

Preventing Ovarian Cancer through early Excision of Tubes and late Ovarian Removal

(PROTECTOR): protocol for a prospective non-randomised multicentre trial

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ABSTRACT

Background

Risk-reducing-salpingo-oophorectomy is gold standard for preventing tubo-ovarian-cancer in women at increased risk. However when performed in premenopausal women, it results in premature-menopause and associated detrimental health consequences. This, along with acceptance of the central role of the fallopian-tube in etiopathogenesis of high-grade serous carcinoma, by far the most common type of tubo-ovarian-cancer, has led to risk-reducing early-salpingectomy with delayed-oophorectomy being proposed as a two-step surgical alternative for pre-menopausal women declining/delaying oophorectomy.

Primary-objective

To evaluate impact on sexual function of risk-reducing-early-salpingectomy, within a two-step risk-reducing early-salpingectomy with delayed-oophorectomy tubo-ovarian-cancer prevention strategy in premenopausal women at increased risk of tubo-ovarian-cancer.

Hypothesis

Risk-reducing-early-salpingectomy is non-inferior for sexual and endocrine function compared to controls; risk-reducing-early-salpingectomy is superior for sexual/endocrine function, non-inferior in terms of quality-of-life, and equivalent in satisfaction compared to the standard risk-reducing-salpingo-oophorectomy.

Trial-design

Multi-centre, observational cohort trial with three arms: risk-reducing-early-salpingectomy with delayed-oophorectomy; risk-reducing-salpingo-oophorectomy; controls (no surgery). Consenting individuals undergo an ultrasound, serum CA125 and FSH and provide information on medical history, family-history, quality-of-life, sexual function, cancer worry, psychological well-being and satisfaction/regret. Questionnaire follow-up takes place annually for three years. Risk-reducing

early-salpingectomy women can undergo delayed-oophorectomy at a later date of their choosing or definitely by menopause.

Major inclusion/exclusion criteria

Inclusion-criteria: Premenopausal; ≥ 30 years; at increased risk of tubo-ovarian-cancer (mutation carriers or on the basis of a strong family-history); completed their family (for surgical arms).

Exclusion-criteria: Postmenopausal; previous bilateral salpingectomy or bilateral oophorectomy; pregnancy; previous tubal/ovarian/peritoneal malignancy; <12 months post cancer treatment; clinical suspicion of tubal/ovarian cancer at baseline.

Primary endpoint

Sexual-function measured by validated questionnaires.

Sample size

1000 (333 per-arm).

Estimated dates for completing accrual and presenting results

It is estimated recruitment will be completed by 2023 and results published by 2027.

Trial registration

ISRCTN registry: 25173360 (<https://doi.org/10.1186/ISRCTN25173360>).

INTRODUCTION

BRCA1/BRCA2 mutation carriers have a 17%-44% lifetime risk of tubo-ovarian-cancer and a 69-72% lifetime risk of breast-cancer.¹ Primary surgical prevention in the form of risk-reducing-salpingo-oophorectomy is the most effective option and gold-standard for tubo-ovarian-cancer risk reduction, particularly given the absence of an effective national screening programme. Premenopausal risk-reducing-salpingo-oophorectomy leads to premature surgical menopause which has detrimental long-term health sequelae (increased risk of coronary-heart-disease, osteoporosis, vasomotor symptoms, sexual dysfunction, neurocognitive decline), especially if individuals are unable to use hormone-replacement-therapy.²⁻⁵ Widespread acceptance of a central role for the fallopian tube as the site of origin of most high-grade-serous-carcinomas, by far the most common and aggressive subtype of adnexal malignancy, from a precursor known as serous-tubal-intraepithelial-carcinoma has led to the attractive proposal of a two-step alternative tubo-ovarian-cancer surgical prevention strategy in premenopausal women who have completed their family but decline or wish to delay risk-reducing-salpingo-oophorectomy. This involves risk-reducing early-salpingectomy as the first-step followed by delayed-oophorectomy after menopause. Risk-reducing early-salpingectomy with delayed-oophorectomy has the advantage of providing some level of risk-reduction whilst conserving ovarian function and avoiding the negative health consequences of premature menopause. Lack of clarity on several key issues strengthens the case to offer risk-reducing-early-salpingectomy-with-delayed-oophorectomy solely within a research setting. The precise estimate of tubo-ovarian-cancer risk reduction and long-term health outcomes with risk-reducing-early-salpingectomy remain unclear. Salpingectomy will not prevent tubo-ovarian-cancer arising outside the tube. Residual fimbrial tissue may remain on the ovarian surface after salpingectomy in 9.8% of cases,⁶ and could be a potential site for malignant transformation which could also theoretically arise from tubal-type tissue within the ovarian stroma (endosalpingiosis/cortical inclusion-cysts). The etiopathogenesis of tubo-ovarian-cancer is complex and our current understanding incomplete. It has been suggested that there are different types of serous-tubal-intraepithelial-carcinoma and the

