC risk management for patients with a pathogenic mutation in the BRCA genes are outlined in a diagram (ANNEX 5).

3.2.7.1. Biomarker CA125

Cancer Antigen 125 or Carbohydrate Antigen 125 is a high molecular weight mucinous glycoprotein found on the surface of OC cells (16). The CA125 expression pattern differ among the distinct subtypes of ovarian cancer. Overall, serous tumours have higher CA125 concentrations, in contrast with mucinous tumours, that have the lowest (16).

CA125 is present in multiple locations; it can be found in cervical mucus from healthy women, in the amniotic fluid and chorionic membrane from the foetus, in human milk and in some structures related with the respiratory system, among others (16). During embryogenesis, CA125 is present in the ovary, but eventually disappears. Nevertheless, if the development of an ovarian neoplasia occurs, it is expressed again (16).

Likewise, it is worth noting the main factors influencing concentrations of the mentioned biomarker (Table 4). Thus, the elevation of CA125 in multiple conditions, whether benign or malignant, results in a high rate of false positive results (16) and limitation of its specificity (37).

Table 4. Main factors influencing serum CA125 concentrations (16). – BMI: Body Mass Index

Factors associated with high [CA125]	Factors associated with low [CA125]
Premenopausal status	African and Asian women
Caucasian women	Routine caffeine consumption
Previous cancer diagnosis	Smoking
Endometriosis	Previous hysterectomy
Menstruation	Women over 45-50 years of age
Increased BMI	Osteoporosis
Cirrhosis	Osteoarthritis
Coronary artery disease	Hypercholesterolemia
Multiple non-ovarian malignancies (such as lung and breast cancer)	

Regarding the interpretation of results, the threshold is set at 35 U/mL; concentrations below this value are considered normal (16). Moreover, a recent systematic review concerning biomarkers in ovarian cancer, reports a sensitivity of 81% and specificity of 75% for CA125 in distinguishing benign from malignant tumours in pre- and postmenopausal women, even though the reported specificity for only premenopausal population was lower (14). These values

are consistent with previous studies (16,39,41). Lastly, it is relevant to highlight that, because of the low prevalence of OC, the ideal screening test must have a sensitivity over 75% and a specificity of at least 99.6% (16).

3.2.7.2. When are the follow-up tests considered altered?

Although there are no uniform criteria, in our setting it is considered tests as altered if:

- **CA125 marker** has values **above 35 U/mL** and/or,
- **TVUS** shows altered ovarian images (**O-RADS ≥ 2**) (3) (ANNEX 3).

These findings are considered an indication for individualized surgery since the patient has a very high risk of harbouring a malignant tumour due to her genetic predisposition (3).

3.2.8. SCREENING STRATEGIES

Multiple studies have been conducted concerning the population screening for ovarian cancer. The results of these projects have not been favourable in terms of decreasing mortality from OC and improvement in survival of the screened population (13,42,43). Consequently, consensus among medical and public health organizations is that screening for OC in the general population is not recommended (13,42,43). Despite the evidence and as discussed in previous sections, a follow-up strategy should be offered to asymptomatic women carrying a pathogenic mutation in the BRCA1 or BRCA2 genes that refuse or postpone RRS (43). Currently, the scientific and clinical groups continue working to develop an effective screening strategy.

3.2.9. PROGNOSIS AND SURVIVAL

Numerous studies of OC survival in patients with BRCA1/2 mutation compared to free-mutation patients suggest that short-term survival is better in carrier women (4,5,44). In a large study in Canada and the United States, *McLaughlin et al.* reported that in the first 2 years after diagnosis, annual mortality rates were lower for the carriers than for non-carriers, but between years 3 to 10, mortality rates were higher for women with BRCA1/2 mutation than for the free-mutation group (44). These results suggest that patients with high-risk mutations have different survival patterns. Some studies proposed that these differences may reflect a better acute response to chemotherapy in carrier women (44). Thus, for women with invasive OC, the short-term survival advantage of carrying BRCA1/2 mutation does not prompt into a long-term survival benefit (4,44).

3.3. PAPSEEK

3.3.1. CONCEPT AND BASIS

The PapSEEK test is a type of liquid biopsy developed to detect early stage endometrial and ovarian cancer, based on the combined analysis of somatic mutations and aneuploidy from samples collected from the endocervical and intrauterine cavity through routine Papanicolaou (Pap) tests (45,46). The underlying technology of the test is a multiplex PCR (45,46).

The PapSEEK test takes as a reference the analysis of the Pap smear in the context of cervical cancer screening and how its application has shown a decrease in mortality from cervical cancer in the screened population (45). It is relevant to highlight the recent advances that have replaced the traditional Pap smear by a liquid-based cytology, which enables both cytological analysis and DNA collection for the detection of the HPV, the causative agent of cervical cancer (37).

Currently, there has been evidence showing that ovarian cancer cells exfoliate from the primary ovarian malignancy and descend through the cervical os and into the vagina, where they can be collected for subsequent analysis (45,47). Moreover, a recent proof-of-principle study showed that neoplastic cells from ovarian tumours peel off and accumulate in the cervix, allowing the detection of tumour DNA collected in the fluids that are usually analysed in routine Pap tests (37,45).

3.3.2. PROCEDURE

The technique includes the following procedures (Figure 6):

- **Sample collection** using the Pap test, which can be performed with two distinct medical devices: the Pap brush or the Tao brush (45,46). The main difference between the two methods is the anatomical location of the sample collection. The Pap brush collects samples from the endocervical canal, while the Tao brush collects intrauterine samples (48), closer to the anatomical sites of the tumour (45,46).
    - Pap brush is an endocervical brush used for cytology sampling, mostly in the context of cervical cancer screening (49). Samples can be collected from the endocervical canal as well as from the ectocervix, depending on the type of endocervical brush (49). Cervical samples are collected by a gynaecologist using a Cervex brush (Rovers® Medical Devices) (50).
    - Tao brush is a patented device and FDA approved, used for endometrial histology and cytology sampling (48). It allows to collect endometrial tissue to a depth of 1.5-2 mm and brushes the interior of the uterus to obtain a complete

sampling of the endometrium (48). With the patient in lithotomy position and after pelvic examination, the brush with the protection sheath is inserted into the uterus. When the device is properly disposed, the sheath is pulled out, and the collection of the tissue is made by the 5-times rotation of the brush. Next, the sheath is replaced to avoid contamination and loss of the sample. Finally, the brush is removed (48). The use of the Tao brush may require the intervention of experienced and trained healthcare professionals, due to the complexity of its use compared to the Pap brush (46).

- **Sample processing** by submerging the brush into preservative fluid (45,46). Cervical samples are suspended in 20 mL of ThinPrep liquid-based solution, as performed in the cervical cancer screening program in Catalonia (50).
- **DNA purification and analysis** of the fluid using a polymerase chain reaction (PCR) based multiplex test and Next Generation Sequencing to assess genetic alterations that commonly occur in OC (45,46).

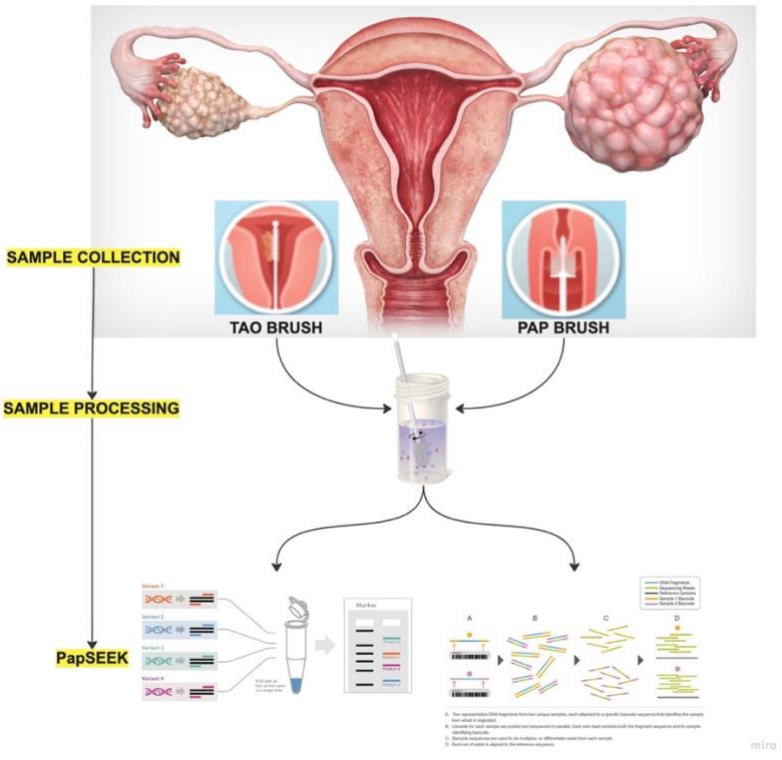

Figure 6. PapSEEK procedures. *Images taken from (51–53).*

3.3.3. MARKERS AND ANALYSIS

The PapSEEK test is comprised by two distinct markers:

- **Somatic mutations**.
    - Target. Pinpointing of specific somatic mutations in the collected samples.
    - Method. A sensitive PCR-based error-reduction technology called Safe-Sequencing System (Safe-SeqS) is used, since the DNA from the tumour cells is expected to be the smallest fraction of the total DNA collected from the Pap samples, with the DNA fraction from normal cells being predominant, and allowing to pinpoint low-frequency mutations (45). DNA from the samples is amplified in three multiplex PCR with 139 primers pairs that were designed to amplify 110 base pairs to 142 base pairs segments (45). The analysis is performed in the following **18 genes**: *AKT1, APC, BRAF, CDKN2A, CTNNB1, EGFR, FBXW7, FGFR2, KRAS, MAPK1, NRAS, PIK3CA, PIK3R1, POLE, PPP2R1A, PTEN, RNF43 and TP53* (45).
    - Interpretation of results. A sample is scored as positive if any somatic mutation is detected in the specified genes.
- **Aneuploidy**.
    - Target. Pinpointing of altered chromosome arms in the collected samples.
    - Method. A PCR-based method is used to amplify about 38.000 loci of long interspersed nucleotide elements (LINEs) with a single primer pair. LINEs are a group of non-long terminal repeat retrotransposons that are widespread in the genome and are found on all 39 non-acrocentric autosomal arms (45). After sequencing, the data are processed to identify gains or losses on single chromosome arms and allelic imbalance on 39 chromosome arms using the Within-Sample AneupLoidy DetectiOn (WALDO) software (45). This software includes a support vector machine (SVM) to distinguish between euploid and aneuploid samples (45). The most frequently altered arms in OC are ***4p, 7q, 8q*** and ***9q*** (45).
    - Interpretation of results. A sample is scored as positive (aneuploid) if gains or losses of chromosomes arms are identified, particularly 7q and 8q (45).

3.3.4. INTERPRETATION OF THE RESULTS

PapSEEK scores a sample as positive if it holds either a mutation or an abnormal chromosome arm number (45).

3.3.5. *Diagnostic yield*

		Total sample (n = 245)			Stages I and II (n = 89)			E (n = 714)
		S	PPV	NPV	S	PPV	NPV	
Pap brush	Somatic mutations	29.4%	22.6	0.71	28.1%	21.6	0.73	**98.7%**
	Aneuploidy	11.0%	55	0.89	14.6%	73	0.86	**99.8%**
	Either	33.1%	23.6	0.68	33.7%	24.1	0.67	**98.6%**
		Total sample (n = 51)			Stages I and II (n = 15)			E (n = 125)
		S	PPV	NPV	S	PPV	NPV	
Tao brush	Somatic mutations	41.2%	n.c.	0.59	40.0%	n.c.	0.60	**100%**
	Aneuploidy	33.3%	n.c.	0.67	13.3%	n.c.	0.87	**100%**
	Either	45.1%	n.c.	0.55	46.7%	n.c.	0.53	**100%**

Table 5. Diagnostic yield of the PapSEEK test in ovarian cancer (total sample and early stages) – (46).

S: sensibility, E: specificity, PPV: positive predictive value; NPV: negative predictive value, n.c.: not calculable.

