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# Preventive Strategies for Ovarian Cancer

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Additional information is available at the end of the chapter

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## 1. Introduction

According to the American Cancer Society, in 2012 ovarian cancer is expected to account for 3% (22,280) of all new cases and 6% (15,500) of all female cancer deaths in the United States. The proportion of ovarian cancer among gynaecological cancers is increasing, also because of the decrease in cervical cancer as a result of pap smear screening programmes. On the other hand, survival from ovarian cancer is the poorest of all gynaecological cancers, with a five-year relative survival rate of 44% for all stages [1,2]. The main reasons for this poor survival are the lack of early detection strategies and an unfavourable anatomical situation. Thus, the vast majority of ovarian cancer is diagnosed at an advanced stage and therapy for this pathology is very complex [3-5]. Reduction in mortality rates could be gained both with new screening strategies and with ameliorations in surgical and medical treatments. However, neither of these approaches will affect cancer incidence, thus, it is clear that the prospects for making a major impact on the mortality from ovarian cancer lie more in the area of prevention.

The purpose of this chapter is to identify the evidence for the appropriate practical strategies to prevent ovarian cancer or the detection of cancer in the early stages in order to improve the overall survival. The search was restricted to full reports and guidelines published in English between 2000 and May 2012, in an attempt to summarize the principal findings regarding primary and secondary ovarian cancer prevention.

## 2. Primary prevention for ovarian cancer in general population

Primary prevention aims to prevent the disease before its biological onset, thus it is based on avoiding risk factors and increasing protective factors.

A summary of the most significant risk and protective factors, with relative hazard ratio, for epithelial ovarian cancer is summarized in Tables 1a and 1b,

<b>AUTHORS</b>	<b>RISK FACTORS</b>	<b>RR (95% CI)</b>
Schorge JO et al. [6]	White race	1.35 (1.08-1.50)
Schouten LJ et al. [7]	Height $\geq$ 160 cm	1.38 (1.16-1.65)
Lahmann PH et al. [8]	BMI $\geq$ 25	1.33 (1.05-1.68)
Camargo MC et al. [9]	Asbestos exposure	1.77 (1.37-2.28)
Cramer DW [10]	early age at menarche	1.74 (1.28-2.18)
Cramer DW [10]	late menopause	1.61 (1.15-2.08)
Beral V et al. [11]	HRT	1.20 (0.98-1.32)
Melin A et al. [12]	Endometriosis	1.43 (1.19-1.71)
Chen S et al. [13]	BRCA1	42.4 (15-119.6)
	BRCA2	20.6 (7.75-57.2)
Watson P et al. [14]	MMR	19 (5.0-30.0)
(a)		
<b>AUTHORS</b>	<b>PROTECTIVE FACTORS</b>	<b>HR (95% CI)</b>
Trudel D et al. [15]	green tea components	0.66 (0.54-0.80)
Collaborative Group on Epidemiological Studies of Ovarian Cancer [16]	hormonal contraceptive use	0.73 (0.70-0.76)
Ness RB et al. [17]	Multiparity( $\geq$ 4 pregnancies)	0.40 (0.30-0.50)
Danforth KN et al. [18]	breastfeeding	0.98 per month ( 0.97-1.00)
Hankinson SE et al. [19]	bilateral tube ligation	0.33 (0.16-0.64)
Hankinson SE et al. [19]	hysterectomy	0.67 (0.45-1.00)
(b)		

**Table 1.** (a) Main significant risk factors for ovarian cancer. (b) Main significant protective factors for ovarian cancer.

The average age at diagnosis is approximately 60 years, but the overall incidence of ovarian cancer rises with increasing age up to 75-84 years, due to the accumulation of random genetic alterations, before declining slightly among women beyond 84 years [20]. Women residing in North America, Northern Europe or in any industrialized Western country have a higher risk of developing ovarian cancer. Conversely, women residing in developing countries have shown the lowest rate [6]. The exact reasons for this distribution are unknown but discrepancies in parity, rates of gynaecologic surgery and dietary habits may account for some differ-

ences [21]. In particular, regarding dietary habits, a comprehensive meta-analysis of the observational studies published up to September 2011 provided no evidence of a material association between alcohol drinking and epithelial ovarian cancer risk [22]. Finally, a recent study provided some suggestion that soy and phytoestrogen consumption may decrease ovarian cancer risk, although the results did not reach statistical significance [23].

Exposure to radiation may increase the risk of ovarian cancer and the risk increases with increasing dose. The Life Span Study incidence data for ovarian cancer demonstrated a borderline significant association [24], and mortality data showed a significant positive association between exposure to radiation and ovarian cancer [25].

With regards to reproductive factors, early age at menarche and late menopause have been consistently associated with an increased risk of ovarian cancer, likely due to an increase in ovulation and in oestrogen exposure [26]. The effect of combined hormonal contraceptive use on the risk of ovarian cancer has been long discussed. In 2007, the IARC review concluded that women who had at least for a period used combined hormonal contraceptives orally had an overall reduced risk for ovarian cancer, which persists for at least 20 years after cessation of use, and an inverse relationship was observed with duration of use [27]. These results have been confirmed by the Collaborative Group on Epidemiological Studies of Ovarian Cancer [16] that reported an overall reduction in ovarian cancer risk in users versus non-users of 27%, which was not confined to any particular type of oral formulation nor to any histological type of ovarian cancer, although it was less consistent for mucinous than for other types of ovarian cancer. On this basis, the "incessant menstruation" hypothesis was postulated, which concludes that the use of oral contraceptives (OC) should be favoured for prolonged periods of time, especially in women with endometriosis, a population at doubled risk of ovarian cancer [28]. On the other hand, in the Million Women Study HRT after menopause was shown to increase the risk of ovarian cancer [11].

Women who have never had children are at increased risk of developing ovarian cancer [29]. Regarding fertility drug use, previous studies have provided conflicting results. Recent data demonstrated that fertility drug use does not significantly contribute to ovarian cancer risk among the majority of women. However, women who despite their use remain nulliparous may have an increased risk [30]. The role of breastfeeding as a protective factor against ovarian cancer has been long discussed. Finally, the risk of ovarian cancer decreases in women who underwent bilateral tube ligation or hysterectomy, probably because these surgical interventions do not allow the carcinogenic agents to enter the body from the vagina and reach the ovaries [19, 31]. For instance, a number of observational studies (largely case-control) conducted over the last two decades suggested an association between use of talc powders on the female perineum and increased risk of ovarian cancer, although the weak statistical associations observed in a number of epidemiological studies do not support a causal association between cosmetic talc use and ovarian cancer [32,33].