natural history, progression rates, outcomes and rate limiting step in development of tubo-ovarian-cancer associated with each type remains unknown.⁷ In addition, there are a proportion of high-grade-serous-carcinoma with histologically normal tubes (even after complete examination using a sectioning-and-extensively-examining-the-fimbriated-end protocol) and serous-tubal-intraepithelial-carcinoma may not be the precursor of all high-grade-serous-carcinoma.⁸ The long-term impact of salpingectomy on sexual-function, endocrine-function and onset of menopause is unknown. There are concerns from clinicians regarding attrition from delayed-oophorectomy and a proportion of patients who do not undergo delayed-oophorectomy may develop tubo-ovarian-cancer. In addition, uncertainties remain around cost-effectiveness.

We present the protocol for the 'Preventing Ovarian Cancer through early Excision of Tubes and late Ovarian Removal' (PROTECTOR) Trial which evaluates risk-reducing-early-salpingectomy-with-delayed-oophorectomy in UK-women who are at increased risk of tubo-ovarian-cancer. The full PROTECTOR protocol can be found at <http://protector.org.uk/> (ISRCTN25173360). Our hypotheses are: 1) risk-reducing-early-salpingectomy is non-inferior for sexual and endocrine function compared to controls and 2) risk-reducing-early-salpingectomy is superior for sexual/endocrine function, non-inferior in terms of quality-of-life, and equivalent in satisfaction compared to the standard risk-reducing-salpingo-oophorectomy.

METHODS

Trial-design

Multi-centre, observational cohort trial with three-arms: risk-reducing-early-salpingectomy-with-delayed-oophorectomy; risk-reducing-salpingo-oophorectomy; controls (no surgery). Eligible individuals self-select which of the three-arms they wish to opt for. Randomisation to a control-arm

involving no surgery is unethical in high-risk women as is randomisation to a risk-reducing-early-salpingectomy-with-delayed-oophorectomy arm given the lack of clarity on tubo-ovarian-cancer risk reduction. Furthermore, randomisation is unacceptable to women and reported to be a barrier to participation in a similar clinical-trial amongst *BRCA1/BRCA2*-carriers and gynaecological-oncologists/geneticists.⁹

A pragmatic way forward is a prospective observational cohort study based on a standardized nationally acceptable protocol, with a well-designed patient information-sheet (highlighting advantages and limitations) and comprehensive evaluation of short and long-term outcomes. This is a UK-wide study with 41 sites planned (30 currently active and 11 in set up).

Funding: The study is funded by Barts Charity and Rosetrees-Trust.

Participants

Inclusion-criteria include premenopausal women ≥ 30 years who have completed their family (surgical-arms), and are at increased risk of tubo-ovarian-cancer. Women may be at increased risk of tubo-ovarian cancer if they carry a pathogenic or likely pathogenic mutation in the *BRCA1/BRCA2/RAD51C/RAD51D/BRIP1* gene or based on a strong family history of tubo-ovarian cancer (*BRCA*-negative or *BRCA*-unknown with ≥ 2 first-degree-relatives with tubo-ovarian-cancer or ≥ 3 relatives with tubo-ovarian-cancer (affected relatives must be on the same (maternal/paternal) side of the family)).

Exclusion-criteria include, postmenopausal women (FSH >40), women who have undergone previous bilateral-salpingectomy/bilateral-oophorectomy, those with clinical suspicion of tubo-ovarian cancer at baseline, women with a history of tubo-ovarian/peritoneal malignancy, women <12 months post cancer treatment, pregnancy, or those unable to provide informed consent.