4. JUSTIFICATION

The identification of the BRCA1 and BRCA2 genes was a relevant milestone to improve risk assessment in families with a high incidence of breast and ovarian cancer (34). The recommendation in the management of OC risk in patients with BRCA1 and BRCA2 mutations is risk-reducing prophylactic bilateral salpingo-oophorectomy (3). Nevertheless, this intervention produces infertility, premature menopause, risk for cardiovascular disease, cognitive decline and osteoporosis (36).

For women who choose to postpone or decline risk-reducing surgery**, a follow-up strategy with more favourable outcomes is lacking** (34). Currently, they are offered a six-monthly follow-up strategy with the performance of a transvaginal ultrasound and the analysis of the serum tumour marker CA125 (3). Notwithstanding, it is not known whether this follow-up strategy actually detect cancer earlier or what the optimal timing should be for high-risk women (36). Furthermore, it is well known that the **false positive rate** for the current screening strategy is significant (34), with a value of **15%** (54) and being higher in premenopausal women (34). Thus, the consequence of this fact is the performance of unnecessary surgeries and the anxiety generated by the diagnostic suspicion of a neoplasia (34). It is estimated, depending on the follow-up strategy modality, that up to eight women will undergo surgery for every woman with OC detected through current screening procedures (34).

PapSEEK is a multiplex PCR-based test developed to detect genetic disorders in the samples collected from endocervical and intrauterine cavity though Pap and Tao brushes, respectively (45). The DNA of tumour cells that are exfoliated from the primary tumour in the intrauterine/endocervical cavity is analysed, and the detection of distinctive genetic alterations of ovarian neoplasia is sought (45). In the diagnostic yield study, the test showed a specificity of 98.6% when the sample is collected with Pap brush, and 100% with Tao brush (46). Thus, these data on the specificity of the PapSEEK test are the starting point of our study. It is relevant to highlight that, overall, the specificity of a test is the probability that an individual who does not have the disease (disease free) will get a negative test result (55).

Our study proposes the incorporation of the PapSEEK test into the current follow-up strategy for the early diagnosis of ovarian cancer in BRCA1 and BRCA2 patients who choose to refuse or postpone RRS. Currently, when a woman with a high-risk pathogenic mutation presents an altered TVUS and/or serum tumour marker CA125, she is candidate for individualized surgery because the suspicion of an ovarian tumour is high, despite the elevated rate of false positive results. In this context, our study raises the realisation of the PapSEEK test prior to surgery with

the aim of showing how many patients with a negative PapSEEK result could have spared surgery, by the comparison of the anatomopathological analysis of the surgical specimen with the results obtained on the PapSEEK test. The potential clinical impact of our study is the diagnostic yield improvement of the current follow-up strategy by avoiding unnecessary surgical interventions and, consequently, reducing the morbidity linked to surgery (ANNEX 6).

To sum up, the aim of this study is to improve the follow-up strategy offered to women with a BRCA1 or BRCA2 pathogenic mutation who refuse or postpone RRS, through decreasing the false-positive rate of screening with the goal of avoiding unnecessary surgeries. The implementation of a new test called PapSEEK with a specificity between 98.6% and 100%, depending on the sampling technique, in previous studies (45), prior to surgery, will allow identifying those women who, despite having an altered TVUS and/or CA125 and, therefore, being candidates for individualized surgery, they do not present a true ovarian neoplasia.

5. HYPOTHESIS

5.1. MAIN HYPOTHESIS

The application of the follow-up strategy based on transvaginal ultrasound, serum tumour marker CA125 and PapSEEK is more specific than the current follow-up strategy based on transvaginal ultrasound and serum tumour marker CA125, in the context of the six-monthly follow-up for the early detection of ovarian cancer in women who carry a pathogenic mutation in the BRCA1 or BRCA2 genes and who are candidates for individualized surgery after presenting a transvaginal ultrasound and/or an altered CA125.

5.2. SECONDARY HYPOTHESES

The application of the follow-up strategy based on transvaginal ultrasound, serum tumour marker CA125 and PapSEEK increases the sensibility, compared with the current follow-up strategy based on transvaginal ultrasound and serum tumour marker CA125.

The incorporation of the PapSEEK test in the six-monthly follow-up strategy for the early detection of ovarian cancer in women carrying a pathogenic mutation in the BRCA1/2 genes is related to a good patient acceptability.

6. OBJECTIVES

The proposed project has the following objectives:

6.1. MAIN OBJECTIVE

The main purpose of this project is to determine whether the application of the combined follow-up strategy based on transvaginal ultrasound, serum tumour marker CA125 and PapSEEK is more specific than the current follow-up strategy based on transvaginal ultrasound and serum tumour marker CA125 , in the context of the six-monthly follow-up for the early detection of ovarian cancer in women who carry a pathogenic mutation in the BRCA1 or BRCA2 genes and who are candidates for individualized surgery after presenting a transvaginal ultrasound and/or an altered CA125.

6.2. SECONDARY OBJECTIVES

To determine whether the application of the follow-up strategy based on transvaginal ultrasound, serum tumour marker CA125 and PapSEEK increases the sensibility, compared with the current follow-up strategy based on transvaginal ultrasound and serum tumour marker CA125.

To assess patient acceptability concerning the incorporation of the PapSEEK test in the six-monthly follow-up strategy for the early detection of ovarian cancer in women carrying a pathogenic mutation in the BRCA1/2 genes, using the TFA questionnaire.

7. MATERIAL AND METHODS

7.1. STUDY DESIGN

The project will be a **cross-sectional** study. It will be carried out in 9 hospitals: "Hospital Universitari Doctor Josep Trueta", "Hospital Universitari Germans Trias i Pujol", "Hospital Universitari Vall d'Hebron", "Hospital Clínic de Barcelona", "Hospital Universitari de Bellvitge", "Hospital Universitari Parc Taulí", "Hospital Universitari Arnau de Vilanova", "Hospital del Mar de Barcelona" and "Hospital de la Santa Creu i Sant Pau"; between December 2022 and December 2025. HUJT will be the reference centre.

7.2. STUDY POPULATION

The target population of this study will be asymptomatic women over 30 years of age, carrying a pathogenic mutation in the BRCA1 or BRCA2 genes without ovarian cancer, who refuse or postpone risk-reducing prophylactic bilateral salpingo-oophorectomy, and who assist to "Unitat d'alt risc oncològic" of the specified hospitals, in Catalonia. All patients must fulfil the following inclusion and exclusion criteria.

7.2.1. INCLUSION CRITERIA

- Women with a **positive genetic study** for a **pathogenic mutation in the BRCA1/2 genes**.
- Women **over 30 years of age**.
- Women who have **postponed or refused risk-reducing surgery**.
- Women who have accepted the current six-monthly follow-up based on TVUS and CA125 for the early detection of OC and have these **tests altered (O-RADS ≥ 2 and/or CA125 > 35 U/mL).**
- **Maximum 45 days term** between the suspected diagnosis and individualized surgery.
- **Accepted and signed informed consent**.

7.2.2. EXCLUSION CRITERIA

- **Previous diagnosis of ovarian cancer**.
- Known diagnosis of **any other neoplasia**.
- Previous **unilateral or bilateral salpingectomy** or **salpingo-oophorectomy**.
- Previous **hysterectomy**.
- **Impossibility to perform speculum examination** due to morphological or functional alteration of the vulva.
- **Non-pathogenic** BRCA1 or BRCA2 gene mutations or of **uncertain significance**.
- Current **pregnancy** or current **puerperium** (8 weeks).
- **Insufficient cytological sample** to perform the PapSEEK test **after two attempts**.

7.3. SAMPLING

7.3.1. SAMPLE SELECTION

Our sample will be obtained through a **consecutive non-probabilistic sampling**. The possibility of entering the study will be offered to women carrying a pathogenic mutation in the BRCA1/2 genes **who have refused or postponed risk-reducing prophylactic bilateral salpingo-oophorectomy and have altered follow-up tests (CA125 and/or TVUS).**

All patients with a pathogenic BRCA mutation who assist to the **"Unitat d'Alt Risc Oncològic"** at the mentioned hospitals and fulfil the inclusion criteria will be asked to participate and will be yielded the information document and the informed consent. Healthcare professionals will underscore the voluntary and confidentiality matters of patients' participation.

7.3.2. SAMPLE SIZE

The sample size was estimated using the **GRANMO software**, and the setting for paired measurements.

In previous studies, the reported false positive rate and specificity of the CA125 marker is **15%** and **85%**, respectively (14,54). The PapSEEK test has specificity values between **99%** and **100%** depending on the technique used to collect the biological sample (45,46). On that basis, we expect to see an **increase in specificity above 99%** with the combined follow-up strategy incorporating the PapSEEK test.

We assumed an alpha risk of 0,05 and a beta risk of 0,2 in a two-sided test. The estimated loss is 0%. Using these variables, GRANMO calculated **62 subjects** to recognize as statistically significant a difference consisting in an initial proportion of 0,85 and a final proportion of 0,99.

7.3.3. ESTIMATED TIME OF RECRUITMENT

The estimated time of recruitment will be **2 years**. Currently, 120 women with a positive genetic study for a pathogenic mutation in the BRCA genes who decide to refuse or postpone risk-reducing surgery are being followed up at HUJT. It is estimated that, each year, 4 of these women undergo individualized surgery to present an altered follow-up.

Taking this data as a reference, we have estimated that we will be able to recruit around **36 women per year**, counting the 9 participating centres. Accordingly, it will require **a total of 2 years** to recruit all the patients to perform the study. This period of time and the indicated participating centres have been established to avoid difficulties in recruiting participants, as explained in subsequent sections.

7.4. VARIABLES AND MEASUREMENTS

7.4.1. STUDY VARIABLE

PapSEEK test prior to surgery

Our study variable is conducting the PapSEEK test prior to surgery, in those patients who undergo the six-monthly follow-up strategy with TVUS plus CA125 and who are candidates for individualized surgery as they obtained abnormal tests results (CA125 > 35 U/mL and/or O-RADS ≥ 2).

PapSEEK will be assessed according to the results obtained from the PCR-multiplex test for the specific somatic mutations and aneuploidy markers described for ovarian cancer, and the test will be considered positive if the sample shows positivity for any of the specified genetic markers.

It will be a **dichotomous qualitative variable**:

- Positive PapSEEK test.
- Negative PapSEEK test.

7.4.2. OUTCOME VARIABLE

Development of ovarian cancer

The development of ovarian cancer will be the **main variable of the study** and the patient will be considered to have an ovarian tumour if she hosts a positive malignant histology according to the pathological anatomy analysis after surgery, excluding borderline tumours.

It will be a **dichotomous qualitative variable**:

- Presence of malignant ovarian tumour.
- Absence of malignant ovarian tumour.

7.4.3. SECONDARY OUTCOMES

Patient acceptability

The Theoretical Framework of Acceptability (TFA) questionnaire will be used (ANNEX 7*)*. It will be a **quantitative discrete variable:**

1. Strongly dislike.
2. Dislike.
3. No opinion.
4. Like.
5. Strongly like.

Table 6. Description of the principal variables included in the study. – OC: ovarian cancer

	Variable	Description	Measurement	Categories
Study variable	PapSEEK test prior to surgery	Qualitative nominal dichotomous	PCR-multiplex test for somatic mutations and aneuploidy markers for OC	- Positive PapSEEK - Negative PapSEEK
Outcome variable	Development of ovarian cancer	Qualitative nominal dichotomous	Histological confirmation by surgical specimen analysis	- Presence of OC - Absence of OC
	Patient acceptability	Quantitative discrete	TFA acceptability questionnaire	- Strongly dislike - Dislike - No opinion - Like - Strongly like

7.4.4. COVARIATES

Covariates may play an important role in the modification of the results because of their influence over the development of ovarian cancer. Thereby, they should be accounted when analysing the results.