Endometriosis represents another considerable risk factor for epithelial ovarian cancer. In particular, self-reported endometriosis was associated with a significantly increased risk of clear-cell, low-grade serous and endometrioid invasive ovarian cancers. No association was noted between endometriosis and risk of mucinous or high-grade serous invasive ovarian

cancers or borderline tumours of either subtype [34]. Also, pelvic inflammatory disease has been suggested to double the risk of epithelial ovarian cancer [35], but few studies have been done and the conclusions are inconsistent.

The most important risk factor still remains a family history of breast or ovarian cancer. Up to 10% of ovarian cancer patients may have inherited a germline mutation that places them at increased risk of the disease. Mutations in the breast and ovarian cancer-susceptibility genes *BRCA1* and *BRCA2* confer an increased lifetime risk of ovarian cancer. *BRCA1* and *BRCA2* are tumour suppressor genes involved in many cellular functions to prevent carcinogenesis [Fig.1].

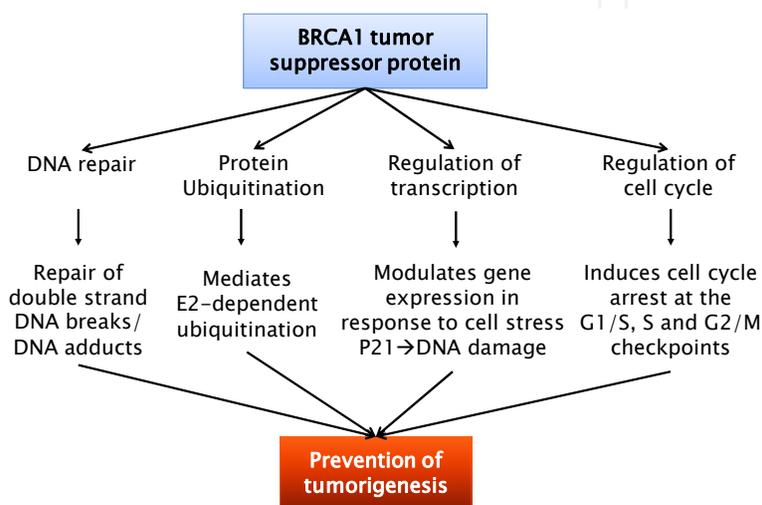


Figure 1. BrCa1 protein functions.

The mechanism to repair the double-strand DNA breaks is shown in Fig.2.

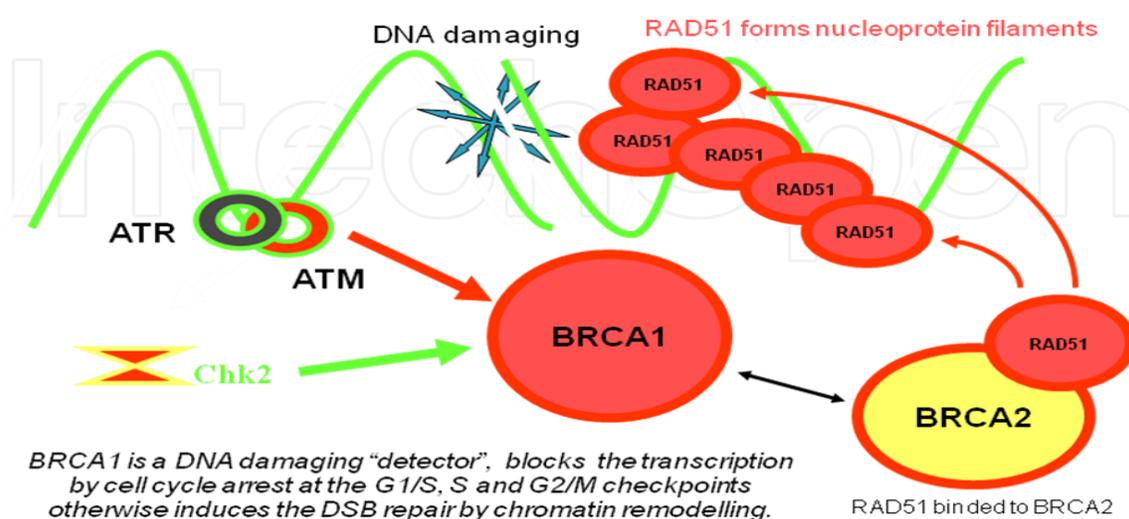
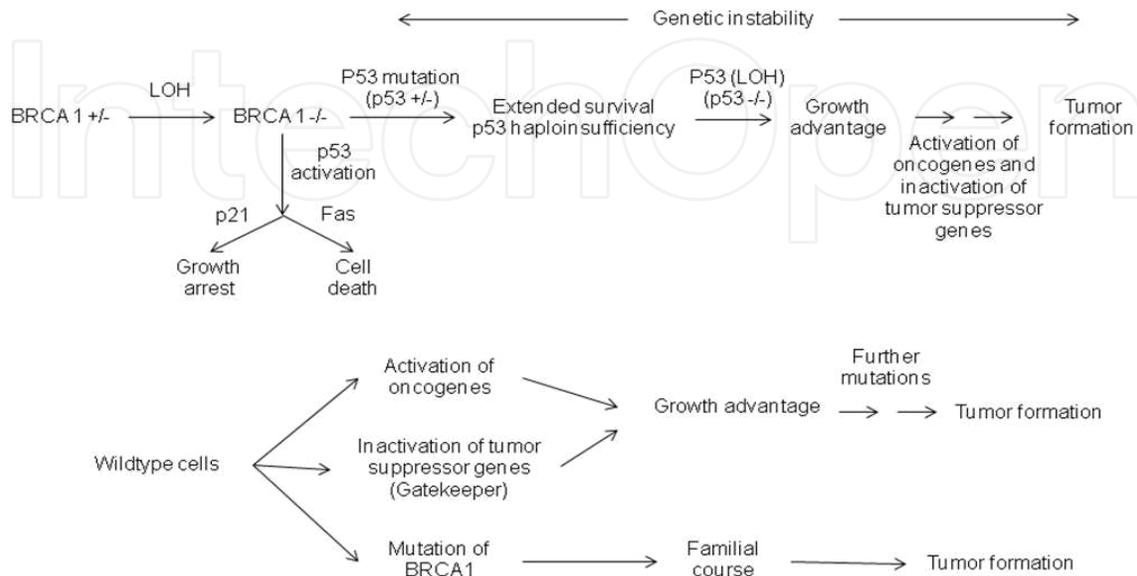


Figure 2. Repair of double-strand DNA breaks by BRCA1 and BRCA2 genes.