Recruitment is undertaken through cancer-genetics, high-risk familial cancer, gynaecological-oncology and general gynaecology outpatient clinics within NHS hospitals and primary-care.

Objectives

The primary objective is to evaluate impact on sexual-function of early-salpingectomy within risk-reducing-early-salpingectomy-with-delayed-oophorectomy, as a two-step tubo-ovarian-cancer prevention strategy in premenopausal women at increased risk of tubo-ovarian-cancer.

Secondary objectives are to evaluate impact of risk-reducing-early-salpingectomy-with-delayed-oophorectomy on endocrine-function; quality-of-life; health and well-being; psychological-health; satisfaction/regret; cancer-risk perception; incidence of tubal in-situ and invasive tubo-ovarian-cancer; surgical-outcomes; to develop utility-scores for early-salpingectomy and determine cost-effectiveness; and establish a national register to facilitate long-term follow-up of women undergoing risk-reducing-early-salpingectomy.

Endpoints

The primary-endpoint is sexual function (measured by the Sexual-Activity-Questionnaire ¹⁰ and Sexual Quality-of-Life 3D questionnaire).¹¹ Secondary-endpoints include (but are not limited to): quality-of-life and psychological-health, incidence of tubal-in situ and invasive tubo-ovarian-cancer, surgical-morbidity and cost-effectiveness.

Interventions

Figure-1 summarises interventions and relevant time-points.

Baseline Screening Tests

A baseline hormonal profile (FSH) is measured for all. Serum CA125 and ultrasound-scans are done for risk-reducing-salpingo-oophorectomy/risk-reducing-early-salpingectomy-with-delayed-oophorectomy arms only.

Interventional-Questionnaires

Questionnaires used in the study have been derived from validated-questionnaires. Questionnaires include the Sexual-Activity-Questionnaire; Sexual-Quality-of-Life 3D questionnaire; endocrine-subscale of the Functional-Assessment of Cancer-Therapy-Endocrine-Symptom questionnaire; EQ5D-5L questionnaire; Hospital-Anxiety-&-Depression Scale; Impact-of-Events-Scale; Decision-Regret-Scale and 1-item from Madalinska 2005, ('I am satisfied with the decision I have made' on a 5-point Likert-scale); cancer-risk perception is assessed with 'Compared with other people of your age and sex, do you think your chances of getting cancer at some point in your life are: much-lower, lower, about-the-same, higher, much-higher?'. An additional risk-item used is 'On a scale from 0-to-100, where 0=no chance at all and 100=absolutely certain, what do you think are the chances that you will get cancer sometime during your lifetime?'. Tubo-ovarian-cancer worry is assessed by a 4-item four-point Likert-scale.

Interviews

A small number of women from each study-arm are invited to one-to-one semi-structured in-depth interviews. Women who elect to have surgery will be followed up with another interview one-year post surgery (risk-reducing-early-salpingectomy and risk-reducing-salpingo-oophorectomy).

Risk-Reducing Surgeries

Volunteers opting for the risk-reducing-salpingo-oophorectomy/risk-reducing-early-salpingectomy-with-delayed-oophorectomy arms undergo bilateral salpingo-oophorectomy or early-salpingectomy with delayed-oophorectomy as per our surgical protocol (Supplementary-1). Timing of delayed-

oophorectomy in the risk-reducing-early-salpingectomy-with-delayed-oophorectomy arm is non-prescriptive and dependent on the wishes of the individual. However, participants will be advised to undergo delayed-oophorectomy once they become post-menopausal. Further counselling will be arranged if women are postmenopausal, and are not complaint with delayed-oophorectomy. Whilst minimal-access surgery is the preferred route, it is not mandatory and the choice of surgical route will be made by the treating clinician. Peritoneal washings are taken during all risk-reducing surgeries and sent for cytology. Participants who are in the control arm are also subsequently (in a few years' time) free to switch to any one of the surgical intervention arms. If any control arm women are using CHC (combined hormonal contraception) at age 50 years, they will be advised to stop the CHC, switch to non-hormonal contraception and have a repeat FSH in 3 months.

Pathological Examination and Central Pathology Review

All ovaries and fallopian tubes are submitted in their entirety for histological examination and the tubes are processed using a sectioning-and-extensively-examining-the-fimbriated-end protocol (Supplementary-2). In addition to local histopathology reporting at recruitment sites, all tubal and ovarian histopathology slides and cytology slides from peritoneal washings are reviewed by an independent team of central specialist gynaecological pathologists. Blocks from consenting participants are stored in a bio-resource facility for future translational work.