- **Age**: it will be a **quantitative continuous variable**, expressed in **years**.
- **Type of mutation**: the information will be acquired from the corresponding genetic study stored in the patient's clinical history. It will be a **qualitative dichotomous variable**:
    - BRCA1 pathogenic mutation.
    - BRCA2 pathogenic mutation.
- **Familiar history of ovarian cancer** (number of first or second-degree affected relatives): the most important risk factor for OC is a family history of breast or ovarian cancer (22). The information will be acquired through the anamnesis. It will be a **quantitative discrete variable.**
- **Parity**: there is evidence to support that parity and pregnancy have a protective effect on the development of OC (22). The information will be acquired through the anamnesis. It will be a **qualitative dichotomous variable**:
    - Nulliparity: the woman has never given birth to a child or has never been pregnant.
    - Multiparity: the woman has given birth to a child or has been pregnant.
- **Endometriosis**: is a chronic gynaecological disease characterized by the implantation and growth of functionally active benign endometrial tissue outside the uterus, which induces a chronic inflammatory reaction. The relationship of endometriosis with the increased risk of developing ovarian cancer is well known (22). It will be a **qualitative dichotomous variable**:
    - Yes: the woman has a diagnosis of endometriosis as clinical, gynaecological examination and imaging tests are compatible.
    - No: the woman has not a diagnosis of endometriosis as clinical, gynaecological examination and imaging tests are not compatible.
- **Tubal ligation**: is a surgical procedure in which fallopian tubes are permanently blocked or clipped. This could be a mechanical barrier for neoplastic cells and interfere with sampling. It will be a qualitative dichotomous variable:
    - Yes: the woman has a tubal ligation.
    - No: the woman has not a tubal ligation.

- **Contraceptives methods**: results of most studies show that the use of oral contraceptive methods is linked with a reduced risk of all histological types of OC (22). It will be a **qualitative dichotomous variable**:
    - Yes: the patient has been using oral contraceptives for more than 5 years.
    - No: the patient has not used oral contraceptives or has been using them for less than 5 years.
- **Obesity**: some studies reported that obesity is associated with an increased risk of ovarian cancer (22). It will be a **qualitative polytomous variable** and measured according to the BMI classification:
    - Underweight: < 18,5 kg/m^2
    - Normal weight: 18,5-24,9 kg/m^2
    - Overweight: 25,0-29,9 kg/m^2
    - Obesity: ≥ 30,0 kg/m^2
- **Hospital**: it will be a **qualitative polytomous nominal variable**.

Table 7. Description of the covariates included in the study

Covariates	Description	Measurement	Categories
Age	Quantitative continuous	Self-referred	
Type of mutation	Qualitative nominal dichotomous	Genetic test	BRCA1 / BRCA2
Familiar history of OC (number of affected relatives)	Quantitative discrete	Self-referred	
Parity	Qualitative nominal dichotomous	Clinical history	Nulliparity / Multiparity

Endometriosis	Qualitative nominal dichotomous	Diagnostic criteria	Yes / No
Tubal ligation	Qualitative nominal dichotomous	Clinical history	Yes / No
Contraceptives methods	Qualitative nominal dichotomous	Clinical history	Yes / No
Obesity	Qualitative ordinal polytomous	BMI	Underweight / Normal weight / Overweight / Obesity
Hospital	Qualitative nominal polytomous		

7.5. MEASURING INSTRUMENTS

To assess patient satisfaction and acceptability, they will be administered the **Theoretical Framework of Acceptability (TFA) questionnaire**. It is a generic questionnaire to assess the acceptability of healthcare interventions by patients. It consists of 7 parts (affective attitude, burden, ethicality, intervention coherence, opportunity costs, perceived effectiveness, and self-efficacy) to identify those features of the interventions that can be improved (ANNEX 7).

7.6. SAFETY

PapSEEK test is considered safe, and no safety issues are expected, given the minimally invasive nature of the technique. Nevertheless, minimal bleeding may occur with sample collection.

7.7. DATA COLLECTION

The creation of a computer database using the SPSS software will be the first step to make a properly information gathering. Data collection will be carried out in accordance with confidential and anonymous treatment of information. To conduct this, the name and personal information of each participant will be coded using an identification number.

First visit

The alteration of the results in the six-monthly follow-up tests (TVUS O-RADS ≥ 2 and/or CA125 > 35 U/mL) is considered the starting point of our study. Thus, the first visit will be considered the one in which these results are delivered and communicated to the patient.

If the patient meets all the inclusion criteria and none of the exclusion criteria, she will be invited to participate. The characteristics and objectives of the study will be explained to the patient. To conclude the appointment, a second visit will be scheduled for the following week to acquaint the patient's decision.

Second visit

If the patient agrees to participate in the study, she will be given the Participant Information Sheet (ANNEX 8) and the Informed Consent Document (ANNEX 9).

The second visit will consist of the following procedures:

- Review of the electronical clinical history through the anamnesis, with the aim of contrasting and expanding it if necessary. All data related to study covariates will be collected. It is of great relevance the consultation and review of the genetic study in which the pathogenic mutation in the BRCA1/2 genes is diagnosed.
- Complete physical examination. Pelvic and rectovaginal examination should be performed.
- Sample collection by brushing the exo and endocervical mucosa (Pap device) and the intrauterine mucosa (Tao device).

Sample delivery and processing

The collected samples will be placed in the preservative liquid in accordance with the description of the technique. The sample containers will then be identified with the patient's code and sent to the laboratory for analysis. The molecular biology laboratory of the HUJT will be designated as the reference laboratory to perform the PapSEEK test.

The PapSEEK test will be performed and analysed according to the technical protocol. Lastly, the introduction of the obtained data will be carried out in accordance with the coding established in the corresponding program.

In the event that a sufficient cytological sample is not obtained to perform the PapSEEK test, it will be scheduled a new visit if the patient is still on the waiting list for individualized surgery, and the samples will be collected again. If a sufficient cytological sample is not obtained for the second time, it will be considered as an exclusion criterion.

The subsequent steps are included in the standard protocol when a patient with a pathogenic mutation in the BRCA1/2 genes present alterations on the follow-up tests.

Extension study and preoperative examination

Since she is a candidate for surgery, the most appropriate extension tests will be agreed upon with the members of the medical team. Imaging tests are usually performed.

A visit with the anaesthesia team will be scheduled to proceed with the preoperative study.

Surgical intervention and histological analysis

The oncological gynaecologist team will perform the corresponding surgical intervention within a maximum period of 45 days from the suspected diagnosis (CA125 > 35 U/mL and/or TVUS O-RADS ≥ 2). The surgical specimen will be sent to the pathological anatomy laboratory according to the usual protocol and respecting the established coding.

The pathologists' team will analyse the piece and prepare a report with the histological findings, which will provide the definitive diagnosis.

Third visit

The oncological gynaecologist team will schedule a visit with the patient to fulfil three objectives:

- Postoperative follow-up (examination of the surgical wounds, pain, etc.)
- Communication and delivery of the histological results.
- Therapeutic strategy and/or follow-up according to the histological results.

The patient's participation in the study ends at this point. The research team will then proceed to analyse the results obtained in the PapSEEK tests relative to the histological diagnoses of the surgical pieces to proceed with the statistical analysis.

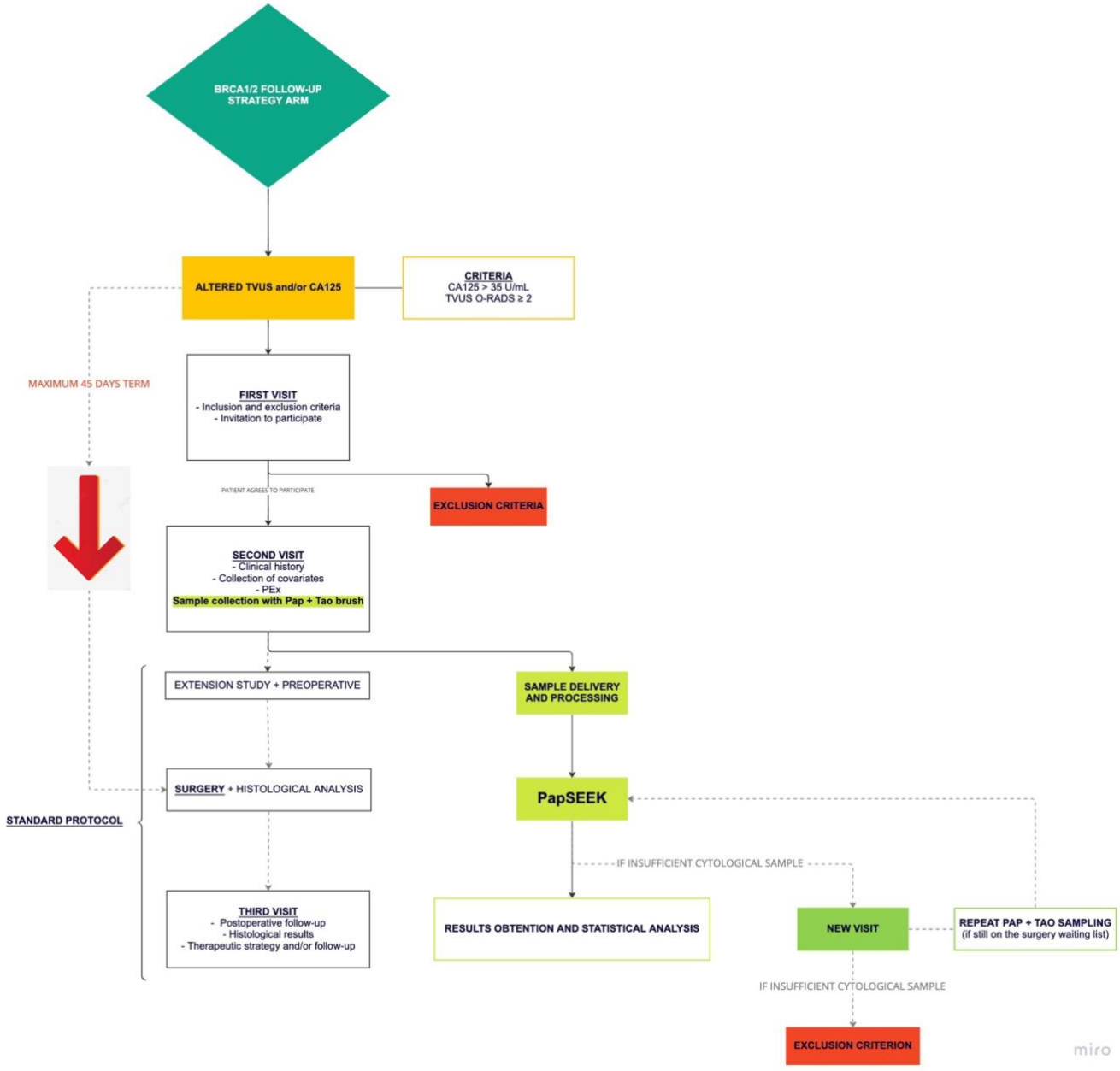

Figure 7. Data collection diagram

8. STATISTICAL ANALYSIS

8.1. DESCRIPTIVE ANALYSIS

The development of ovarian cancer (qualitative dichotomous variable) will be summarized using proportions. Regarding to the patient acceptability score (quantitative discrete variable), it will be summarized using means and SD or medians and interquartile range (IQR) if the variable distribution is normal or non-parametric, respectively.

These analyses will be stratified by the groups defined by positive and negative PapSEEK test. Furthermore, stratification will also be done by the covariates. Lastly, age will be categorized in quartiles.

8.2. BIVARIATE INFERENCE

Specificity and sensitivity are the measures of the diagnostic value of a test. They measure the diagnostic discrimination of a test in relation to a criterion of reference (in this case, the histological analysis, which determine if the patient has or not a malignant ovarian tumour). These indicators allow the comparison between the effectiveness of a test with other.

The sensitivity indicates the ability of the test to detect a sick subject, while the specificity indicates the ability of the test to identify those who are actually healthy (not sick). Specificity and sensibility will be calculated as proportions according to the approach in the following table:

Table 8. Specificity and sensibility calculation

		Histological analysis		
		Disease	Disease-free	TOTAL
Diagnostic test (PapSEEK)	Positive	TP	FP	TP + FP
	Negative	FN	TN	FN + TN
TOTAL		TP + FN	FP + TN	n = TP + FP + FN + TN

Accordingly, sensibility and specificity will be calculated as follow:

$$\text{Sensibility} = \frac{True\ positive}{Total\ sick\ patients} = \frac{TP}{TP+FN}$$

$$\text{Specificity} = \frac{True\ negative}{Total\ healthy\ patients} = \frac{TN}{TN+FP}$$

On the one hand, the proportions between both study groups will be tested to determine whether they are statistically different by using the McNemar's chi-square for paired data.