Heterozygous germline mutation leads to genetic instability as shown in the Fig.3, modified by Brodie *et al.* [36]



**Figure 3.** Genetic instability in the germline and somatic BrCa1 mutations [36].

However, *BRCA* mutations do not account for the entire range of hereditary ovarian cancer syndromes. Other hereditary epithelial ovarian cancers are attributed to Lynch syndrome. Lynch syndrome is an autosomal dominant disorder, which predisposes to colorectal cancer, endometrial cancer, ovarian, gastric, small bowel, biliary/pancreatic, urothelial, skin, and central nervous system cancers. The cumulative risk of ovarian cancer is estimated to be 8–10%, with an average age at onset of 42 years [14]. Moreover, other genes often associated with rare cancer syndromes such as *TP53* and *PTEN*, or *CHEK2* and *PALB2* confer a low to moderate risk of breast and ovarian cancer [37–39]. Recent technological advances have aided in the recognition of additional tumour suppressor genes potentially associated with hereditary breast cancer, such as *RAD51* and *BARD1* [40]. To date, at least 16 genes have been associated with hereditary ovarian cancer, mostly involved in the *FA-BRCA* pathway and the mismatch repair system. However, many families with suspicious pedigrees do not have a specific mutation identified through clinical testing, due to a currently undetectable *BRCA1/2* mutation or a mutation in another susceptibility gene. Although their cancer risks are not as well defined, these families should be considered as part of the hereditary breast and/or ovarian cancer spectrum [13].

However, most of the common risk and protective factors only slightly influence the risk of developing ovarian cancer, thus, to date; the knowledge of these factors has still not been translated into practical strategies to prevent ovarian cancer.

### 3. Primary prevention for ovarian cancer in high risk women

Some women have a high risk of developing ovarian cancer due to hereditary conditions associated to BRCA syndrome and Lynch syndrome. Thus, when one of these forms of hereditary or familial breast and/or ovarian cancer is suspected in clinical practice, the general practitioner should refer the patient to a cancer centre specialising in cancer-specific genetic counselling for the identification, definition and management of risk. Genetic counselling, defined by the American Society of Human Genetics as 'a communication process which deals with the human problems associated with the occurrence or risk of occurrence of a genetic disorder in a family', involves one or more professional figures to help the affected individuals or families [41-44]. Genetic counselling in the oncological setting (cancer-specific genetic counselling) should also provide sufficient information to enable the user to make a fully informed choice as to course of action, particularly with regards to prevention, in the case of the identification of a mutation or of a familial cancer risk [45, 46].

A recent review investigated the impact of cancer genetic risk assessment on outcomes, including perceived risk of inherited cancer and psychological distress. The review found favourable outcomes for patients after risk assessment for familial breast cancer, suggesting that cancer-specific genetic risk assessment services help to reduce distress, improve the accuracy of the perception of risk of ovarian cancer, and increase the knowledge of ovarian cancer and genetics. However, there were too few papers to make any significant conclusions on how best to deliver cancer genetic risk assessment services. Further research is needed, assessing the best means of delivering cancer risk evaluation, by different health professionals, in different ways and in alternative locations [47].

Women at increased risk of breast and ovarian cancer are advised to consider risk-reducing strategies; however, such methods vary in their effectiveness. These strategies include chemoprevention and prophylactic surgery (risk-reducing salpingo-oophorectomy, RRSO). Risk-reducing strategies have been shown to have associations with a lengthening of life expectancy in *BRCA1/2* carriers.

#### 3.1. Risk-reducing salpingo-oophorectomy (RRSO)

Women who have inherited mutations in the *BRCA1* or *BRCA2* genes have substantially elevated risks of breast and ovarian cancer, with a lifetime risk of breast cancer of 56%–84% [48-51]. Breast cancer in *BRCA1/2* mutation carriers also occurs at an earlier age, particularly among the *BRCA1* mutation carriers, than in non-carriers. The risk for ovarian cancer depends on whether the mutation has occurred in *BRCA1* or *BRCA2*, with estimated risks ranging from 34% to 44% for *BRCA1* mutation carriers and from 12% to 25% for *BRCA2* mutation carriers [48, 49, 52-54]. Carriers of *BRCA1/2* mutations are counselled to help them interpret the implications of these elevated risks, choose strategies to reduce these risks, and maximize early detection of cancers. The risk of breast cancer can be reduced either with RRSO and/or mastectomy or non-surgically (i.e. with chemoprevention). However, due to the lack of effective screening for ovarian cancer, RRSO is usually strongly recommended to *BRCA1/2* mutation carriers once childbearing is complete.

RRSO has also been demonstrated to decrease the risk of both breast and ovarian cancer in *BRCA1/2* mutation carriers [55-60]. However, the studies examining the extent of risk reduction have used different designs; some are retrospective case-control studies, while others used a prospective cohort design. In a large, retrospective analysis of 551 *BRCA* carriers, RRSO was found to reduce the risk of ovarian cancer by 96% and breast cancer by 53% at a mean follow-up of 9 years [55]. A multicentre prospective study, Kauff *et al.* [56] found that, during a 3-year follow-up, RRSO was associated with an 85% reduction in *BRCA1*-associated gynaecologic cancer risk and a 72% reduction in *BRCA2*-associated breast cancer risk. Although protection against *BRCA1*-associated breast cancer and *BRCA2*-associated gynaecologic cancer was suggested, neither effect reached statistical significance. The authors postulate that the protection conferred by RRSO against breast and gynaecologic cancers may differ between the carriers of *BRCA1* and *BRCA2* mutations.

Similar findings were observed in a prospective study of 170 *BRCA* carriers. During a mean follow-up of 2 years, the incidence of ovarian or peritoneal cancer and breast cancer was significantly greater amongst those women who selected surveillance than amongst those who chose to undergo RRSO [59.] Even among prospective studies, the inclusion criteria and the definitions of follow-up time differ. In some studies, only unaffected mutation-positive women are included and followed up. In others, particularly when examining ovarian cancer risk, women with breast cancer are included. Such differences in study design can introduce biases (such as the survival bias) and can have an impact on risk reduction estimates. For example, the reported efficacy of RRSO in reducing the risk of ovarian/fallopian tube cancers varies from 71% to 96% [55-61]. Although these estimates imply a substantial reduction in risk, this variability may affect the decisions of premenopausal women who are making a decision about whether to undergo a treatment that will cause abrupt and premature menopause. Patients and their physicians need as much information as possible regarding the efficacy of RRSO in reducing cancer risk to balance this benefit with the health risks caused by premature entry into menopause.

A summary of published studies on RRSO is presented in Table 2.

Reference	Study design	With/Without RRSO	MYFU	OC Risk Reduction (%)	BC Risk Reduction (%)
Rebbeck <i>et al.</i> , 2002 [55]	RC	261/292	8.5	96	53
Kauff <i>et al.</i> , 2008 [56]	PC	509/283	3.2	85	72
Finch <i>et al.</i> , 2006 [57]	RC	1041/779	3.5	80	NA
Chang-Claude <i>et al.</i> , 2007 [58]	RC	55/1601	65,675 PY	NA	44
Rutter <i>et al.</i> , 2003 [60]	RC	5/223	NA	67	NA
Kauff <i>et al.</i> , 2002 [61]	PC	98/72	2.0	85	68

PC = prospective cohort; RC = retrospective cohort; MYFU = mean years of follow-up; PY = person-years; NR = not reported; and NA = not applicable; RRSO = risk-reducing salpingo-oophorectomy.