Management of abnormal histopathology/cytology results

Invasive disease is managed as per local clinical protocols. Participants will be referred to their regional cancer-centre gynaecological-oncology multidisciplinary-team for further investigations, staging and management. Table-1 summarises the management of serous-tubal-intraepithelial-carcinoma lesions. Investigation and treatment outcome data will be obtained by the co-ordinating centre from the treating clinician/regional cancer-centre.

Follow-up:

Participants are followed up actively at 1 month, 3 months (post-surgery in the surgical arms) and annually for three-years (all arms). Patients are followed up directly by the central coordinating centre trials unit as well as the local clinical site. Follow-up compliance will be ensured by direct contact with the patient as well as good communication and liaison with the local site clinical team and the patient's general practitioner. Long-term passive annual follow-up is planned through establishment of a national registry/database as well as linkage via cancer registries, or databases such as Office-for-National-Statistics, Hospital-Episode-Statistics or NHS-Digital.

Data-collection:

Data-collection is standardised and is collected on electronic case-report-forms hosted on a customised study database via a web interface. Figure-1 summarises data-collection time-points. on electronic case-report-forms will be identified by a unique alphanumeric volunteer-reference-number auto generated by the database each time a new participant is enrolled into the trial. The database will enable participant flagging/tracking and electronic data upload/access.

We aim to use the NHS number as the primary identifier when linking to national registries and to track individuals throughout the NHS.

Sample-size:

Sample-size is based on the primary-outcome of Sexual Function, assessed by the Sexual-Activity-Questionnaire. Sample-size is estimated for 90% power and with either $\alpha=0.05$ two-sided (for superiority tests) or $\alpha=0.025$ one-sided (for non-inferiority tests). For a non-inferiority margin (Δ)=0.9 on the Sexual Activity Questionnaire pleasure-scale, between risk-reducing-early-salpingectomy and Controls, the sample-size needed is 266/arm. For testing superiority of risk-reducing-early-salpingectomy compared to risk-reducing-salpingo-oophorectomy, to achieve a mean difference=1 on the Sexual-Assessment-Questionnaire pleasure-scale (SD=3.2-3.5) the sample size needed is 237/ arm.

However, these calculations assume random allocation to the arms. Because participants self-select their arm (non-randomised), it will be necessary to adjust all tests for potential confounders that might relate to both arm and outcome. Assuming inclusion of confounders into a regression model reduces the partial r-squared brought about by treatment arm by 20%, then the necessary sample-size increase to maintain power is by 25%. For our primary-hypotheses, the largest sample-size needed is therefore $266 \times 1.25 = 333$ per-arm, resulting in an overall sample-size of approximately 1000 patients allocated equally between each-arm.

Statistical methods

Baseline characteristics will be calculated using descriptive statistics. Appropriate statistical tests will be used for analyses. Chi-square tests will compare categorical variables and t-Test (parametric) and Mann-Whitney (nonparametric) tests will compare continuous outcome variables between groups. Random-effects-models adjusted for covariates/confounders (including age, family-history, pathogenic-variant type, parity, contraception, body-mass index, subfertility, etc.) will be used to compare outcomes between the different groups over time. Non-inferiority is established when the 97.5% CI does not cross the non-inferiority margin. A two-sided 95% CI will be used to test equivalency of satisfaction. The different non-inferiority/equivalency margins for various outcomes are based on clinically meaningful changes where available or set at no more than 0.5SD worse than values from prior studies.

Utility-scores: Index-values from the Sexual Quality-of-Life-3D questionnaire will be used to generate utility-values for salpingectomy. Utility-values generated will be used to calculate Quality-Adjusted-Life-Years which will be used in an economic-evaluation

Cost-effectiveness: A Markov-model will be developed for cost-effectiveness of risk-reducing early-salpingectomy-with-delayed-oophorectomy. A lifetime horizon will be used to capture all costs-&

benefits and the analysis will be conducted using a healthcare perspective. A 3.5% discount rate will be applied to costs-&-outcomes. Both deterministic and probabilistic sensitivity-analyses will be performed. The incremental-cost-effectiveness-ratio will be calculated and compared with the National-Institute-for-Health-and-Care-Excellence cost-effectiveness willingness-to-pay threshold to determine cost-effectiveness of risk-reducing early-salpingectomy-with-delayed-oophorectomy.