On the other hand, the difference of medians of the patient acceptability score between the groups defined by the PapSEEK test will be tested using the Mann-Whitney's U test.

Lastly, stratification by the covariates will also be done.

8.3. MULTIVARIATE ANALYSIS

To assess the differences in the development of ovarian cancer, according to the groups defined by the PapSEEK test and controlling for the covariates, a logistic regression will be estimated.

The effect of both, the positive and negative PapSEEK groups and of the covariates on the patient acceptability score will be adjusted in a Poisson regression.

9. ETHICAL AND LEGAL CONSIDERATIONS

This study will be conducted under the ethical principles and guidelines established by The World Medical Association in the Declaration of Helsinki, and The Principles of Biomedical Ethics by Beauchamp and Childress of 1979:

- **Beneficence**: it is the moral obligation to act for the benefit of others. All actions must be carried out thinking about what the best for the patient is. In our study this principle is respected as we will implement a test that we hope will help to reduce unnecessary surgical interventions and, consequently, the resulting harm and morbidity.
- **Autonomy**: the values and personal decisions of any individual will be respected all through the study. All participants will be informed by an oncological gynaecologist, member of the research team, and accordingly, they will be provided with the protocol information sheet (ANNEX 8). Written informed consent document (ANNEX 9) is required from all participants to enter the study. Before signing the informed consent, the researchers will emphasize to every individual that they can accept or refuse to participate in the study as well as quit it at any time without prejudice.
- **Justice**: it is based on the equitable distribution of well-being benefits avoiding any discriminatory treatment in terms of access to health resources nor concerning socioeconomic status, ethnicity, or other distinct reasons.
- **Non-maleficence**: no malicious intent is being done to the patients participating in the study. Patients who may be affected by the strategy conducted in the study will be excluded.

Deliberating more extensively on the ethical implications of the present study is key, since it is proposed to perform a minimally invasive test in a priori healthy women with a risk genetic predisposition who present a diagnostic suspicion of ovarian cancer, to avoid unnecessary surgeries. As explained throughout the study, the false-positive rate of the current strategy is 15%, meaning that 15% of the women with altered follow-up tests do not have a malignant tumour. The incorporation of the PapSEEK test in the current strategy, according with the specificity values reported in previous studies, would allow identifying those women who, despite having altered follow-up tests, do not have cancer. Nevertheless, the women included in the study will be operated regardless the PapSEEK result. The reasons that clarify why we will intervene, a priori a percent of healthy women, are founded upon the need of further studies as we cannot assume the hazard of not operating young women with a high risk of wielding an

ovarian neoplasia without having more evidence regarding the specificity of the proposed strategy.

Privacy and confidentially

The processing of personal data as well as the transfer thereof, confidentiality, and communication aspects carried out in this study will be subject to the following legal framework: *The Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of natural persons with regard to the processing of personal data and on the free movement of such data, and repealing Directive 95/46/EC (General Data Protection Regulation)*; the "*Ley Orgánica 3/2018, de 5 de diciembre, de Protección de Datos Personales y Garantía de los derechos digitales*"; and the "*Real Decreto 1720/2007, de 21 de diciembre, por el que se aprueba el Reglamento de desarrollo de la Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal*".

Accordingly, the study will provide anonymity to patients by identifying them with a code number in the database. The data access will only be available to the research team. All data collected will only be used for the intended purpose of this study.

Transparency

All the investigators will have to declare no conflict of interest, and they will also have to agree to publish all data and results with total transparency, including unfavourable data or events. In the event of any rearrangement occurring during the study regarding the initial planning of the project, the research team will contact all participants to inform them in complete transparency and will require the signature of a new informed consent to continue with the study.

10. WORKING PLAN

10.1. PARTICIPATING CENTRES

The study will be conducted from **9 Catalan hospitals**: "Hospital Universitari Doctor Josep Trueta", "Hospital Universitari Germans Trias i Pujol", "Hospital Universitari Vall d'Hebron", "Hospital Clínic de Barcelona", "Hospital Universitari de Bellvitge", "Hospital Universitari Parc Taulí" and "Hospital Universitari Arnau de Vilanova", "Hospital del Mar de Barcelona" and "Hospital de la Santa Creu i Sant Pau".

10.2. RESEARCH TEAM

A multidisciplinary research team will be set up with the following members:

Study coordinator. She/he will handle the monitoring of the study through team coordinating.

Principal investigator. The functions she/he will performed revolve around the design and planning of the study: formulation of the study concept, drafting the protocol, presentation of the protocol to the CEIC for approval, recruitment management of participants, management of the informed consent process and supervision of data collection, analysis, interpretation, and presentation. Plus, distribution of researchers' tasks and evaluation of the team members training. Lastly, she/he will run the execution of the study through coordination tasks and oversee the correct application of the protocol as well as the adequate storage of the data.

Research nurse. She/he will work on managing data collection during the study. Accordingly, she/he should have special training and research experience. Her/his functions will be training staff about the study and assistance to the principal investigator with control, quality assurance, audits, and data management and analysis.

Co-investigators. There will be co-investigators according to the different medical specialities involved in carrying out the study.

- Oncological gynaecologists: they will be in charge of taking cytology samples (cervical and intrauterine), the corresponding surgical intervention, the post-operative control, and the delivery of the histological results of the surgical specimen.
- Molecular biology specialists: they will handle the processing of the cytological samples and performing the PapSEEK test.

- Pathological anatomy specialists: they will deal with the surgical specimen histology analysis.

A **co-investigator coordinator** for each hospital will be appointed, and a meeting will be scheduled with the principal investigator and research nurse every 6 months.

Data manager. She/he will attend to the data collection managing during the study. Her/his tasks will be data processing, quality control and report writing for interim and final data analysis.

Statistic specialist. Her/his function will be carrying out the statistical analysis of the study.

10.3. STUDY STAGES

Stage 0. Study design (December 2022 – January 2023)

First meeting. The study coordinator and the principal investigator attended this first meeting. An agreement was reached to develop and elaborate the present study.

Protocol drafting. The development plan and memorandum of the protocol was drawn up during December 2022 and January 2023.

Stage 1. Ethical assessment (January 2023 – February 2023)

Delivery of the protocol to the CEIC for approval. Any proposed changes will be considered and corrected from the original development plan.

Stage 2. Initial coordination (March 2023)

First research team gathering. The study coordinator, principal investigator, research nurse and co-investigator coordinators will attend the meeting. The meeting objectives will revolve around the distribution of functions and agreement on a work plan and system. Furthermore, uniform criteria will be drawn up to define when follow-up tests (TVUS and CA125) will be considered altered, as there are no standardized guidelines. This will ensure that all participating centres will apply the same criteria.

Staff training. Co-investigators will receive a copy of the protocol and a list of duties to be performed. A training course will be given regarding data collection using SPSS software, by the research nurse. The principal investigator will assess the staff training.

In each specific area, training and refreshing courses will be scheduled on the implementation of specific techniques:

- The use of the Tao brush for taking intrauterine samples and preserving them, aimed at gynaecologists.
- The performance and analysis of the PapSEEK test, aimed at researchers in the molecular biology laboratory.

Stage 3. Participants recruitment and data collection (April 2023 – April 2025)

Participants recruitment. Patients who meet all the inclusion criteria and none of the exclusion criteria will be invited to enter the study, as detailed in previous sections.

Data collection and record. All the procedures specified in prior points and in the data collection diagram will be carried out. Every data collected will be entered into the database in accordance with the training guides and the established systematic method.

Stage 4. Statistical analysis (May 2025 – August 2025)

Statistical analysis (May – June 2025). The statistic specialist will carry out the corresponding analyses with all the information collected.

Interpretation of results (July – August 2025). Thereupon, the results will be interpreted, and the conclusions drawn up. One or more meetings will be scheduled (if needed) to discuss this issue.

Stage 5. Final report and results publication (September 2025 – December 2025)

The final report will be elaborated, and the results published. The principal investigator will give a lecture on the study at the Gynaecology Oncology congress organized by the "Sociedad Española de Ginecología y Obstetricia".

Table 9. Chronogram

STAGE	TASK	PERSONNEL	PERIOD									
			2022	2023				2024	2025			
			Dec	Jan	Feb	Mar	Ap - Dec	Jan - Dec	Jan - Ap	May - Jun	Jul - Aug	Sept - Dec
0. STUDY DESIGN	First meeting	Study coordinator Principal investigator										
	Protocol drafting	Principal investigator										
1. ETHICAL ASSESSMENT	Presentation to CEIC	Study coordinator Principal investigator CEIC										
2. INITIAL COORDINATION	1st research team gathering	Study coordinator Principal investigator Research nurse Co-inv. coordinators										
	Training	All team										
3. RECRUITMENT AND DATA COLLECTION	Participants recruitment	Investigators Co-investigators										
	Data record	Investigators Co-investigators Data manager										
4. STATISTICAL ANALYSIS	Statistical analysis	Statistician										
	Data interpretation	Study coordinator Principal investigator										
5. RESULTS PUBLICATION	Results publication and dissemination	Study coordinator Principal investigator										

11. BUDGET

11.1. NOT INCLUDED COSTS

Follow-up tests (TVUS and CA125), as they are part of the current protocol.

Individualized surgery and histological analysis of the surgical specimen, as they are part of the usual clinical practice.

Staff. The investigators will perform patient recruitment, data collection and interpretation of results as part of their usual work activity.

An insurance will not be taken out as all procedures conducted in the study are included in the usual clinical practice, excluding the performance of the cytology (Pap and Tao sampling).

11.2. INCLUDED COSTS

Subcontracted services. It includes hiring a qualified statistician and a data manager. The required working hours are specified in Table 10.

Materials

- Sampling kit. Cervical and intrauterine sampling, using the Pap and Tao brush, and sample containers with preservative fluid.
- PapSEEK test. Performing the PapSEEK test using a PCR-based multiplex test and Next Generation Sequencing. We have been able to adjust the price to 300€ per sample since the reference laboratory has the necessary equipment and materials to conduct the test. Nevertheless, in the event of obtaining inconclusive genetics and having to conduct an extension of the results, the amount would increase approximately to 500€ per sample. Consequently, a budget line is included to cover these possible contingencies in the laboratory. It has been estimated as 10% of the PapSEEK test budget.

Article publication and **attendance to a national congress** with the related expenses (travel, accommodation, and meals).

Table 10. Budget

EXPENSES	COST PER UNIT	NUMBER OF UNITS	SUBTOTAL
Subcontracted services			
Statistician	30€/hour	1 (50 hours)	1500€
Data management and quality control	50€/patient	62 patients	3100€
Materials			
Sampling kit	2€/kit	62 Pap kits + 62 Tao kits = 124 kits	248€
PapSEEK test	300€/sample	62 Pap samples + 62 Tao samples = 124 samples	37.200€
Laboratory contingencies			3720€
Publication and dissemination			
Article publication	2000€/publication	1 publication	2000€
Attendance to a national congress	600€/congress per attendant	1 congress 2 attendants	1200€
		TOTAL	**48.968€**

12. LIMITATIONS

The limitations of the study are detailed in the following points:

Specificity reference values

As multiple studies on this topic are British, and the use of ultrasound in the UK is not as widespread as in our setting, we encountered difficulties in finding reported values of specificity and false positive rate for the six-monthly combined strategy based on TVUS and CA125. So, given that there are numerous studies on the subject, we have taken the specificity values and false positive rate of the CA125 marker as a reference, although we are aware that in our setting the isolated measurement of the marker does not correspond to the monitoring standard.

Lack of standardized criteria in the follow-up strategy (TVUS and CA125)

Currently, reference criteria for the consideration of follow-up tests (TVUS and CA125) have not been described. Thereby, each hospital can adopt distinct guidelines regarding the interpretation of the findings. We recognize this fact as a limitation and in order to solve it, the research team has developed specific criteria that determine when follow-up tests should be considered altered. These guidelines must be implemented in all participating centres.

Participating centres

Since this is a multicentric study, information collection bias can occur, as it is complicated to develop a standardized protocol. It is also difficult to have an implementation control of the protocol in all the participating centres. To avoid this, regular meetings will be scheduled to assure the correct development of the study. In addition, in the multivariate analysis we will control for heterogeneity between hospitals including hospital as an additional covariate. However, an associated strength is that the multicentric nature of our design increases the external validity.