**Table 2.** Published studies on risk-reducing salpingo-oophorectomy in *BrCa1* and/ or *BrCa2* mutation carriers.

A synopsis of different management strategies available for *BRCA1* and *BRCA2* mutation carriers is shown in Table 3.

Management options Gynecologic cancer	Strategy	Advantage	Limitation
Chemoprevention	OC	Likely 30-60% reduction in ovarian cancer risk	Potential increase in risk of breast cancer
Screening	TVUS, CA 125	Avoids RRSO	Unproven efficacy
Risk-reducing surgery	Bilateral salpingo-oophorectomy	Substantial decrease in risk of ovarian and fallopian tube cancers	Premature menopause and iatrogenic infertility

TVUS= Transvaginal Ultrasound; RRSO = risk-reducing salpingo-oophorectomy

**Table 3.** Synopsis of different prevention strategies for *BRCA1* and *BRCA2* mutation carriers.

The National Comprehensive Cancer Network (NCCN) guidelines and other institutions concerning this method, recommend RRSO “for women with a known *BRCA1/2* mutation, ideally between 35 and 40 years or upon completion of child bearing” or at an adjusted age based on earliest age of ovarian cancer diagnosis in the family” [62].

Also ACOG, the Committee on Genetics and the Society of Gynecologic Oncologists, recommends RRSO for women with *BRCA1/2* mutations, by the age of 40 years or when childbearing is complete [63].

The National Cancer Institute (NCI) [64] on the clinical management of *BRCA* mutation carriers considers, besides salpingo-oophorectomy, bilateral salpingectomy as an interim procedure to reduce risk in *BRCA* mutation carriers. There are no data available on the efficacy of salpingectomy as a risk-reducing procedure. The procedure preserves ovarian function and spares the premenopausal patient the adverse effects of a premature menopause. It can be performed using a minimally invasive approach, and a subsequent bilateral oophorectomy could be deferred until the patient approaches menopause. While the data make the compelling argument that some pelvic serous cancers in *BRCA* mutation carriers originate in the fallopian tube, clearly, some cancers arise in the ovary. Furthermore, bilateral salpingectomy could give patients a false sense of security that they have eliminated their cancer risk as completely as if they had undergone a bilateral salpingo-oophorectomy. A small study of 14 young *BRCA* mutation carriers documented the procedure as feasible [65]. However, efficacy and impact on ovarian function was not assessed in this study. Future prospective trials are needed to establish the validity of the procedure as a risk-reducing intervention.

For the European Society of Medical Oncology ESMO [66], RRSO is associated with a reduction in risk of breast cancer in premenopausal *BRCA* mutation carriers, a reduction in risk of ovarian cancer, and there is evidence of a reduction in overall mortality [67]. RRSO is recommended after the age of 35 and when childbearing decisions are complete.

The significantly reduced risk of breast cancer by RRSO seems to be higher in *BRCA2* mutation carriers than in *BRCA1* carriers. Several reports have addressed this question although additional research is required [56]. Short-term HRT after RRSO seems not to decrease the overall benefit of this strategy for breast cancer risk reduction [68].

However, it should be noted that the NCCN and other institutions couch these recommendations within a multidisciplinary consultative process in which reproductive desires, assessment of cancer risk, and the pros and cons of surgery along with the potential sequelae of surgery are fully discussed.

The recommendations of different organizations regarding surgical primary prevention for *BRCA1/2* mutation carriers are shown in Table 4.

Management options	NCCN [62]	ACOG Committee on Genetics and the Society of Gynecologic Oncologists [63]	National Cancer Institute (NCI) [64]	ESMO [65]
RRSO	Between 35 and 40 years or upon completion of child bearing	By the age of 40 years or when childbearing is complete	Considered but age is not indicated	After age 35 and when childbearing decisions are complete
Bilateral salpingectomy	-	-	Considered but age is not indicated	-

**Table 4.** Recommendations of several organizations regarding primary prevention for BRCA mutation carriers.

### 3.2. Chemoprevention

Women at increased risk, based on their personal or family history of breast and/or ovarian cancer including *BRCA1/2* mutation carriers, may join a cancer prevention clinical trial or a chemoprevention trial. OC have been the most widely studied chemopreventive agents in ovarian cancer. Recently, Iodice *et al.* conducted a meta-analysis updated to March 2010 on the association between OC use and breast or ovarian cancer in *BRCA1/2* mutation carriers [69]. Based on 18 studies a total of 2855 breast cancer cases and 1503 ovarian cancer cases carrying an ascertained *BRCA1/2* mutation were included. As previously noted, use of OC at any point during one’s life was associated with a 50% reduction in relative risk of developing ovarian cancer for *BRCA1/2* mutation carriers. Looking specifically at duration of use, each 10-year period of OC use resulted in a 36% relative risk reduction in the development of ovarian cancer. However, the meta-analysis showed no evidence of a significant association between OC use and breast cancer risk. Notably, formulations used before 1975 correlated with an increased risk of breast cancer, but there was no correlation with the use of more recent formulations. A summary of the association between OC use and ovarian cancer risk in mutation carriers is shown in Table 5.

EXPOSURE	GENES WITH MUTATION	CATEGORIES OF EXPOSURE	No. of STUDIES	No. of CASES	HR (95% CI)
	BRCA 1/2		5	1503	0.50 (0.33-0.75)
OC USERS	BRCA 1	Users	5	1251	0.51 (0.40-0.65)
	BRCA 2		4	286	0.52 (0.31-0.87)
DURATION OF USE	BRCA 1/2	1 year			0.96 (0.94-0.97)
		5 years	4	1336	0.80 (0.73-0.88)
		10 years			0.64 (0.53-0.78)

**Table 5.** Summary of the association between OC use and ovarian cancer risk in mutation carriers

Another meta-analysis of cohort, case-control and case-case studies published in English up to December 2009 confirmed a significantly decreased ovarian cancer risk in *BRCA1/2* mutation carriers associated with the use of OC, while a significantly increased risk in breast cancer was only shown in a subset of cohort studies on *BRCA1* mutation carriers. To conclude, OC use can be considered as an alternative strategy in the chemoprevention of ovarian cancer in *BRCA1* mutation carriers who do not accept RRSO above the age of 30 years [70].

Other chemopreventative agents such as retinoids, vitamin D, cyclo-oxygenase inhibitors and peroxisome proliferator activated receptor-gamma ligands have shown promise in early investigations of disease prevention [71].