DISCUSSION

This trial protocol describes a prospective non-randomised multicentre UK cohort-trial evaluating the impact of risk-reducing-early-salpingectomy-with-delayed-oophorectomy in pre-menopausal women, at increased risk of tubo-ovarian-cancer. PROTECTOR ensures that an early-salpingectomy tubo-ovarian-cancer prevention strategy can be offered to high-risk UK-women who choose to decline/delay oophorectomy, within a safe clinical-study setting, with strict protocols, proper consent, monitoring and independent oversight. Risk-reducing-early-salpingectomy permits women to retain their natural hormones for longer and limit harmful consequences of premature menopause. Risk-reducing-early-salpingectomy also enables women who have completed childbearing but too young for oophorectomy by current clinical guidelines, the option of undergoing risk-reducing surgery. The trial will provide long-term outcome data to address knowledge-gaps which currently exist including impact of risk-reducing-early-salpingectomy on sexual/endocrine function, quality-of-life, psychosocial consequences, utility-scores and cost-effectiveness. Data will be collected on attrition from delayed-oophorectomy and interval cancers. This study will generate new insights to inform provision of NHS care and tubo-ovarian-cancer prevention guidelines in women at increased tubo-ovarian-cancer risk. The bio-resource generated will facilitate translational research and secondary-studies to provide further insights into disease biology.

Currently, there are four other non-randomised trials investigating different aspects of risk-reducing-early-salpingectomy-with-delayed-oophorectomy being undertaken in France (Fimbriectomy trial),¹² the Netherlands (TUBA- Tubectomy with delayed oophorectomy to improve quality-of-life as alternative for risk-reducing salpingo-oophorectomy in *BRCA1/2* mutation-carriers),¹³ and US (PSDO– Prophylactic-Salpingectomy with Delayed Oophorectomy;¹⁴ WISP- Women Choosing Surgical Prevention Trial).¹⁵ The ongoing trials vary with respect to primary outcomes, design and sample sizes. The Fimbriectomy trial is powered on tubo-ovarian/primary-peritoneal cancer incidence, while the others are powered on menopause related quality-of-life (TUBA), delayed-oophorectomy uptake (PSDO) and sexual-function (WISP). The Fimbriectomy trial does not involve delayed-oophorectomy. delayed-oophorectomy is undertaken in the TUBA trial at 40-45 years in *BRCA1* and 45-50 years in *BRCA2* carriers and in the PSDO trial three years after risk-reducing-early-salpingectomy. Delayed-oophorectomy is undertaken in premenopausal women well before onset of menopause in the TUBA and WISP studies. Similarly, in the WISP study, women are given the choice as to when to undergo delayed-oophorectomy but are encouraged to have this done between 40-50 years. While the TUBA and the PSDO trials only include *BRCA* carriers, the Fimbriectomy trial also includes women ascertained using family history. The WISP trial in addition offers risk-reducing-early-salpingectomy-with-delayed-oophorectomy to *PALB2/BARD1/MSH2/MSH6/MLH1/PMS2/EPCAM* mutation carriers. However, validated data linking *BARD1/EPCAM/PMS2* mutations with increased tubo-ovarian-cancer risk are currently lacking. In addition, mutations in the Lynch syndrome genes are not thought to be associated with an increased risk of high-grade-serous-carcinoma but with ovarian endometriosis-related neoplasms, such as endometrioid/clear-cell carcinoma which typically present at earlier-stages with a better prognosis.