Difficulties in recruiting participants

It has been estimated that about 4 women have alterations in the follow-up tests, so that solely 4 patients can be recruited per year at each hospital. Since only a few patients can be recruited, this could lead to difficulties in recruiting participants to make up the entire sample in the established time. Besides, on the assumption of obtaining insufficient cytological samples to perform the PapSEEK test, the participant would be excluded from the study, hampering the

recruitment. To prevent this, one more hospital has been embodied in the study to dispose of additional participants if necessary.

Aspects related to the samples' logistics

The molecular biology laboratory of the HUJT has been designated as the reference laboratory of this study, so all samples collected among the different participating centres must be sent to the same laboratory. This can cause logistical difficulties, such as loss of samples (wrong shipment, break or opening of sample containers, etc.), among others. To avoid this, a sample sending protocol will be drawn up to always process it in the same way.

13. IMPACT

Ovarian, fallopian tube and peritoneal cancers represent the seventh leading cause of cancer in women and the eighth leading cause of cancer death in women worldwide. Despite not being the cancer with the highest incidence, it has a very elevated mortality rate, positioning it as the most lethal gynaecological cancer.

Currently, the scientific and medical world is working to improve the understanding of risk factors and their influence on prognosis with the aim of developing prevention strategies. Meanwhile, research is being done to develop an effective screening strategy that allows the diagnosis of the neoplasia at early stages to achieve a decrease in mortality.

The best characterized risk factors are a family history of breast and ovarian cancer and having a positive genetic test for a pathogenic mutation in the BRCA1/2 genes. As discussed throughout the present study, a screening strategy with better results for the diagnosis of ovarian cancer in this patient group is lacking.

Our study seeks to improve the specificity of the proposed monitoring strategy, as the tests performed have a false positive rate of 15%. This leads to the performance of unnecessary surgical interventions with the morbidity derived from these procedures. Thus, we hope that the incorporation of the PapSEEK test will allow us to identify those women who do not have a malignant ovarian tumour despite having altered standard follow-up tests, opening a great opportunity for women carrying a pathogenic mutation in the BRCA1/2 genes.

To sum up, the present study will allow the achievement of the following milestones:

- Decrease in the number of surgeries performed and the non-performance of surgical interventions in those women who do not have the disease.
- Reduction of mental disorders resulting from the negative psychological effects linked to the suspicion of cancer, with less anxiety and discomfort, both for the patient and her family.
- Possibility of an effective and minimally invasive alternative in contrast to the great aggressiveness of the procedures we currently have. Consequently, we would achieve lower morbidity in young women, prolonging their fertile life and thus, give them the possibility to fulfil their gestational desire. At the same time, we would avoid causing a premature menopause and all the secondary effects that derive from it.
- Reduction in public health costs, since the incorporation of a single test will save the cost of surgeries, treatments and monitoring that usually need to be established

afterwards to control the premature menopausal state. Medical visits to other specialists will probably also be saved, since the patient will not present early problems related to osteoporosis, cardiovascular disease, and mental disorders, among others.

If favourable results are obtained, this study could lay the foundation to broaden the implementation of the PapSEEK test. We expect that a similar study could be considered to evaluate the application of the PapSEEK test within the context of undetermined adnexal masses in the general population, with the aim of having a minimally invasive test to determine the benign or malignant nature of adnexal masses. Moreover, we look forward to new approaches regarding the improvement of a screening strategy in asymptomatic women in which the PapSEEK test could have a significant role. Thus, we reckon that the PapSEEK test could be applied in the context of ovarian cancer screening both in the general population and in patients with a pathogenic mutation in the BRCA1/2 genes.

14. FEASIBILITY

The present study will be conducted in 9 hospitals: “Hospital Universitari Doctor Josep Trueta”, “Hospital Universitari Germans Trias i Pujol”, “Hospital Universitari Vall d’Hebron”, “Hospital Clínic de Barcelona”, “Hospital Universitari de Bellvitge”, “Hospital Universitari Parc Taulí”, “Hospital Universitari Arnau de Vilanova”, “Hospital del Mar de Barcelona” and “Hospital de la Santa Creu i Sant Pau”.

A sample of 62 patients was estimated to conduct the study. Strictly speaking, with 8 of the 9 participating hospitals, the entire sample could be obtained in 2 years. Nevertheless, to improve the study feasibility, we have decided to incorporate one more participating hospital assuming that we will not be able to include a few patients for not obtaining sufficient cytological sample to perform the PapSEEK test (exclusion criterion).

This project will be carried out from the “Unitat d’Alt Risc Oncològic” for gynaecological cancer, comprised for a multidisciplinary team, in which gynaecologists, oncologists, radiotherapists, radiologists, pathologists and nursing staff are part of it. These healthcare professionals have great training and experience for the tasks to be developed throughout the project.

Regarding the rest of the staff, it will be required to hire a data manager, for data management and quality control, and a statistical specialist to perform the statistical analysis.

In relation to healthcare practice, the current follow-up strategy will remain the same with the only addition of taking cytology samples in an extra visit, so that the timings and procedures with the patient will be similar.

In summary, we consider this a feasible project due to the short time it will take to recruit participants, the centralized analysis in a single reference laboratory and the expertise of the responsible team.

15. BIBLIOGRAPHY

1. Berek JS, Renz M, Kehoe S, Kumar L, Friedlander M. Cancer of the ovary, fallopian tube, and peritoneum: 2021 update. Int J Gynecol Obstet. 2021 Oct;155(S1):61–85.

2. Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, et al. Ovarian cancer statistics, 2018: Ovarian Cancer Statistics, 2018. CA Cancer J Clin. 2018 Jul;68(4):284–96.

3. Protocol clínic síndromes de càncer hereditari amb alt risc de càncer ginecològic. Girona: Hospital Universitari Doctor Josep Trueta; 2020. 1–23 p.

4. Yoshida R. Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer. 2021 Nov;28(6):1167–80.

5. Aguirre Ortega E, Alés Martínez JE, Andrés Conejero R, Balaguer Guill J, Balmaña Gelpí J, Beristain Mendizabal E, et al. Cáncer hereditario. Madrid: Sociedad Española de Oncología Médica; 2019. 472 p.

6. Lledó Matoses S, Sala Felis T, Chover Lara D, Garcés Honrubia V, García Garijo M, Payá Roma A, et al. Guía de práctica clínica en cáncer hereditario. 2a. ed. Valencia: Conselleria de Sanitat; 2009. 91 p.

7. Stewart C, Ralyea C, Lockwood S. Ovarian Cancer: An Integrated Review. Semin Oncol Nurs. 2019 Apr;35(2):151–6.

8. Azribi F, Abdou E, Dawoud E, Ashour M, Kamal A, Al Sayed M, et al. Prevalence of BRCA1 and BRCA2 pathogenic sequence variants in ovarian cancer patients in the Gulf region: the PREDICT study. BMC Cancer. 2021 Dec;21(1):1–8.

9. Dilley J, Burnell M, Gentry-Maharaj A, Ryan A, Neophytou C, Apostolidou S, et al. Ovarian cancer symptoms, routes to diagnosis and survival – Population cohort study in the 'no screen' arm of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Gynecol Oncol. 2020 Aug;158(2):316–22.

10. Gaba L. What is ovarian cancer? In: Portal Clínic [Internet]. Barcelona: Hospital Clínic de Barcelona; 2020. Available from: https://www.clinicbarcelona.org/en/assistance/diseases/ovarian-cancer

11. Domingo del Pozo S, Lago Leal V, Coronado Martín PJ, Tejerizo García Á, Mancebo Moreno G, Sánchez Iglesias JL, et al. Cancer de ovario 2022. Guía de Asistencia Práctica. Rev Of Soc Esp Ginecol Obstet. 2022;90–131.

12. Prat J, D'Angelo E, Espinosa I. Ovarian carcinomas: at least five different diseases with distinct histological features and molecular genetics. Hum Pathol. 2018 Oct;80:11–27.

13. Nebgen DR, Lu KH, Bast RC. Novel Approaches to Ovarian Cancer Screening. Curr Oncol Rep. 2019 Aug;21(8):1–17.

14. Davenport C, Rai N, Sharma P, Deeks JJ, Berhane S, Mallett S, et al. Menopausal status, ultrasound and biomarker tests in combination for the diagnosis of ovarian cancer in

symptomatic women. Cochrane Gynaecological, Neuro-oncology and Orphan Cancer Group, editor. Cochrane Database Syst Rev. 2022 Jul 26;2022(9):1–447.

15. Crum CP, Drapkin R, Miron A, Ince TA, Muto M, Kindelberger DW, et al. The distal fallopian tube: a new model for pelvic serous carcinogenesis. Curr Opin Obstet Gynecol. 2007 Feb;19(1):3–9.

16. Charkhchi P, Cybulski C, Gronwald J, Wong FO, Narod SA, Akbari MR. CA125 and Ovarian Cancer: A Comprehensive Review. Cancers. 2020 Dec 11;12(3730):1–29.

17. Kroeger PT, Drapkin R. Pathogenesis and heterogeneity of ovarian cancer. Curr Opin Obstet Gynecol. 2017 Feb;29(1):26–34.

18. Piek JMJ, Kenemans P, Verheijen RHM. Intraperitoneal serous adenocarcinoma: A critical appraisal of three hypotheses on its cause. Am J Obstet Gynecol. 2004 Sep;191(3):718–32.

19. Cooper GM. Tumor Suppressor Genes. In: The Cell: A Molecular Approach [Internet]. 2nd edition. Sunderland (MA): Sinauer Associates; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK9894/

20. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209–49.

21. Xiao Y, Bi M, Guo H, Li M. Multi-omics approaches for biomarker discovery in early ovarian cancer diagnosis. eBioMedicine. 2022 May;79(104001):1–12.

22. Momenimovahed Z, Tiznobaik A, Taheri S, Salehiniya H. Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health. 2019 Apr;Volume 11:287–99.

23. Rahmani K, Moradi-Lakeh M, Mansori K, Bidokhti F, Asadi-Lari M. Global Inequalities in Incidence and Mortality of Ovarian Cancer and Associated Factors: An Ecological Study. SM J Neurol Disord Stroke. 2018;4(1):1–5.

24. Fiscutean A. Ovarian cancer outlook. Crunching de numbers. Nature. 2021 Dec 16;600:48–9.

25. Cancer Facts & Figures 2018. Atlanta: American Cancer Society; 2018. 76 p.

26. Stirling D, Evans DGR, Pichert G, Shenton A, Kirk EN, Rimmer S, et al. Screening for Familial Ovarian Cancer: Failure of Current Protocols to Detect Ovarian Cancer at an Early Stage According to the International Federation of Gynecology and Obstetrics System. J Clin Oncol. 2005 Aug 20;23(24):5588–96.

27. Philpott S, Raikou M, Manchanda R, Lockley M, Singh N, Scott M, et al. The avoiding late diagnosis of ovarian cancer (ALDO) project; a pilot national surveillance programme for women with pathogenic germline variants in *BRCA1* and *BRCA2*. J Med Genet. 2022 Nov 1;1–10.

28. Walker M, Sobel M. Diagnosing ovarian cancer. Can Med Assoc J. 2018;190(42):E1259.

29. Colombo N, Van Gorp T, Parma G, Amant F, Gatta G, Sessa C, et al. Ovarian cancer. Crit Rev Oncol Hematol. 2006 Nov;60(2):159–79.

30. Roett M, Evans P. Ovarian cancer: an overview. Ovarian Cancer. 2009;80(6):609–16.

31. Andreotti RF, Timmerman D, Strachowski LM, Froyman W, Benacerraf BR, Bennett GL, et al. O-RADS US Risk Stratification and Management System: A Consensus Guideline from the ACR Ovarian-Adnexal Reporting and Data System Committee. Radiology. 2020 Jan;294(1):168–85.

32. 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 Feb;19(2):191–226.

33. Sessa C, Balmaña J, Bober SL, Cardoso MJ, Colombo N, Curigliano G, et al. Risk reduction and screening of cancer in hereditary breast-ovarian cancer syndromes: ESMO Clinical Practice Guideline. Ann Oncol. 2023 Jan;34(1):33–47.