Retinoids, a class of compounds comprising vitamin A, its natural derivatives, and synthetic analogs, have been extensively studied in both the prevention and treatment of gynaecologic malignancies [72]. One of the most promising retinoids to be used in chemoprevention trials is the synthetic amide of retinoic acid fenretinide, *N*-4-hydroxyphenyl retinamide (4-HPR). 4-HPR has been found to have significant chemopreventive action in a large variety of in vitro and in vivo systems. Since both fenretinide and its major metabolite, 4-methoxyphenyl retinamide (MPR), selectively accumulate in the human breast, evaluation of 4-HPR as a chemopreventive agent in breast cancer has been particularly attractive [73]. The most important clinical trials with 4-HPR are mentioned in Table 6.

The most important study where 4-HPR was administered was a multicentric phase III randomized trial, coordinated by the Istituto Nazionale dei Tumori in Milan, which started in 1987. Most notably, the younger the women were, the greater the benefit of 4-HPR. Such a benefit was associated with a remarkable 50% risk reduction in women aged 40 years or younger, whereas it disappeared after 55 years of age. Interestingly, the incidence of ovarian cancer during the 5-year intervention period was significantly lower in the treatment arm [74].

The role of analgesic drug use in the development of ovarian cancer is still widely discussed.

STUDY	DESIGN	TREATMENT	END POINTS	OUTCOMES
Costa et al., 1989 [75]	Phase I, Randomized, Placebo controlled (60)	Orally: 100, 200 and 300mg x 6 months subsequently at 200mg for another 6 months.	Tolerability	Recommended dose for chemoprevention trials of HPR is 200mg/die.
Formelli et al., 1989 [76]	Phase II, Randomized, Placebo controlled (60)	Orally: 100, 200 and 300mg x 6 months subsequently at 200mg for another 6 months.	Pharmacokinetic	HPR treatment lowers retinol and RPB plasma concentrations. Effect related to HPR levels and reversible on cessation of HPR administration.
Veronesi et al., 1999 [77]	Phase III, Randomized (2867)	Orally 200mg versus no treatment x 5 years.	Second breast cancer prevention	No statistically significant effect but a possible benefit in premenopausal women.
Veronesi et al., 2006 [78]	Phase III, Randomized, 15-year follow-up (1879)	Orally 200mg versus no treatment x 5 years: 15-years followup.	Second breast cancer prevention	4-HPR induces a significant reduction of risk of second breast cancer in premenopausal women, which is remarkable at younger ages, and persists several years after treatment cessation.

HPR: fenretinide, RBP: retinol-binding protein.

**Table 6.** Clinical trials with 4-HPR [74].

A recent population-based case-control study, carried out in Denmark in the period 1995-1999, analysed the association between analgesic drug use and ovarian cancer risk using multiple logistic regression models. The study showed that regular use of non-aspirin non-steroidal anti-inflammatory drugs (NA-NSAID), paracetamol or other analgesics did not decrease ovarian cancer risk. In contrast, use of any analgesics (OR = 0.72; 95% CI 0.53-0.98) or aspirin (OR = 0.60; 95% CI 0.36-1.00) resulted in a statistically significant decreased risk of serous ovarian cancer but not mucinous or other ovarian tumours [79]. On the other hand, recent data reported by the Multiethnic Cohort Study did not find compelling evidence to support an association between use of NSAID and risk of ovarian and endometrial cancers in a multiethnic population. The RR (95% CI) for ovarian cancer associated with aspirin, non-aspirin NSAID, and acetaminophen were 0.87 (0.68, 1.14), 0.97 (0.74, 1.26), and 0.86 (0.67, 1.12), respectively. No heterogeneity across ethnic groups ( $P$ 's  $\geq 0.29$ ) or dose-response relation with increased duration of use ( $P$ 's for trend  $\geq 0.16$ ) was observed [80]. Finally, in an attempt to review and summarize the evidence provided by longitudinal studies on the association between NSAID use and ovarian cancer risk, a comprehensive literature search for articles published up to December 2011 was performed (Table 7). The meta-analysis found no evidence of an association between aspirin or NA-NSAID use and ovarian cancer risk, based on a random-effects

model or a fixed-effects model. Furthermore, the analysis did not show strong association between frequency or duration of NA-NSAID use and ovarian cancer, leading to the conclusion that there is no strong evidence of an association between aspirin/NA-NSAID use and ovarian cancer [81].

	No. Of studies	Fixed-effects model		Random-effects model		P-value
		RR	(95% CI)	RR	(95% CI)	
<b>ASPIRIN USE</b>						
All studies	17	0,94	(0,87-1,01)	0,91	(0,82-1,01)	0,046
C-C studies	14	0,94	(0,87-1,02)	0,90	(0,79-1,03)	0,015
Cohort studies	3	0,92	(0,77-1,09)	0,92	(0,77-1,10)	0,456
Regular Use	7	0,86	(0,73-1,03)	0,83	(0,65-1,05)	0,119
Irregular Use	7	1,07	(0,96-1,20)	1,07	(0,96-1,21)	0,421
Duration > 5 yrs	5	0,91	(0,67-1,24)	0,89	(0,63-1,25)	0,332
<b>NA-NSAID use</b>						
All studies	7	0,86	(0,76-0,98)	0,89	(0,74-1,08)	0,089
C-C studies	4	0,88	(0,75-1,03)	0,97	(0,73-1,28)	0,042
Cohort studies	3	0,82	(0,64-1,04)	0,89	(0,74-1,08)	0,283
Regular Use	3	1,45	(1,07-1,98)	1,47	(0,95-2,27)	0,153
Irregular Use	3	0,96	(0,69-1,33)	0,93	(0,49-1,76)	0,038
Duration > 5 yrs	3	1,65	(1,13-2,41)	1,56	(0,92-2,65)	0,21

NA-NSAID: non aspirin- non-steroidal anti-inflammatory drugs

**Table 7.** Metanalysis of longitudinal studies on the association between NSAID use and ovarian cancer risk

#### 4. Secondary prevention for ovarian cancer

This is based on diagnosing and treating extant disease in the early stages before it causes significant morbidity. CA125 (or MUC16) glycoprotein is the most studied tumour marker, alone and/or in combination with other biomarkers, for ovarian cancer screening. However, false positive CA125 levels can occur in women with benign conditions, including menstruation, appendicitis, benign ovarian cysts, endometriosis and pelvic inflammatory disease, as well as with other malignancies, including breast, lung, endometrial and pancreatic cancers. Thus, a large number of false-positive screening tests can occur, potentially leading to unnecessary surgeries and subsequent issues of morbidity and cost [82]. Consequently, multimodal strategies, in particular the combination of CA125 with pelvic ultrasound, have been examined, in order to improve sensitivity and positive predictive value of ovarian cancer screening.