In conclusion risk-reducing-salpingo-oophorectomy remains gold-standard for preventing tubo-ovarian-cancer in women at high-risk women. However, when performed in premenopausal women,

it increases risk of coronary-heart-disease, osteoporosis, neurocognitive-decline, vasomotor-symptoms and sexual-dysfunction. Use of hormone-replacement-therapy until natural menopause mitigates risks and there is data supporting safety of short-term hormone-replacement-therapy use in *BRCA*-carriers without a personal history of breast-cancer. Acceptance of the central role of the fallopian-tube in tubo-ovarian-cancer etiopathogenesis of together with health consequences of premature menopause from early-oophorectomy has led to risk-reducing early-salpingectomy-with-delayed-oophorectomy being proposed as a two-step surgical alternative for pre-menopausal women who have completed childbearing but prefer to decline/delay oophorectomy. It is essential that this is robustly evaluated in clinical trials to address various knowledge-gaps and inform future practice. PROTECTOR, TUBA and WISP are three trials offering risk-reducing early-salpingectomy-with-delayed-oophorectomy which are currently open to recruitment. These studies will generate important data and provide an evidence base to inform future international practice with respect to risk-reducing-early-salpingectomy-with-delayed-oophorectomy in high-risk women. International collaboration is warranted to pool outcome data from these studies to better understand the benefits and safety profile (including reduction in tubo-ovarian cancer risk) with risk-reducing-early-salpingectomy with delayed-oophorectomy and inform policy and management guidelines in the future.

Contribution to authorship

Trial conception and design: RM

Protocol development: RM, UM, GE, NS, FG, MB, RL, WGM, RG, NW, GB, GR

Pathology committee: NS, WGM, NW, RG, GB, GR, RA, RM, FG

Trial management: RM, FG, SR, CT, NS, UM, GE, ES, HH

Preparation of tables and figures: FG, RM

Initial draft of manuscript: FG, RM

Statistical aspects: MB, RM, RL

Manuscript writing and approval: All authors.

Disclaimers/Conflict of interest statement

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Figure-1: PROTECTOR Trial Flowchart

FU – follow up; USS – ultrasound scan; RRSO – risk reducing salpingo-oophorectomy; RRESDO – risk reducing early salpingectomy and delayed oophorectomy; ES – early salpingectomy; DO – delayed oophorectomy; PIS – participant information sheet; FSH – follicular stimulating hormone

Table-1: Management and follow up of STIC lesions (without invasion)

Management	Histopathology and Cytology		
	STIC* with positive cytology	STIC* with negative cytology	STIC* with missing cytology
Staging CT Chest, abdomen, pelvis	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Surgical staging**	<input checked="" type="checkbox"/>	Not indicated unless abnormality on CT suggesting otherwise	Not indicated unless abnormality on CT suggesting otherwise
Panel genetic testing***	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

*All cases of isolated STIC identified at salpingectomy alone (patients undergoing early salpingectomy) should have completion oophorectomy

**Hysterectomy, omentectomy, pelvic/para-aortic lymphadenectomy (excision of all visible disease)

***BRCA1/BRCA2/RAD51C/RAD51D/BRIP1 testing if not previously undertaken

Supplementary material-1: Surgical Protocol for Risk Reducing Surgery for Ovarian Cancer Prevention in Women at Increased Risk

1. While a minimal access (laparoscopic/robotic) approach is preferable, this is not mandatory for the study.
2. Inspection of abdomino-pelvic cavity is required and this is best done laparoscopically.
3. Peritoneal washings for cytology are mandatory as malignant cells have also been reported – even in the absence of an identifiable ovarian/tubal cancer.
4. In women having a vaginal hysterectomy for benign pathology, the ovaries, tubes and the pelvic cavity should be inspected laparoscopically at the start of the procedure.
5. If there are adhesions between the adnexa and adjacent structures, careful dissection should be performed to ensure complete removal of the ovaries and fallopian tubes.
6. Peritoneal/ Omental biopsies should be taken if indicated.
7. Routine curettage of the uterus is less evidence based, and is advisable in all women on Tamoxifen.
 - 1.1 Hysterectomy is not usually advocated unless there are other indications. If the woman is taking Tamoxifen it is important to ensure that the risk of endometrial cancer has been discussed as the woman may wish to opt for a hysterectomy, although the standard recommendation is that this is not required.
8. Damage to the fimbrio-ampullary end of the tubes by electrosurgery or crushing should be avoided as this can cause artefacts which make diagnosing occult cancer/carcinoma insitu and dysplasia difficult. Monopolar diathermy should be avoided. Use of low power settings and short application times is advisable to reduce inadvertent thermal tissue injury.
9. In women undergoing risk reducing salpingo-oophorectomy or delayed oophorectomy, the infundibulopelvic ligament should ideally be ligated 2cm from the ovarian hilum to reduce the risk of remnant ovarian syndrome.
10. It is important to avoid fragmentation or morcellation of the specimen as this makes systematic histopathological evaluation difficult.
11. Right and left sided specimens should be sent in separate containers/pots. In case of multiple fragments, care should be taken to avoid mixing of right and left sided fragments.