34. Jacobs I. Screening for Familial Ovarian Cancer: The Need for Well-Designed Prospective Studies. J Clin Oncol. 2005 Aug 20;23(24):5443–5.

35. Rosenthal AN, Fraser LSM, Philpott S, Manchanda R, Burnell M, Badman P, et al. Evidence of Stage Shift in Women Diagnosed With Ovarian Cancer During Phase II of the United Kingdom Familial Ovarian Cancer Screening Study. J Clin Oncol. 2017 May 1;35(13):1411–20.

36. Jacobson M, Coakley N, Bernardini M, Branco KA, Elit L, Ferguson S, et al. Risk reduction strategies for BRCA1/2 hereditary ovarian cancer syndromes: a clinical practice guideline. Hered Cancer Clin Pract. 2021 Dec;19(39):1–7.

37. Kinde I, Bettegowda C, Wang Y, Wu J, Agrawal N, Shih IM, et al. Evaluation of DNA from the Papanicolaou Test to Detect Ovarian and Endometrial Cancers. Sci Transl Med. 2013 Jan 9;5(167):1–21.

38. Zhang M, Cheng S, Jin Y, Zhao Y, Wang Y. Roles of CA125 in diagnosis, prediction, and oncogenesis of ovarian cancer. Biochim Biophys Acta BBA - Rev Cancer. 2021 Apr;1875(2):1–11.

39. Moss EL. The role of CA125 in clinical practice. J Clin Pathol. 2005 Mar 1;58(3):308–12.

40. Problems after ovarian cancer surgery. In: Cancer Research UK [Internet]. London: Cancer Research UK; 2022. Available from: https://www.cancerresearchuk.org/about-cancer/ovarian-cancer/treatment/surgery/problems-after

41. Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res. 2019 Dec;12(1):1–9.

42. Nash Z, Menon U. Ovarian cancer screening: Current status and future directions. Best Pract Res Clin Obstet Gynaecol. 2020 May;65:32–45.

43. Henderson JT, Webber EM, Sawaya GF. Screening for Ovarian Cancer: Updated Evidence

Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2018 Feb 13;319(6):595–606.

44. McLaughlin JR, Rosen B, Moody J, Pal T, Fan I, Shaw PA, et al. Long-Term Ovarian Cancer Survival Associated With Mutation in BRCA1 or BRCA2. JNCI J Natl Cancer Inst. 2013 Jan 16;105(2):141–8.

45. Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, et al. Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Sci Transl Med. 2018 Mar 21;10(433):1–22.

46. Ramos García V, Rivero Santana A, Perestelo Pérez L, Duarte Díaz A, Torres Castaño A, Álvarez Pérez Y, et al. Biopsia líquida para la detección de cáncer de ovario y/o endometrio en muestras tomadas del frotis de Papanicolaou: PapSEEK. In: Detección Temprana de Tecnologías Sanitarias Nuevas y Emergentes en la RedETS. Madrid: Ministerio de Sanidad, Gobierno de Canarias, Red Española de Agencias de Evaluación de Tecnologías y Prestaciones del Sistema Nacional de Salud; 2021. p. 71.

47. Erickson BK, Kinde I, Dobbin ZC, Wang Y, Martin JY, Alvarez RD, et al. Detection of Somatic TP53 Mutations in Tampons of Patients With High-Grade Serous Ovarian Cancer. Obstet Gynecol. 2014 Nov;124(5):881–5.

48. Kumar A. Tao Brush. J Obstet Gynecol India. 2017 Aug;67(4):304–5.

49. Meherbano K. Pap Smear Collection and Preparation: Key Points. Cytojournal. 2022;19(24):1–23.

50. Peremiquel-Trillas P, Paytubi S, Pelegrina B, Frias-Gomez J, Carmona Á, Martínez JM, et al. An Integrated Approach for the Early Detection of Endometrial and Ovarian Cancers (Screenwide Study): Rationale, Study Design and Pilot Study. J Pers Med. 2022 Jun 29;12(1074):1–14.

51. Pettersson K. Rapid Test for Ovarian Cancer Detection Developed at the University of Turku. In: University of Turku [Internet]. Turku (Finlandia); 2020. Available from: https://www.utu.fi/en/news/press-release/rapid-test-for-ovarian-cancer-detection-developed-at-the-university-of-turku

52. Mobley I. What is multiplex PCR? In: Front Line Genomics [Internet]. Hampshire: Front Line Genomics; 2021. Available from: https://frontlinegenomics.com/what-is-multiplex-pcr/

53. Costas L, Frias-Gomez J, Guardiola M, Benavente Y, Pineda M, Pavón MÁ, et al. New perspectives on screening and early detection of endometrial cancer. Int J Cancer. 2019 Dec 15;145(12):3194–206.

54. Lentz SE, Powell CB, Haque R, Armstrong MA, Anderson M, Liu Y, et al. Development of a longitudinal two-biomarker algorithm for early detection of ovarian cancer in women with BRCA mutations. Gynecol Oncol. 2020 Dec;159(3):804–10.

55. Parikh R, Mathai A, Parikh S, Sekhar GC, Thomas R. Understanding and using sensitivity,

speciÞcity and predictive values. Indian J Ophthalmol. 2008;56(1):45–50.

56. Adhikari L, Hassell LA. Ovary General WHO classification (2020). In: Pathology Outlines [Internet]. 2021. Available from: https://www.pathologyoutlines.com/topic/ovarytumorwhoclassif.html

57. Köbel M, Kang EY. The Evolution of Ovarian Carcinoma Subclassification. Cancers. 2022 Jan 14;14(416):1–16.

58. Sekhon M, Cartwright M, Francis JJ. Development of a theory-informed questionnaire to assess the acceptability of healthcare interventions. BMC Health Serv Res. 2022 Dec;22(279):1–12.

16. ANNEXES

ANNEX 1. WHO HISTOLOGICAL CLASSIFICATION

Table 11 WHO histological classification. Adapted from (56).

EPITHELIAL TUMOURS	Serous tumours	- Serous cystadenoma, adenofibroma and surface papilloma - Serous borderline tumour - Low grade serous carcinoma - High grade serous carcinoma
	Mucinous tumours	- Mucinous cystadenoma and adenofibroma - Mucinous borderline tumour - Mucinous carcinoma
	Endometrioid tumours	- Endometrioid cystadenoma and adenofibroma - Endometrioid borderline tumour - Endometrioid carcinoma
	Clear cell tumours	- Clear cell cystadenoma and adenofibroma - Clear cell borderline tumour - Clear cell carcinoma
	Seromucinous tumours	- Seromucinous cystadenoma and adenofibroma - Seromucinous borderline tumour
	Brenner tumours	- Brenner tumour - Borderline Brenner tumour - Malignant Brenner tumour
	Other carcinomas	- Mesonephric-like adenocarcinoma - Undifferentiated and dedifferentiated carcinoma - Carcinosarcoma - Mixed carcinoma
MESENCHYMAL TUMOURS	Endometrioid stromal sarcoma	- Low grade - High grade
	Smooth muscle tumours	- Leiomyoma - Smooth muscle tumour of uncertain malignant potential - Leiomyosarcoma
	Ovarian myxoma	
MIXED EPITHELIAL AND MESENCHYMAL	- Adenosarcoma	
SEX CORD STROMAL TUMOURS	Pure stromal tumours	- Fibroma - Thecoma - Sclerosing stromal tumour - Microcystic stromal tumour - Signet ring stromal tumour - Leydig cell tumour - Steroid cell tumour - Fibrosarcoma
	Pure sex cord tumours	- Adult granulosa cell tumour - Juvenile granulosa cell tumour - Sertoli cell tumour

		- Sex cord tumour with annular tubules
	Mixed sex cord stromal tumours	- Sertoli-Leydig cell tumour - Sex cord stromal tumour - Gynandroblastoma
GERM CELL TUMOURS	- Teratoma - Dysgerminoma - Yolk sac tumour - Embryonal carcinoma - Choriocarcinoma - Mixed germ cell tumour	
	Monodermal teratomas and somatic type tumours arising from a dermoid cyst	- Stroma ovarii - Stromal carcinoid - Teratoma with malignant transformation - Cystic teratoma
	Germ cell sex cord stromal tumours	- Gonadoblastoma - Mixed germ cell sex cord stromal tumour
MISCELLANEOUS TUMOURS	- Rete cystadenoma, adenoma and adenocarcinoma - Wolffian tumour - Solid pseudopapillary tumour - Small cell carcinoma of the ovary, hypercalcemic type - Wilms tumour	
TUMOUR-LIKE LESIONS	- Follicle cyst - Corpus luteum cyst - Large solitary luteinized follicle cyst - Hyperreaction luteinalis - Pregnancy luteoma - Stromal hyperplasia and hyperthecosis - Fibromatosis and massive edema - Leydig cell hyperplasia	

ANNEX 2. HYSTOTYPES AND MOLECULAR SUBTYPES OF EOC

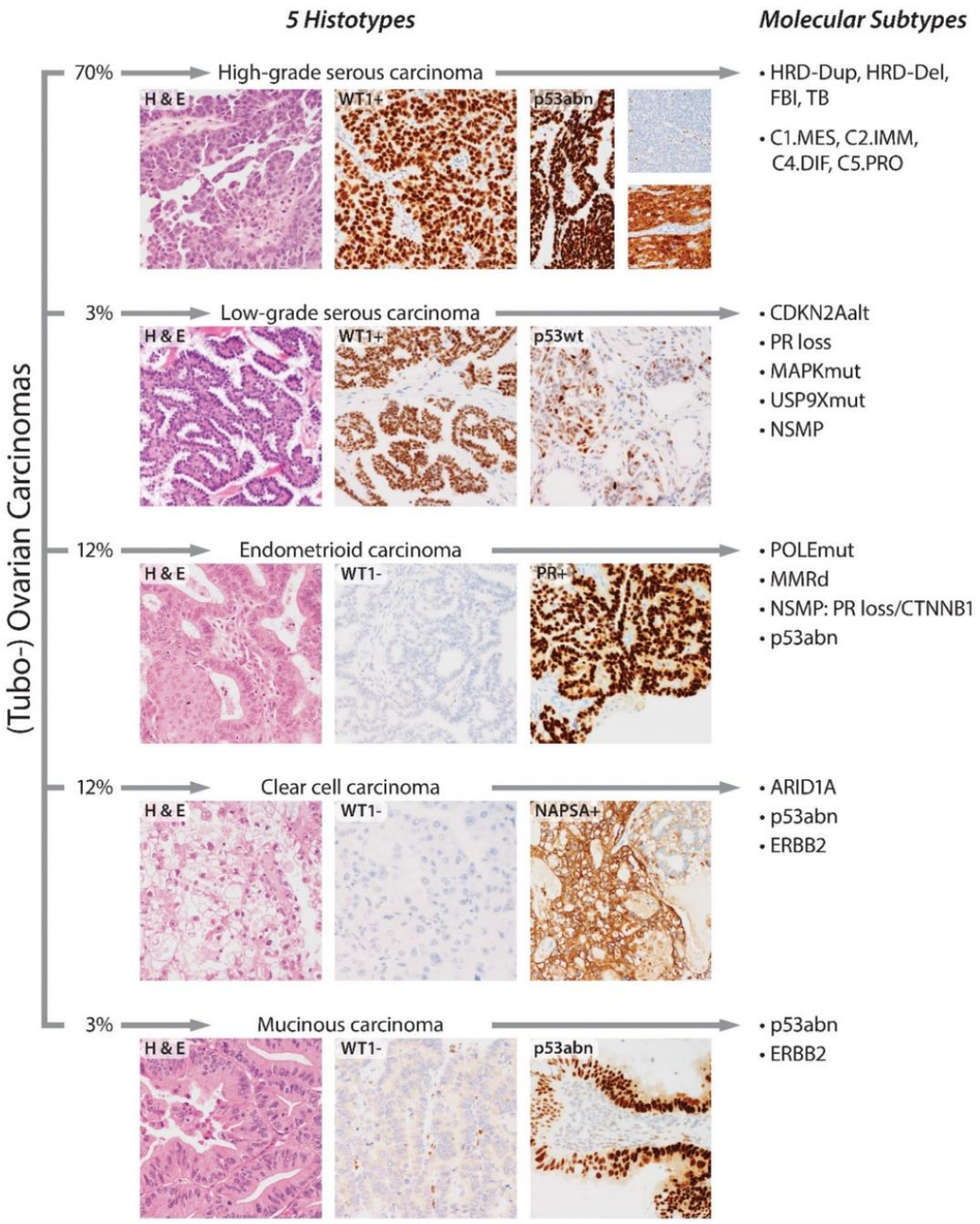

Figure 8. Histotypes and molecular subtypes of EOC (57).