#### 4.1. Transvaginal ultrasound

In the general population, TVUS appears to be superior to transabdominal ultrasound in the preoperative diagnosis of adnexal masses. Both techniques have lower specificity in premenopausal women than in postmenopausal women due to the cyclic menstrual changes in premenopausal ovaries (e.g., transient corpus luteum cysts) that can cause difficulty in the interpretation. The randomized prospective Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial found no reduction in mortality with the annual use of combined TVUS and CA125 in screening asymptomatic, postmenopausal women at average risk of ovarian cancer [83].

Data are limited regarding the potential benefit of TVUS in screening women at inherited risk of ovarian cancer. A number of retrospective studies have reported experiments with ovarian cancer screening in high-risk women using TVUS with or without CA125 [84-90].

However, there is little uniformity in the definition of high-risk criteria and compliance with screening, and in whether the cancers detected were incident or prevalent. One of the largest reported studies included 888 *BRCA1/BRCA2* mutation carriers who were annually screened with TVUS and CA125. Ten women developed ovarian cancer; five of the ten developed interval cancers after normal screening results within 3 to 10 months before diagnosis. Five of the ten ovarian cancers were screen-detected incident cases, which had had normal screening results within 6 to 14 months before diagnosis. Out of these five cases, four were stage IIIB or IV [85].

A similar study reported the results of annual TVUS and CA125 combined-screening in a cohort of 312 high-risk women (152 *BRCA1/BRCA2* mutation carriers) [86]. Out of four cancers detected because of abnormal TVUS and CA125, all cases were symptomatic, and three had an advanced-stage disease. Annual screening of *BRCA1/BRCA2* mutation carriers with pelvic ultrasound, TVUS, and CA125 failed to detect early-stage ovarian cancer among 241 women in a study from the Netherlands [87]. Three cancers were detected over the course of the study, all advanced stage IIIC disease. Finally, a study of 1,100 moderate- and high-risk women who underwent annual TVUS and CA125 combined screening reported that ten out of 13 ovarian tumours were detected due to screening. Only five out of ten were stage I or II [88]. There are limited data related to the efficacy of semiannual screening with TVUS and CA125 [89].

The first prospective study of TVUS and CA125 with survival as the primary outcome was completed in 2009. Out of 3,532 high-risk women screened, 981 were *BRCA* mutation carriers, of which 49 developed ovarian cancer. The 5- and 10-year survival was 58.6% (95% CI, 50.9–66.3) and 36% (95% CI, 27–45), respectively, and there was no difference in survival between carriers and non-carriers. A major limitation of the study was the absence of a control group. Despite these limitations, this study suggests that annual surveillance by TVUS and CA125 level appears to be ineffective in detecting tumours at an early stage to substantially influence survival [90].

#### 4.2. Serum CA125

Serum CA125 screening for ovarian cancer in high-risk women has been evaluated in combination with TVUS in a number of retrospective studies, as described in the previous section [84-90].

The National Institutes of Health (NIH) Consensus Statement on Ovarian Cancer recommended against routine screening of the general population for ovarian cancer with serum CA125. The NIH Consensus Statement did, however, recommend that women at inherited risk of ovarian cancer undergo TVUS and serum CA125 screening every 6 to 12 months, beginning at the age of 35 years [91]. The Cancer Genetics Studies Consortium task force recommends that female carriers of a deleterious *BRCA1* mutation undergo annual or semi-annual screening using TVUS and serum CA125 levels, beginning at age of 25 to 35 years [92]. Both recommendations are based solely on expert opinion and best clinical judgment.

NCCN for those patients who have not chosen RRSO, consider concurrent TVUS (preferably day 1-10 menstrual cycle women in premenopausal women) + CA125 (preferably after day 5 of menstrual cycle women in premenopausal women) every 6 months starting at the age of 30 years or 5-10 years before the earliest age of first diagnosis of ovarian cancer in the family [93].

Although there are retrospective data indicating that annual ovarian cancer screening using TVUS and measurement of serum CA125 levels is neither an effective strategy for the early detection of ovarian tumours nor a reasonable substitute for a bilateral RRSO, the effectiveness of these interventions is limited to six-monthly screening. Investigational imaging and screening studies may be considered for this population.

#### 4.3. Proton Magnetic Resonance Spectroscopy (MRS)

MRS has proved to be a reliable technique for probing metabolic patterns, biochemical effects of tumour microenvironment, and the action of therapy in cancer cells, both in vivo and in vitro [94]. In particular, an increase in the total choline-containing compounds (tCho) content allows to distinguish malignant from benign lesions in the breast [95].

Moreover, some studies have also shown alterations of the phospholipid metabolism in vitro using epithelial ovarian carcinoma cell lines [96,97], and demonstrated the feasibility of 3D CSI MRS to detect a choline peak in ovarian lesions in vivo at 1.5 T.

Then, the metabolic meaning of a high concentration of choline in ovarian tumours merits some consideration. This topic has been extensively reviewed by Podo *et al.* in 2007 [98]. The high choline concentration of ovarian tumours can be considered as the result of an inappropriate storage attributable to metabolic deregulation associated with clinical indicators of increased malignancy. The possibility of using a spatially resolved approach for MRS of ovarian masses opens an intriguing prospect for the diagnosis of early-stage tumours, with potential impact on the overall survival. This is especially true for carriers of a *BRCA* mutation, with a lifetime risk of 39% to 46% among women with the *BRCA1* mutation and a risk of 12% to 20% among those with the *BRCA2* mutation [99].

Based on peer-reviewed published data, several institutions established the Guidelines to facilitate clinical management of patients with a suggestive personal or family history of breast and/or ovarian cancer, in particular individuals from a family with a known deleterious *BRCA1/2* mutation. Screening options include transvaginal ultrasonography (TVUS), and serum CA125, while prevention options include medical therapy with drugs and surgery such

as RRSO. The guidelines, summarized in Table 8, include age ranges for which these options should be begun and how often screening should take place. [53].

Management options	NCCN [62]	ACOG Committee on Genetics and the Society of Gynecologic Oncologists [63]	National Cancer Institute (NCI) [64]	ESMO [65]
<b>Surveillance</b>				
TVU+CA125	Every 6 months starting at age 30 years	Periodic screening beginning between the ages 30 years and 35years	Every 6 to 12 months, beginning at age 35 years	Not considered
<b>Surgery</b>				
RRSO	Between 35 and 40 years or upon completion of child bearing	By age 40 years or when childbearing is complete	Considered but age is not indicated	After age 35 and when childbearing decisions are complete
Bilateral salpingectomy	-	-	Considered but age is not indicated	-
<b>Chemoprevention</b>	Considered	Considered	Considered	Not considered
<b>Investigational imaging and screening studies</b>	Considered	Considered	Considered	Not considered

**Table 8.** Published Guidelines/Consensus Statements for the management of BRCA mutation carriers.

#### 4.4. Human Epididymis Protein 4 (HE4)

Additional potential serum biomarkers have been studied for the detection of ovarian cancer. For instance, human epididymis protein 4 (HE4) is a secreted glycoprotein over-expressed by serous and endometrioid ovarian cancers and expressed by 32% of ovarian cancers lacking CA125 expression.