Supplementary material-2: Protocol for Histopathological processing

Both tubes/ovaries are entirely embedded and microscopically examined following serial transverse sectioning at 2 mm intervals.

Ovaries:

1. After standard recording of size and macroscopic appearance, each ovary should be serially sectioned transversely at 2 mm intervals perpendicular to longest axis and processed in toto. If the ovary is enlarged by 'benign disease', sampling should follow recommended protocols of a minimum of one section per centimetre of maximum diameter.

Fallopian tubes:

2. The overall length (including the fimbrial end) and macroscopic appearance of each fallopian tube should be stated.
3. The distal 15 mm (approximately), including the infundibulum and fimbrial end of the tube, is sliced *longitudinally* at 2 mm intervals to maximise exposure and histological examination of the tubal epithelium in this region. If possible this should be processed in 1-2 cassettes to reduce laboratory effort if serial sectioning and/or immunohistochemistry are required; however the cassette(s) must not be overcrowded.
4. The proximal portions of the tube, i.e. isthmus and ampulla, are *transversely* sliced in serial sections at 2 mm intervals. The mid and proximal portions can be processed in separate cassettes with multiple slices in one cassette, avoiding overcrowding.
5. Overall the entire tube can be sampled in 3 or 4 cassettes including any mesosalpinx.
6. Immunohistochemistry (IHC) is only required if there are atypical or abnormal H+E findings and the appropriate block should be selected. All IHC findings must be reported. In general, if STIC or any other atypical tubal mucosal lesion is identified, immunohistochemistry for p53 and the proliferation marker Ki67/ MIB1 should be performed. Other markers, for example WT1, can be performed at the pathologist's discretion.
7. A single level of each tissue block is sufficient, although additional levels can be undertaken at the discretion of the pathologist.
8. When reporting an invasive tumour of STIC, it must be made clear whether lesions are unifocal or multifocal. The largest diameter of STIC/invasion must be reported.
9. Lesions less than STIC may also be reported. However, if reported, the pathologist must state on the issued report that these lesions have no clinical significance.

Peritoneal /Omental biopsies:

10. If submitted, these should be processed in their entirety.

Peritoneal Washings:

11. Cytological examination of fluid obtained after instillation of normal saline into the peritoneal cavity.

Criteria for diagnosis of 'Serous Tubal Intraepithelial Carcinoma' (STIC), Serous tubal intraepithelial lesion (STIL), p53 signature

1. **Serous tubal intraepithelial carcinoma (STIC).** The histologic diagnosis of STIC is based on a combination of features, including variably stratified epithelium with increased nuclear to cytoplasmic ratio, nuclear enlargement, prominent nucleoli, loss of cell polarity, mitotic activity and loss of cilia. Immunohistochemically, these areas exhibit abnormal mutation-type staining with p53 (either diffuse intense nuclear positivity in more than 80% of the lesional cells or completely negative staining in all lesional cells) and expression of the proliferation marker Ki67/ MIB1 in greater than 10% of the cells. STIC most commonly involves the fimbria but may also have a non-fimbrial location.
2. **Serous tubal intraepithelial lesion (STIL).** Lesions less than STIC may also be reported. However, if reported, the pathologist should state on the report that these lesions have no established clinical significance. The preferred designation for these lesions is STIL. This diagnosis should be made sparingly and the criteria are variable but include cases where
 - the morphological features are in keeping with STIC but p53 staining is wild-type and/ or Ki67/ MIB1 is less than 10%,
 - cases where the morphology is suspicious of STIC but p53 staining is wild-type or Ki67/ MIB1 is less than 10%,
 - cases where the morphology is atypical but not considered suspicious of STIC but p53 exhibits mutation-type staining and Ki67/ MIB1 is greater than 10%.
3. **p53 signatures.** These are stretches of morphologically normal non-ciliated/ secretory tubal epithelium exhibiting mutation-type staining with p53. They will only be picked up if p53 staining is undertaken. They are commonly seen in the fallopian tubes and are of no clinical significance. They should not be reported.