ANNEX 3. O-RADS SCORE SYSTEM

O-RADS Score	Risk Category [IOTA Model]	Lexicon Descriptors		Management	
				Pre-menopausal	Post-menopausal
0	Incomplete Evaluation [N/A]	N/A		Repeat study or alternate study	
1	Normal Ovary [N/A]	Follicle defined as a simple cyst ≤ 3 cm		None	N/A
		Corpus Luteum ≤ 3cm			
2	Almost Certainly Benign [< 1%]	Simple cyst	≤ 3 cm	N/A	None
			> 3 cm to 5 cm	None	Follow up in 1 year. *
			> 5 cm but < 10 cm	Follow up in 8 - 12 weeks	
		Classic Benign Lesions	See **Figure 3** for separate descriptors	See **Figure 3** for management strategies	
		Non-simple unilocular cyst, smooth inner margin	≤ 3 cm	None	Follow up in 1 year * If concerning, US specialist or MRI
			> 3 cm but < 10 cm	Follow-up in 8 - 12 weeks If concerning, US specialist	US specialist or MRI
3	Low Risk Malignancy [1-<10%]	Unilocular cyst ≥ 10 cm (simple or non-simple)		US specialist or MRI Management by gynecologist	
		Typical dermoid cysts, endometriomas, hemorrhagic cysts ≥ 10 cm			
		Unilocular cyst, any size with irregular inner wall <3 mm height			
		Multilocular cyst < 10 cm, smooth inner wall, CS = 1-3			
		Solid smooth, any size, CS = 1			
4	Intermediate Risk [10- < 50%]	Multilocular cyst, no solid component	≥ 10 cm, smooth inner wall, CS = 1-3	US specialist or MRI Management by gynecologist with GYN-oncologist consultation or solely by GYN-oncologist	
			Any size, smooth inner wall, CS = 4		
			Any size, irregular inner wall and/or irregular septation, any color score		
		Unilocular cyst with solid component	Any size, 0-3 papillary projections, CS = any		
		Multilocular cyst with solid component	Any size, CS = 1-2		
		Solid	Smooth, any size, CS = 2-3		
5	High Risk [≥ 50%]	Unilocular cyst, any size, ≥ 4 papillary projections, CS = any		GYN-oncologist	
		Multilocular cyst with solid component, any size, CS = 3-4			
		Solid smooth, any size, CS = 4			
		Solid irregular, any size, CS = any			
		Ascites and/or peritoneal nodules**			

Figure 9. O-RADS score system (31).

ANNEX 4. FIGO STAGING OF OVARIAN, FALLOPIAN TUBE AND PERITONEUM CANCER

Table 12. FIGO staging of ovarian, fallopian tube and peritoneum cancer (12).

STAGE	ANATOMIC DISTRIBUTION
I	Tumour confined to ovaries of fallopian tube(s).
A	Tumour limited to one ovary (capsule intact) or fallopian tube. No tumour on ovarian or fallopian tube surface. No malignant cells in the ascites or peritoneal washings.
B	Tumour limited to both ovaries (capsules intact) or fallopian tubes. No tumour on ovarian or fallopian tube surface. No malignant cells in the ascites or peritoneal washings.
C	Tumour limited to one or both ovaries or fallopian tubes, with any of the following:
1	Surgical spill intraoperatively.
2	Capsule ruptured before surgery or tumour on ovarian or fallopian tube surface
3	Malignant cells in the ascites or peritoneal washings.
II	Tumour involves one or both ovaries or fallopian tubes with pelvic extension (below pelvic brim).
A	Extension and/or implants on the uterus and/or fallopian tubes and/or ovaries.
B	Extension to other pelvic intraperitoneal tissues.
III	Tumour involves one or both ovaries, fallopian tubes, or primary peritoneal cancer, with microscopically confirmed spread to the peritoneum outside the pelvis or metastasis to the retroperitoneal lymph nodes.
A	Metastases to the retroperitoneal lymph nodes with or without microscopic peritoneal involvement beyond the pelvis.
1	Positive retroperitoneal lymph nodes only. Microscopically proven. (i) Metastasis ≤ 10 mm in greatest dimension. (ii) Metastasis > 10 mm in greatest dimension.
2	Microscopic extrapelvic (above pelvic brim) peritoneal involvement with or without positive peritoneal lymph nodes.

	B	Macroscopic peritoneal metastasis beyond the pelvic brim 2 cm or less in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes.
	C	Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes.
IV		Distant metastasis excluding peritoneal metastases.
	A	Pleural effusion with positive cytology.
	B	Metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside the abdominal cavity).

ANNEX 5. OVARIAN CANCER RISK MANAGEMENT: CURRENT STRATEGIES

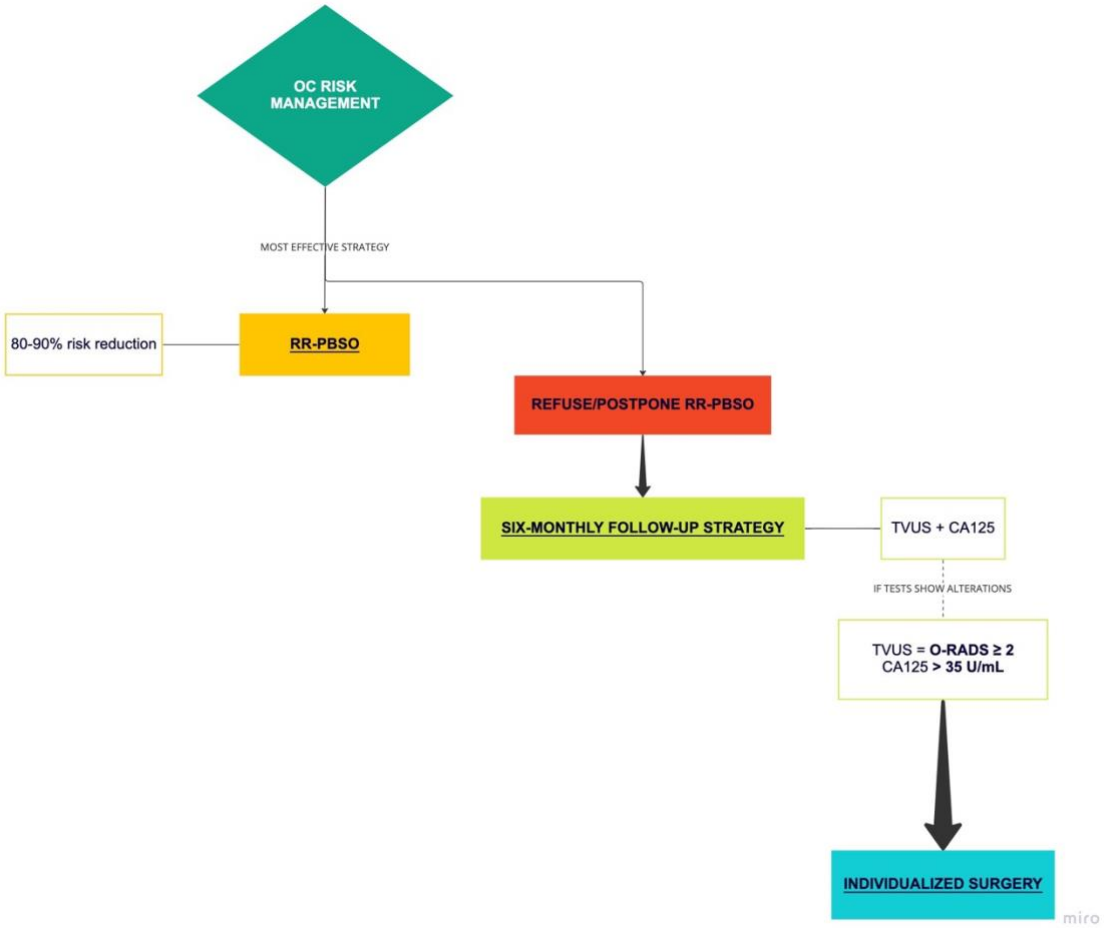

Figure 10. OC risk management diagram: current strategies – RR-PBSO: Risk Reducing Prophylactic Bilateral Salpingo-Oophorectomy; TVUS: TransVaginal UltraSound

ANNEX 6. OVARIAN CANCER RISK MANAGEMENT: STUDY STRATEGY

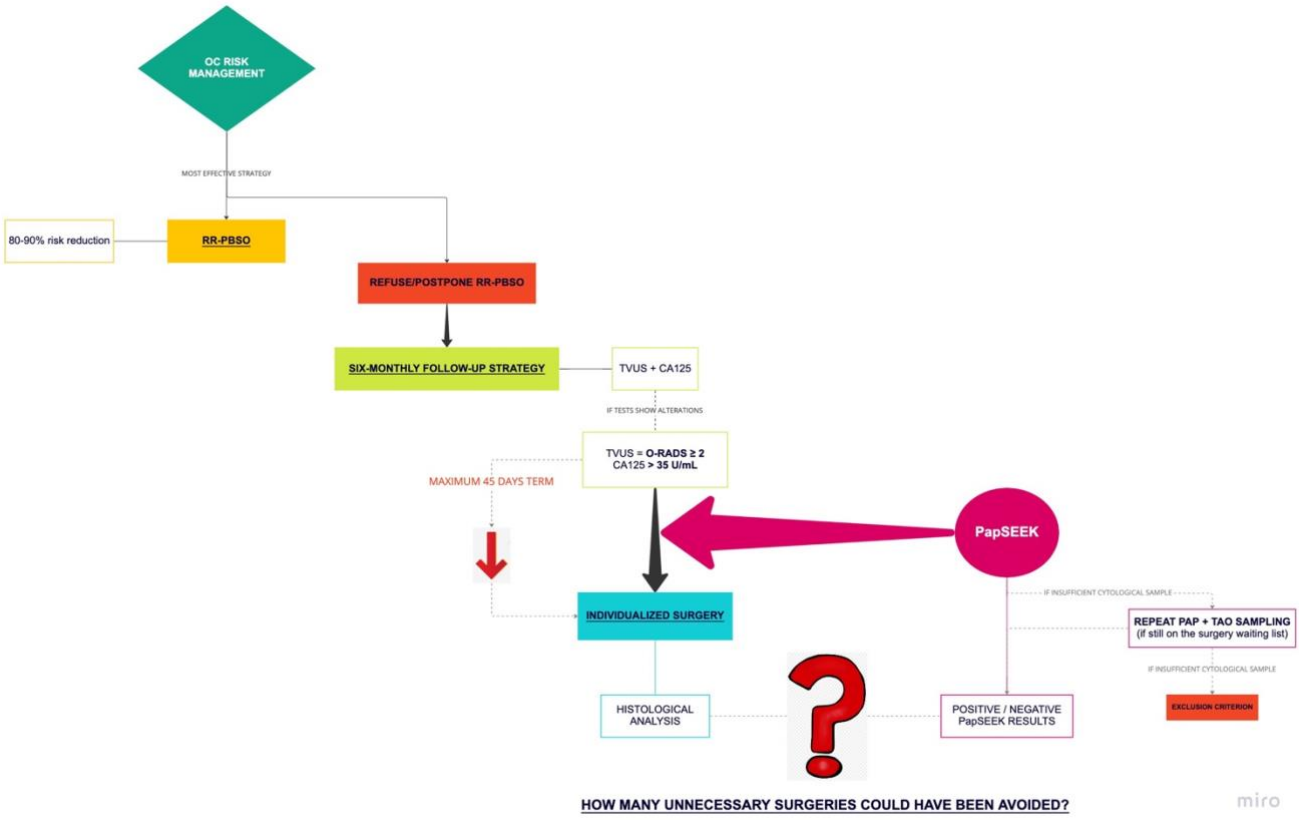

Figure 11. OC risk management: study strategy – RR-PBSO: Risk Reducing Prophylactic Bilateral Salpingo-Oophorectomy; TVUS: TransVaginal UltraSound