To define the clinical utility of HE4, a comprehensive assessment of HE4 protein expression in benign and malignant ovarian and non-ovarian tissues by immunohistochemistry was performed and published in 2005. In comparison with normal surface epithelium, which does not express the protein, HE4 was widely found in cortical inclusion cysts lined by metaplastic Mullerian epithelium. These findings suggested that the formation of Mullerian epithelium is a prerequisite step in the development of some types of epithelial ovarian cancer. Moreover, the expression was restricted to certain histologic subtypes: 93% of serous and 100% of endometrioid epithelial ovarian cancers expressed HE4, while only 50% and 0% of clear cell carcinomas and mucinous tumours, respectively, were positive. HE4 protein expression is restricted in normal tissue to the reproductive tracts and respiratory epithelium. In fact, tissue microarrays revealed that the majority of non-ovarian carcinomas do not express HE4 [100].

In 2008 the Food and Drug Administration (FDA) approved HE4 to monitor disease recurrence and this marker was recently incorporated into the clinical evaluation of ovarian cancer

patients. Recently, Moore *et al.* published a series of papers that used a combination of CA125, HE4 and menopausal status to predict the presence of a malignant ovarian tumour and developed the Risk of Ovarian Malignancy Algorithm (ROMA), a simple biomarker based algorithm, which requires US [101, 102].

In the last few years, several multi-modal screenings of women at high risk, combining different approaches, were carried out to improve ovarian cancer diagnostic test performance [103,104]. In 2010, a prospective case-control study was designed to evaluate the independent contributions of HE4, CA125 and the Symptom Index (SI) to predict ovarian cancer status in a multivariate model [105]. The SI is a screening tool that evaluates specific symptoms in conjunction with their frequency and duration to identify women who are at risk of ovarian cancer [106]. The SI, HE4 and CA125 all made significant independent contributions to ovarian cancer prediction. A rule for the positive cut-off based on anyone of the three tests being positive had a sensitivity of 95% with specificity of 80%. A rule based on any two of the three tests being positive had a sensitivity of 84% with a specificity of 98.5%. The SI alone had sensitivity of 64% with specificity of 88%. If the SI index is used to select women for CA125 and HE4 testing, specificity is 98.5% and sensitivity is 58% using the 2-of-3-positive positive cut-off rule. A comparison between different markers in ovarian cancer early diagnosis is presented in Table 9.

SCREENING TESTS	DESCRIPTION
<b>Single Markers</b>	
CA125	CA125 was dichotomized at 95th percentile in the control group. Subjects with a marker value above that threshold were considered to be positive for CA125.
HE4	HE4 was dichotomized at 95th percentile in the control group. Subjects with a marker value above that threshold were considered to be positive for HE4.
Symptom Index (SI)	The SI was considered to be positive if the patient had at least one of the following symptoms for less than one year but more than 12 times per month: bloating or increased abdominal size, abdominal or pelvic pain, difficulty eating or feeling full quickly.
<b>Marker Combinations</b>	
CA125 or HE4	Screen considered positive if CA125, HE4 or both were positive.
SI or CA125	Screen considered positive if either the SI or CA125 was positive, or if both were positive.
SI or HE4	Screen considered positive if either the SI or HE4 was positive, or if both were positive.
Any 1 of 3 tests positive	Screen considered positive if any one of the SI or CA125 or HE4 was positive, or if two or more tests were positive.
Any 2 of 3 tests positive	Screen considered positive if both the SI and CA125 were positive, or if both the SI and HE4 were positive, or if both CA125 and HE4 were positive, or if all three tests were positive.
SI and at least 1 additional test positive	Classified as positive if SI was positive in addition to either a positive CA125 or a positive HE4, or if all three tests were positive.

**Table 9.** Description of screening tests and biomarker combinations.

#### 4.5. Proteomic profiling of ovarian cancer for biomarker discovery

Unfortunately, current diagnostic tools have had very limited success in early detection. The search for an ovarian cancer screening method with improved specificity and sensitivity has led to the examination of serum biomarker patterns using new 'omic' technologies [107-110]. In recent years, the advancing techniques for proteomics have accelerated the research for ovarian cancer biomarkers. Numerous proteomics-based molecular biomarkers/panels have been identified and hold great potential for diagnostic applications, but they need further development and validation.

Several studies have analysed the proteomic profiles of ovarian tumour tissue, cell lines, urine, ascites fluid and blood samples from ovarian cancer patients (Table 10) [111- 114].

AUTHORS	IDENTIFIED BIOMARKER	REGULATION IN CANCER
An et al.,(2006)[111]	NM23-H1	↑
	Annexin-1	↑
	Protein phosphatase-1	↑
	Ferritin light chain	↑
	Proteasome alpha-6	↑
	NAGK (N-acetyl glucosamine kinase)	↑
Petri et al., (2009) [112]	fibrinogen alpha fragment	↑
	collagen alpha 1 (III) fragment	↑
	fibrinogen beta NT fragment	↑
Li et al., (2009) [113]	prx-II	↓
	prx-III	↑
	hsp27	↑
	hsp60	↑
	mitochondrial short-chain enoyl-CoA hydratase	↑
Cortesi et al.,(2011) [114]	Prohibitin	↑
	Annexin-5 (ANXA5)	↓
	Phosphatidylethanolamine-binding protein 1 (PEBP)	↓
	glutathione S-transferase A2 (GSTA2)	↓
	galectin-3 (LEG3)	↓
	protein S100-A8-calgranulin A (S100A8)	↑
	retinol binding protein (RET1)	↓

**Table 10.** Promising biomarkers discovered by proteomic technology for ovarian cancer diagnosis.

An *et al.* [111] identified that different histologic subtypes of ovarian malignant epithelial tumours showed distinctly different protein expression profiles. The potential candidate biomarkers screened in ovarian tumours and found to be significantly up-regulated in comparison to normal tissues were: NM23, annexin-1, protein phosphatase-1, ferritin light chain, proteasome R-6, and NAGK (N-acetylglucosamine kinase). More recently, Petri *et al.* [112] examined whether urine could be used to measure specific ovarian cancer proteomic

profiles and whether one peak alone or in combination with CA125 or other peaks had the sensitivity and specificity to discriminate between ovarian cancer pelvic mass and benign pelvic mass. Twenty-one significantly different peaks ( $p < 0.001$ ) were examined and the three most significant peaks were identified as fibrinogen alpha fragment, collagen alpha 1 (III) fragment and fibrinogen beta NT fragment. These results supported the feasibility of using urine as a diagnostic tool and suggested the enhanced prediction performance of combined marker analysis. Li *et al.* [113] performed a comparative proteomic study of normal ovarian epithelial and ovarian epithelial serous cystadenocarcinoma tissue and identified six proteins significantly differentially expressed. In particular, Prx-II expression was found to be linearly decreased from normal ovarian tissue, to benign ovarian lesions, and ovarian malignancies. No statistical difference between carcinoma groups in different clinical stages, differentiation status, and histological type was seen, suggesting that the decreased level of Prx-II is a common marker for ovarian malignancies. This was the first report on the altered expression of Prx-II in ovarian cancer.

A recent comparative proteomic study investigated and defined protein expression patterns associated with advanced stage ovarian cancer, to define a panel of diagnostic and/or prognostic markers. The study also investigated proteins secreted by the cancer cell into the interstitial fluid, as cancer growth and progression also depends on stromal factors present in the tumour microenvironment. Moreover, many biomarkers present in biopsied cancer tissues can also be found in blood serum, representing potential biomarkers of the disease. Proteomic profiling of differentially expressed proteins in cancer ovarian tissue, tumoral interstitial fluid (TIF) and ascitic fluid, compared with healthy tissue samples and normal interstitial fluid (NIF), allowed the identification of protein spots consistently differentially expressed between normal and cancer samples. Protein expression/identification was evaluated by 2-DE (two-dimensional gel electrophoresis) and MS (mass spectrometry) analysis and was confirmed by immunohistochemistry. Six proteins showed differential expression in tumoral interstitial fluid and tumour tissue compared to normal interstitial fluid and healthy tissue. Differential protein expression between tumoral and normal ovarian tissue is presented in Table 11.

PROTEIN NAME	FOLD CHANGE TUMORAL VERSUS NORMAL TISSUE	P-value	FOLD CHANGE TIF VERSUS NIF	P-value
ANXA5	-1,88 ± -0,48	<0,0001	-5,605 ± -3,29	<0,01
PEBP	-4,21 ± -2,90	<0,01	-2,82 ± -0,69	<0,0001
GSTA2	-4,67 ± -1,88	<0,0001	-27,39 ± -21,24	<0,01
LEG3	-2,19 ± -0,69	<0,0001	-5,10 ± -4,42	<0,05
S100A8	3,67 ± 1,50	<0,001	3,58 ± 1,11	<0,0001
RET1	-6,33 ± -3,30	<0,001	-5,01 ± -4,28	<0,05

The fold change indicates the direction and the magnitude of the change in expression level. Data are expressed as mean ± standard deviation.

**Table 11.** Modification in protein expression in tumoral tissue and interstitial fluid

Five were found to be down-regulated and identified as galectin 3, glutathione S-transferase A-2, retinol binding protein 1, phosphatidylethanolamine-binding protein and annexin 5, while the calgranulin, was significantly up-regulated in all pathological samples, including the ascitic fluid. This is the first study to report an over-expression of calgranulin by 2-DE analysis combined with MS/MS on surgical biopsy. As previously reported, the reduced expression of galectin 3 and retinol binding protein 1 in cystic fluid and serum of patients with early stage disease is confirmed in this study. The results highlight alterations in proteins that control cell-cycle progression and apoptosis, as well as factors that modulate the activity of signal transduction pathways. Moreover, this study suggests that calgranulin expression may be used as a diagnostic and/or prognostic biomarker [114].

However, critical assessment of the results has shown significant shortcomings and uncertainties with regard to the reproducibility of the findings and identity of the proteins behind the peak patterns, thus, the validation of the newly discovered biomarkers still remains the most challenging aspect of clinical proteomics. The advancing techniques for proteomics have shown promise in a variety of studies and have provided new insights into ovarian cancer diagnosis, but few have turned out to be useful in the clinic. At present, the development of an effective strategy for early detection of ovarian cancer is still a work in progress [110].

## 5. Discussion

Primary and secondary prevention of ovarian cancer play a crucial role in the attempt to improve the overall survival from the disease. In particular, primary prevention is based on avoiding risk factors and increasing protective factors. Despite the identification of several risk and protective factors among the general population, most of the common factors described to date only slightly influence the risk of developing ovarian cancer, thus, the knowledge of these factors has still not been translated into practical strategies to prevent ovarian cancer.

On the other hand, primary prevention could represent a good opportunity for high-risk women. Women who inherit a mutation in either the *BRCA1* or *BRCA2* gene have greatly elevated lifetime risks of ovarian cancer, fallopian tube cancer and breast cancer. Surveillance for ovarian and fallopian tube cancer has not been proven to be effective. For this reason, preventive surgical removal of the ovaries and fallopian tubes (salpingo-oophorectomy) is actively recommended to these women by the age of 35 or 40 years, often prior to natural menopause, to prevent cancer. Moreover, women at increased risk may join a cancer prevention clinical trial or a chemoprevention trial. In particular, oral contraceptive use can be considered as an alternative strategy in the chemoprevention of ovarian cancer in *BRCA1* mutation carriers who do not accept RRSO above the age of 30 years. Other chemopreventive agents such as retinoids, analgesic drugs, vitamin D, cyclo-oxygenase inhibitors and peroxisome proliferator activated receptor-gamma ligands have shown promise in early investigations of disease prevention.

Regarding radiological methods to investigate ovaries and their adnexes, new techniques besides TVUS need to be explored. Pelvic Magnetic Radiological Imaging could be of interest

even if it is difficult to imagine such an expensive technique being employed in the screening of high-risk women. For high-risk women, recommended cancer screening strategies, which need to be adjusted depending on the earliest age of onset in a family, have not been assessed by randomized trials or case-control studies. Ovarian cancer screening relies on a combination of annual or semi-annual pelvic examination, annual or semi-annual transvaginal ultrasound examination with colour Doppler, and annual measurement of serum CA125 concentrations.

Current approaches are a futile attempt to detect ovarian cancer in the early stages, but future research should be directed to better characterizing critical pathways in ovarian carcinogenesis and to identifying appropriate surveillance programs based on biomarker tests and/or radiological investigations, in order to improve overall survival, which dramatically decreases in the first 5 years. Due to the fact that an analysis of potentially thousands of proteins which could be simultaneously altered is necessary, comparative proteomics is a promising mode of potential biomarker discovery for cancer detection and monitoring. A better estimation of the biological importance of certain proteins with regard to the progression from pre-neoplastic tissue alterations to malignant tumours, as well as the prediction of the metastasis-forming potential by biomarkers, will be a necessary prerequisite to provide a more detailed insight and understanding of tumour progression.

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