Supplementary material-3: PROTECTOR collaborators and participating centres

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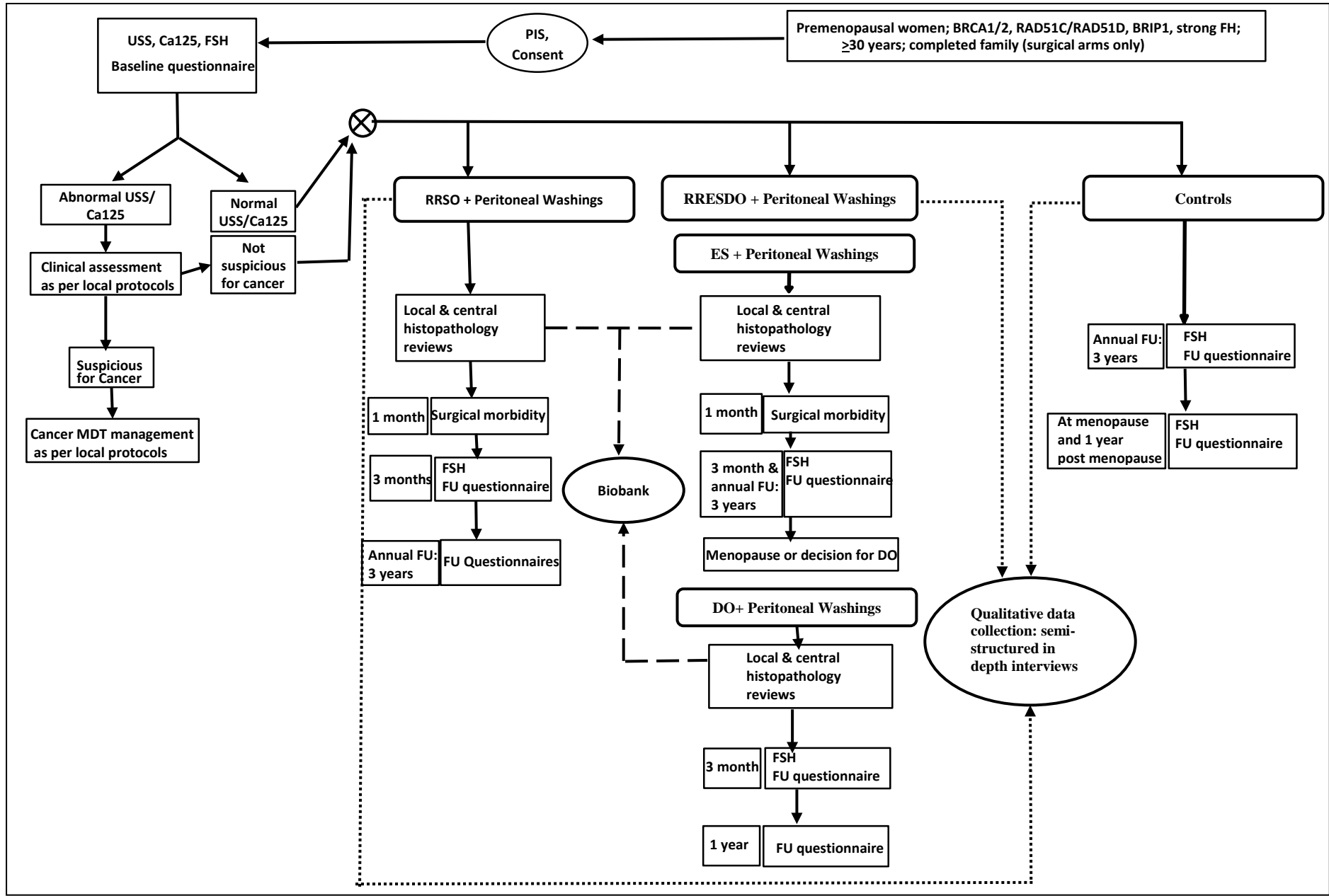
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Figure-1: PROTECTOR Trial Flowchart



FU – follow up; USS – ultrasound scan; RRSO – risk reducing salpingo-oophorectomy; RRESDO – risk reducing early salpingectomy and delayed oophorectomy; ES – early salpingectomy; DO – delayed oophorectomy; PIS – participant information sheet; FSH – follicular stimulating hormone