ANNEX 7. TFA ACCEPTABILITY QUESTIONNAIRE

Table 1 Generic form of TFA acceptability questionnaire

TFA Construct	Questionnaire item
Affective attitude *How an individual feels about the intervention*	**Affective attitude:** Did you like or dislike [intervention]? Strongly dislike (1) | Dislike (2) | No opinion (3) | Like (4) | Strongly like (5) ***OR*** How comfortable did you feel [behaviour *e.g. to engage with*] [intervention]? Very uncomfortable (1) | Uncomfortable (2) | No opinion (3) | Comfortable (4) | Very comfortable (5)
Burden *The amount of effort required to participate in the intervention*	How much effort did it take [behaviour *e.g. to engage with*] [intervention]? No effort at all (1) | A little effort (2) | No opinion (3) | A lot of effort (4) | Huge effort (5)
Ethicality *The extent to which the intervention has good fit with an individual's value system*	**Ethical Consequences:** There are moral or ethical consequences [behaviour *e.g. to engage with*] [intervention] Strongly disagree (1) | Disagree (2) | No opinion (3) | Agree (4) | Strongly agree (5) ***OR*** How fair is [Intervention] for [people/ participants/ recipients] with [condition]? Very unfair (1) | Unfair (2) | No opinion (3) | Fair (4) | Very fair (5)
Perceived effectiveness *The extent to which the intervention is have achieved its intended purpose*	The [intervention] has improve [behaviour/ condition/ clinical outcome]: Strongly disagree (1) | Disagree (2) | No opinion (3) | Agree (4) | Strongly agree (5)
Intervention coherence *The extent to which the participant understands how the intervention works*	It is clear to me how [intervention] will help [manage/ improve] my [behaviour/ condition/clinical outcome] Strongly disagree (1) | Disagree (2) | No opinion (3) | Agree (4) | Strongly agree (5) **Please tell us more about your views*
Self -efficacy *The participants confidence that they can perform behaviour(s) required to participate in the intervention*	**Self-efficacy:** How confident did you feel about [*behaviour e.g. engaging with*] [intervention]? Very unconfident (1) | Unconfident (2) | No opinion (3) | Confident (4) | Very confident (5)
Opportunity costs *The benefits, profits or values that were given up to engage in the intervention*	**Opportunity Costs:** [Behaviour *e.g. engaging in]* [intervention] interfered with my other priorities Strongly disagree (1) | Disagree (2) | No opinion (3) | Agree (4) | Strongly agree (5)
General Acceptability	How acceptable was the [intervention] to you? Completely unacceptable (1) | Unacceptable (2) | No opinion (3) | Acceptable (4) | Completely acceptable (5)

Note: [intervention] should be replaced with the name of the healthcare intervention (e.g. Did you like or dislike the [feedback materials]?

[Engaging with/ engaging with intervention] should be replaced with the specific behaviour participants are required to complete to engage with the intervention (e.g. how much effort did it to take you to [book your own appointment]? [clinical condition] should be replaced with the name of the clinical condition associated with the intervention (e.g. it is clear to me how [feedback reports] will result in improvements in [blood transfusion practice].

Figure 12. TFA acceptability questionnaire (58)

ANNEX 8. PARTICIPANT INFORMATION SHEET

FULL D'INFORMACIÓ PEL PARTICIPANT

NOM DE L'ESTUDI: *Could unnecessary surgeries for suspected ovarian cancer be avoided in women with a pathogenic mutation in the BRCA1/2 genes?*

INVESTIGADOR PRINCIPAL: Maria Ràfols Pérez

CENTRE DE REFERÈNCIA: Hospital Universitari Doctor Josep Trueta

Ens dirigim a vostè per convidar-la a participar en un estudi d'investigació. Aquest estudi ha estat aprovat pel Comitè d'Ètica i Investigació Clínica (CEIC) de l'Hospital Universitari Doctor Josep Trueta, conforme amb la legislació vigent i els principis postulats en la declaració de Hèlsinki.

La intenció del present document és que vostè rebi la informació necessària sobre l'estudi per tal que pugui decidir si vol participar-hi, de forma completament lliure i voluntària. Li preguem que llegeixi detingudament aquest document i, en cas de sorgir-li qualsevol dubte o pregunta, es dirigeixi a l'investigador principal o als membres de l'equip de recerca per tal d'aclarir-los.

Quin és l'objectiu de l'estudi?

El present estudi té com a objectiu principal avaluar la incorporació d'una prova basada en l'anàlisi del material genètic obtingut de mostres de citologia cervical i intrauterina mitjançant un test de PCR múltiple en dones portadores d'una mutació patogènica en els gens BRCA1/2.

Actualment, la mesura de reducció del risc de càncer d'ovari amb més evidència en relació a la disminució de la mortalitat és la salpingo-ooforectomia bilateral profilàctica reductora de risc. Aquesta intervenció ha demostrat una disminució del risc d'aparició d'una neoplàsia ovàrica en un 80-90%. No obstant, algunes dones decideixen rebutjar o posposar la intervenció quirúrgica, especialment pels efectes posteriors que comporta (infertilitat, menopausa prematura, osteoporosi, risc de malaltia cardiovascular, entre d'altres).

Amb l'estudi que es realitzarà, es pretén avaluar l'eficàcia d'una prova mínimament invasiva per millorar l'estratègia de seguiment alternativa a la cirurgia reductora de risc, amb l'objectiu últim d'evitar procediments quirúrgics innecessaris. Actualment, si una dona presenta alteracions en les proves de seguiment, es considera candidata a cirurgia individualitzada, ja que el risc de presentar una neoplàsia ovàrica és molt elevat. No obstant, aquestes proves tenen una taxa de resultats falsos positius significativa, estimada del 15%. Això implica que un 15% de les dones amb un resultat positiu en les proves no tindrà un tumor ovàric maligne, sotmetent-les per tant, a una intervenció quirúrgica que no seria necessària. D'aquesta forma, amb l'aplicació del test

PapSEEK pretenem identificar aquelles dones que, tot i presentar resultats alterats en les proves de seguiment estàndard i ser candidates a cirurgia, no presenten un tumor maligne. No obstant i donat que l'eficàcia del test PapSEEK està en investigació, les dones que participin en aquest estudi seran sotmeses al procediment quirúrgic individualitzat seguint amb l'estàndard actual, ja que considerem que no seria ètic no realitzar la cirurgia donat que el risc de presentar un tumor ovàric maligne és elevat. Aquest estudi aportarà evidència que recolzi la futura incorporació del test PapSEEK en l'estratègia de seguiment semestral i amb la finalitat última d'evitar cirurgies innecessàries en un futur.

Descripció general i activitats de l'estudi

L'estudi inclourà un total de 62 pacients portadores d'una mutació patogènica en els gens BRCA1/2 que no acceptin sotmetre's a la salpingo-ooforectomia bilateral profilàctica reductora de risc i que hagin presentat uns resultats alterats en les proves realitzades en el context de l'estratègia actual de seguiment semestral per a la detecció precoç de càncer d'ovari.

Un cop la pacient accepti participar a l'estudi, es duran a terme els següents procediments:

- Visita amb un ginecòleg oncològic, membre de l'equip de recerca, per la recollida de les mostres. Aquesta consistirà en un examen pelvià i posterior realització de dues citologies (cervical i intrauterina). Es processaran les mostres i s'enviaran al laboratori per la realització del test PapSEEK. En cas de no obtenir mostra suficient per a l'anàlisi, es recitarà a la pacient per prendre noves mostres. Si no s'obté prou mostra la segona vegada, la pacient serà exclosa de l'estudi.

Els següents procediments formen part de la pràctica clínica habitual:

- Estudi d'extensió amb TC d'abdomen i pelvis +/- tòrax. Programació de la cirurgia i visita preoperatòria amb l'equip d'anestèsia.
- Intervenció quirúrgica corresponent en un termini màxim de 45 dies des de la sospita diagnòstica i enviament de la peça quirúrgica al laboratori d'anatomia patològica per al seu anàlisi.
- Visita amb un ginecòleg oncològic, membre de l'equip de recerca, per l'entrega i comunicació dels resultats de l'anàlisi histològic de la peça quirúrgica. Seguiment post-operatori.

L'equip de recerca analitzarà els resultats del test PapSEEK i els compararà amb el diagnòstic histològic de la peça quirúrgica, per mostrar quantes intervencions quirúrgiques es podrien haver evitat.

La meva participació implica riscos?

No, el test PapSEEK es considera segur i no s'esperen problemes de seguretat d'acord amb la naturalesa mínimament invasiva de la prova. No obstant, la recollida de les mostres de citologia pot provocar un petit sagnat, com a màxim d'un dia de duració.

Confidencialitat i tractament de les dades personals

La informació obtinguda serà totalment confidencial, recollida i analitzada anònimament, d'acord amb la *Llei Orgànica de Protecció de Dades de Caràcter Personal i Garantia de Drets Digitals (3/2018) i el Reglament 2016/679 del Parlament i Consell Europeu*.

Les dades personals seran tractades de forma confidencial i només els investigadors de l'estudi hi tindran accés. La informació serà sempre utilitzada amb finalitats d'investigació.

Què se'n farà de la informació obtinguda de l'estudi?

En cas de publicar els resultats a través de publicacions i/o congressos, les dades personals seran tractades de forma anònima sense que sigui possible la identificació dels participants.

És obligatòria la participació?

La participació a l'estudi és completament voluntària. En el supòsit d'acceptar participar, vostè té el dret de revocar el consentiment en qualsevol moment, sense necessitat d'explicar-ne els motius i sense que això provoqui perjudicis en la seva assistència mèdica.

Quina és la compensació econòmica?

No s'ofereix cap compensació econòmica per participar a l'estudi, i tampoc suposa cap cost addicional pel pacient.

Contacte en cas de dubtes

Pot posar-se en contacte amb l'investigador principal i els altres membres de l'equip de recerca si al llarg de l'estudi li sorgeixen nous dubtes o necessita més informació. Se li proporcionarà un document amb les dades de contacte corresponents.

ANNEX 9. INFORMED CONSENT DOCUMENT

CONSENTIMENT INFORMAT

TÍTOL DE L'ESTUDI:

Jo, _____,

amb DNI _____, de nacionalitat _____, major d'edat, amb domicili _____

Declaro que:

- He rebut i llegit el Full Informatiu pel Participant sobre l'estudi, que se m'ha entregat.
- He rebut la informació suficient del membre responsable de l'equip investigador anomenat a sota, en relació a les característiques, objectius i possibles riscos de l'estudi.
- He pogut formular les preguntes que he considerat oportunes sobre l'estudi i que, aquestes han estat respostes satisfactòriament per l'investigador responsable.
- He estat informat/ada per l'investigador _____.
- Entenc que la meva participació és totalment voluntària.
- Entenc que puc revocar el consentiment informat sobre la meva participació a l'estudi en qualsevol moment, sense haver d'especificar-ne les raons i sense que això repercuteixi a la meva assistència sanitària.
- Dono permís perquè les dades de la meva història clínica siguin usades per l'equip investigador per fins relacionats amb aquest estudi. He estat informat/ada sobre l'ús de caire científic que es farà de les meves dades personals.
- Entenc que es respectarà la confidencialitat de les meves dades personals i que puc sol·licitar la retirada i eliminació d'aquestes en qualsevol moment de l'estudi.
- Declaro que se m'ha entregat una còpia del Full d'Informació pel Participant i una còpia d'aquest Consentiment Informat.
- D'acord amb la informació rebuda fins el moment, ACCEPTO voluntàriament la meva participació a l'estudi especificat.

A _____, _____ de _____ 20__

SIGNATURA DE L'INVESTIGADOR/A: SIGNATURA DEL PARTICIPANT:

REVOCACIÓ DEL CONSENTIMENT

Jo, _____,

amb DNI _____, revoco el consentiment prèviament signat de participar en l'estudi anteriorment especificat.

A _____, _____ de _____ 20__

SIGNATURA DE L'INVESTIGADOR/A: SIGNATURA DEL PARTICIPANT: