PROTOCOL

Full/long title of the project

Characterisation of the STEroid Metabolome in mucinous OVArian cancer.

Short title/acronym

STEMOVA

Protocol version number and date

Protocol version number:	1.0
Protocol version date:	28th February 2025

Research reference numbers

IRAS number:	338830
Sponsor/RG number:	RG_24-085
REC reference number:	25/YH/0085
Public registry number:	
Funder number:	EVE0038 / BWHGRP004

This protocol has regard for the HRA guidance.

Signature page

The undersigned confirm that the following protocol has been agreed and accepted, and that the CI agrees to adhere to the signed University of Birmingham's sponsorship CI declaration.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the project will be given; and that any discrepancies from the project as planned in this protocol will be explained.

Full project title:	Characterisation of the steroid metabolome in mucinous ovarian cancer.
Protocol version number:	1.0
Protocol version date:	28th February 2025

Chief Investigator (CI)	
Name:	Prof Sudha Sundar
Date:	28th February 2025
Signature:	

Sponsor statement

Where the University of Birmingham takes on the sponsor role for protocol development oversight, the signing of the IRAS form by the sponsor will serve as confirmation of approval of this protocol.

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Key contacts

Role/function	Contact details
Chief Investigator	Prof Sudha Sundar Professor of Gynaecological Cancer Institute of Cancer and Genomic Sciences University of Birmingham Vincent Drive B15 2TT s.s.sundar@bham.ac.uk 0121 414 44021
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Project summary

Dysregulation in steroid hormones, the immune system and gene expression have been implicated in ovarian cancer progression, but their true role and underlying mechanisms remain to be elucidated. We plan to use gas chromatography – mass spectrometry (GC-MS) and other technologies to assess biomarkers that can identify early cancer from healthy and benign disease. To do so we will recruit participants with ovarian tumours and obtain urine samples from them. We will analyse a subset of recruits with confirmed mucinous histology type as well as a set of recruits with other histology types of ovarian tumours for novel markers. Previous work by Dr David Jeevan has already identified some markers that we will test, we will also attempt to identify other novel markers.

We will also investigate if these markers can identify patients with early recurrence of mucinous cancer.

To do this, participants who have undergone surgery or biopsy and confirmed as any stage mucinous ovarian cancer will be recruited to donate serial urine samples during the first two years after diagnosis.

Participants with mucinous appendiceal pseudomyxoma peritonei cancer will also be recruited to donate urine samples. This third population will determine if urinary steroid analysis can differentiate between primary mucinous ovarian cancer and mucinous appendiceal pseudomyxoma peritonei cancer – this is a common clinical conundrum and has implications for treatment and outcomes.

Study Title	Characterisation of the steroid metabolome in mucinous ovarian cancer.
Internal ref. no. (or short title)	STEMOVA
Study Design	Prospective study limited to working with human tissue samples and data.
Study Participants	The study population will involve participants with confirmed or suspected mucinous ovarian cancer prior to and following surgery/biopsy. Participants with diagnoses of mucinous appendiceal pseudomyxoma peritonei cancer will also be invited to participate.
Planned Size of Sample (if applicable)	340 i. Recruiting participants at first surgery or biopsy - Diagnostic sub-study: 265 ii. Recruiting participants with a confirmed diagnosis of mucinous cancer at follow-up sub-study: 50 iii. Recruiting participants with a suspected or confirmed diagnosis of appendiceal/ pseudomyxoma peritonei cancer sub-study: 25
Follow up duration (if applicable)	Two years
Planned Study Period	01 June 2025 to 30 June 2029
Research Question/Aim(s)	We aim to characterise the steroid metabolome in mucinous ovarian cancer to investigate the hypothesis that the steroid pathway is involved in the aetiopathogenesis of mucinous ovarian cancers. Primary Objectives: 1) Investigate the urine steroid metabolome in patients with mucinous carcinomas, mucinous borderline tumours, and benign ovarian tumours and other histology types of ovarian tumours and appendiceal pseudomyxoma peritonei tumours. 2) Investigate urine changes in steroids and other biomarkers in appendiceal/pseudomyxoma peritonei tumours.

Funding and support in kind

Funder(s)	**Financial and non-financial support given**
Birmingham Women's Hospital Charity	£99,951
The Eve Appeal	£87,261.50

Role of sponsor and funder

The sponsor of this study, the University of Birmingham, assumes overall responsibility for the initiation and management of the project but has no direct role in the study design, conduct, data analysis, interpretation of results, manuscript writing, or dissemination of findings.

Roles & responsibilities of management committees/groups & individuals

There are no study management committees or groups involved in this study.

Patient & public involvement group

The CI has presented the planned research to a focused patient group of three individuals with Mucinous Ovarian Cancer (MOC) and to a larger audience at Eve Appeal cancer charity events. The study received enthusiastic support, as patients expressed concerns about the lack of research on this rare cancer. They welcomed the exploration of novel biomarkers to improve the accurate identification of women with MOC, given the limited evidence available to guide diagnosis and treatment.

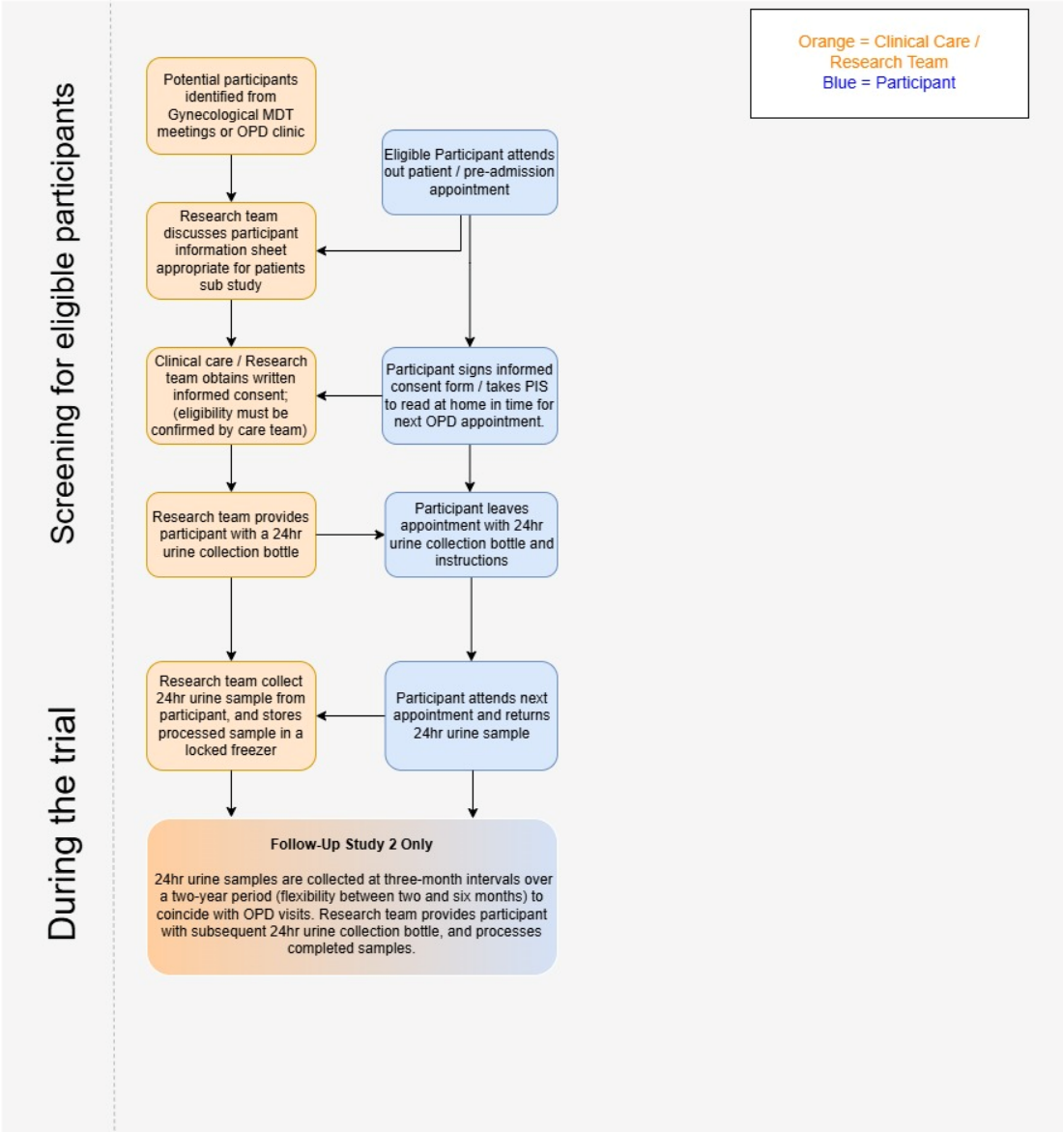
Protocol contributors

The protocol contributors are: Mr Alex St. John (Study Coordinator, University of Birmingham), Professor Sudha Sundar (Chief Investigator, Professor of Gynaecological Cancer and Consultant Gynaecological Oncology Surgeon for Pan Birmingham Gynaecology Cancer Centre), and Dr Tejumola Olaoye (Clinical Research Fellow, University of Birmingham).

Key words

Ovarian cancer, biomarkers, diagnosis, metabolomics, genomics, immunotherapy, appendiceal and pseudomyxoma peritonei cancer.

Project flow chart



Protocol

Characterisation of the steroid metabolome in mucinous ovarian cancer.

1. Background

Ovarian cancer is the most lethal gynaecological cancer. 1 in 52 women will be diagnosed with the disease in their lifetime and survival from the disease is highly dependent on the stage at diagnosis [1]. At stage 1, 90% of women will survive their cancer for 5 years or more after diagnosis. At stage 3 this figure drops to 30% and then to just 15% at stage 4 [2,3]. Due to the non-specific nature of symptoms (bloating, abdominal discomfort) most affected women (70%) are diagnosed at advanced stage 3 or 4. The current blood test CA-125 is poor and only identifies 50% of Stage 1 ovarian cancers [4]. Therefore, novel diagnostic tools to detect ovarian cancer are urgently needed. Consequently, there is an urgent need for innovative diagnostic tools.

A rare type of ovarian cancer is mucinous ovarian cancer which is diagnosed in approximately 3% of women with ovarian cancer overall, the proportion rises to approximately 9 – 10% in women who undergo surgery initially for ovarian cancer [5]. Mucinous tumours are more common in a younger population (median range, 36-50 years) than the more common types of invasive ovarian cancer [4]. The key symptoms are due to an expanding pelvic mass, which is often very large, averaging 16 to 19 cm in diameter, which may provide an initial diagnostic clue [6]. This study seeks to characterise the steroid metabolome and other biomarkers in mucinous ovarian cancer (MOC) and mucinous ovarian tumours to investigate the hypothesis that the steroid pathway is involved in the aetiopathogenesis of mucinous ovarian cancers.

Although mucinous ovarian cancer is frequently diagnosed at early stages with excellent prognosis (90% five-year survival), when diagnosed late prognosis is extremely poor (median survival of 12-33 months). Treatment options are extremely limited for women with advanced stage or recurrent cancer; this histology type does not respond well to platinum-based chemotherapy. Currently no MOC-specific biomarkers exist and CA125 has very limited accuracy for use in mucinous cancer diagnosis or to identify recurrence.

The urinary steroid metabolome in ovarian cancer was previously investigated as a novel diagnostic marker [7]. This work built on successful research in steroid profiling to discriminate adrenal benign tumours from adrenal cancer for diagnosis and monitoring established by Prof W Arlt at University of Birmingham [8,9]. The ovaries and adrenal glands arise from the same urogenital ridge at embryogenesis and therefore findings from adrenal cancer could also have relevance for ovarian cancer [10]. A multi-centre observational study was conducted to recruit both healthy women and women undergoing surgery or biopsy for suspected or confirmed ovarian cancer from twelve Gynaecology cancer units and centres across NHS England between January 2018 and March 2020. Urinary steroid metabolite excretion was analysed from 24-hour urine collections for 32 steroid metabolites using GC-MS [11].

Data were analysed across healthy controls, benign ovarian tumours, Type 1 cancer (endometrioid, clear cell, mucinous) and Type 2 cancer (high-grade serous, carcinosarcomas). Borderline tumours (BOT) were grouped with either Type 1 or Type 2 cancers based on mucinous or serous histology. There is a strong rationale to combine mucinous BOTs with MOC given their precursor relationship supported by genomic and morphological similarities [12]. A model of progression has recently been generated from benign to mucinous BOTs to localised low-grade MOC and progressively through to high-grade and/or metastatic MOC [12].

Results showed that five urinary steroid metabolites: 5- Pregnanediol (5-PD), Pregnanediol (PD), 5-Pregnanetriol (5-PT), Pregnanetriol (PT) and 17-Hydroxyprogesterone (17-HP) can differentiate women with Type 1 from Type 2 ovarian cancers, benign ovarian tumours, and healthy ovaries. Of the Type 1 group, distinct steroid metabolite differences were seen only in patients with mucinous histology but not in other histology types. The five steroid metabolites, 5-PT, 5-PD, PD, 17-HP and PT were all consistently elevated in mucinous BOTs and mucinous carcinomas with median excretion values elevated above the 95th centile of normal in either the mucinous BOTs or mucinous carcinomas or both. Importantly, there were incremental increases in some of these metabolites from healthy to benign to borderline to mucinous ovarian cancer.

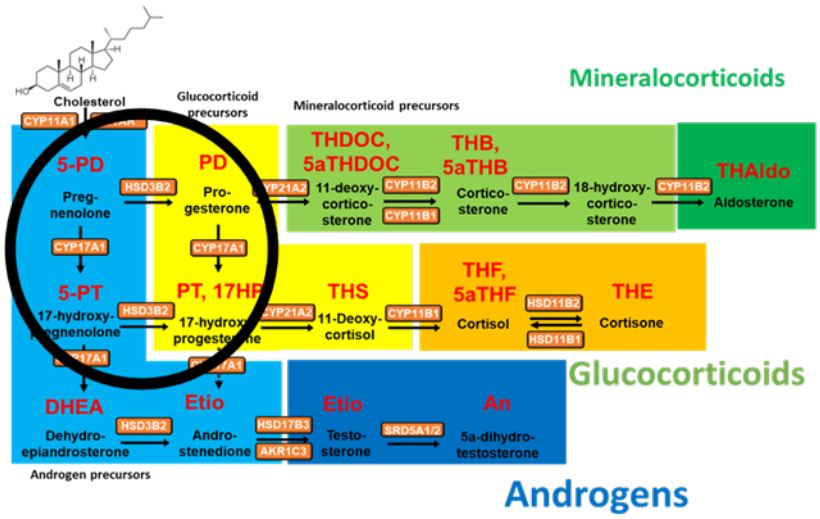


Figure 1. Steroid pathways showing the five urinary steroid metabolites elevated in mucinous ovarian cancers and mucinous BOTs [7].

Glucocorticoid precursors (PD, PT, 17-HP) and androgen precursors (5-PD, 5-PT) were significant urinary steroid metabolites identified from the Type 1 group. This could represent early immature steroidogenesis and a key pathological change in early mucinous ovarian carcinogenesis.

2. Rationale

Dysregulation in steroid hormones [13], the immune system and gene expression have been implicated in ovarian cancer progression, but their true role and underlying mechanisms remain to be elucidated. We plan to use several technologies to search for previously determined biomarkers [7] that may spot the disease early. To do so we will recruit patients with ovarian tumours and obtain urine samples from them and analyse a subset of recruits with confirmed mucinous histology type for novel markers. We will also analyse samples from women recruited with other types of ovarian tumours as a control group.

Additionally, we will analyse urine samples from women with a known diagnosis of mucinous ovarian cancer who are being kept under surveillance in clinic.

Finally, we will also recruit women from Christie Hospital which is a centre for specialist cancer surgery who are undergoing surgery for specific types of appendiceal tumours, and a specific type of cancer called pseudomyxoma peritonei to donate a urinary sample. Primary mucinous ovarian cancer is challenging to differentiate from ovarian metastases from these tumours at histopathology analysis due to similarities in morphology and immunohistochemistry profile. It is for this reason, low grade appendiceal neoplasm and pseudomyxoma peritonei as a comparator disease profile is an appropriate choice.

Novel technologies that will be used include mass spectrometry [11]. Hormonal therapy has been used successfully in the management of ovarian cancer (e.g granulosa cell tumours and hormone receptor positive platinum resistant ovarian cancer). Nevertheless, steroid pathways have not been fully characterised in epithelial ovarian cancer/ mucinous ovarian cancer.

3. Theoretical framework

The cornerstone of treatment for all ovarian cancer is surgery and chemotherapy. However, patients who initially respond will relapse within 18-24 months and need further therapies to prolong survival. Effective targeted therapy options are limited. Early diagnosis is crucial to save lives. There is an urgent unmet need to improve early diagnosis in ovarian cancer and identify better therapies. Steroid hormones have been implicated in general ovarian cancer development, but their true role and underlying mechanisms remains unexplained.

Novel and revolutionary techniques are now available and affordable to discover the answers to these questions. The combination of mass spectrometry-based profiling and computational data analysis is a breakthrough in allowing potential discovery of new biomarkers. It can comprehensively find all metabolites and analytes produced in a disease of interest.

Ovarian tissue has the ability for steroid hormone production. Analysis of the genes that control the enzymes for hormone production is known to be altered in ovarian cancer and we believe that by looking at patient's urine with mass spectrometry and other biomarkers, we can detect those changes to improve ovarian cancer

diagnosis initially, detect early recurrence and distinguish between certain types of appendiceal, pseudomyxoma peritonei, and ovarian cancer.

This research will lead to better understanding of the disease and identification of novel targets. Providing proof-of concept for the use of mass spectrometry as a novel detection tool for ovarian cancer would represent a significant advance.

4. Research question/aims

We aim to characterise the steroid metabolome and other novel targets in mucinous ovarian cancer to investigate the hypothesis that the steroid pathway is involved in the aetiopathogenesis of mucinous ovarian cancers.

4.1. Objectives

a) Investigate urinary metabolome and other novel targets in women with mucinous carcinomas, mucinous borderline tumours, benign ovarian tumours, and mucinous appendiceal /pseudomyxoma peritonei tumours and other histology types of ovarian tumours.

b) Investigate urinary steroids and other biomarkers in women undergoing follow-up with known mucinous ovarian cancer to characterise changes seen in women with recurrence.

c) Investigate urinary steroids and other biomarkers in women undergoing surgery for specific types of appendiceal and pseudomyxoma peritonei cancer to identify key differences between these tumour types and mucinous ovarian cancer.

4.2. Outcome

The primary outcome measures for the study will be expression of steroidogenic genes, enzymes and metabolites from the ovaries.

5. Design and methods of data collection and data analysis

Study design: Prospective study limited to working with human urine samples and data.

Urine samples will be subjected to gas chromatography-mass spectrometry and other technologies to search for steroid metabolites to elicit a pattern that correlates with subtypes of ovarian cancer [12]. Those identified patterns will then be compared to patterns seen in mucinous appendiceal and pseudomyxoma peritonei cancer.

5.1. Data Collection

All data collected will be first recorded on a Case Report From by the clinical care staff, or research nurses and then transferred to REDCap. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

Demographic data including ethnicity, BMI at diagnosis, smoking status, and deprivation status (by postcode) will be collected for all participants. Surgical data including date of operation, surgery details, appendectomy performed, operating clinician, and residual disease will be collected for all participants. Clinical data including CA125 at diagnosis, CEA at diagnosis (if performed), CA19-9 at diagnosis (if performed), Laterality, and Peri-operative Endoscopy (if performed) will be collected for participants in study 1 (Diagnostic arm). Histopathological data including FIGO stage, grade, histological subtype will be collected for participants in study 1 (Diagnostic arm).

Adjuvant chemotherapy treatment data including adjuvant chemotherapy planned, adjuvant chemotherapy type planned, date of recurrence (if any), serum tumour markers at recurrence, and treatment of recurrence will be collected for participants in study 2 (Follow-up arm).

Diagnostic data including diagnosis of additional cancer, additional cancer primary site, and date of additional cancer diagnosis will be collected for participants in study 2 (Follow-up arm).

5.2. Prospective study – Diagnostic Study 1

The first sub-study is a multicentre prospective study to investigate urinary steroid profiling in mucinous cancer, mucinous benign and borderline tumours. 265 individuals will be recruited in total: for an eventual diagnosis and analysis of ~25 individuals with mucinous ovarian cancer and a control number of samples from individuals with other types of ovarian tumours.

The urinary steroid metabolome will be profiled via GC-MS and other technologies to determine the “steroid fingerprint” in MOC, MBT, and benign ovarian tumours.

24-hour urine samples will be collected from all recruited participants however, a subset of participants with confirmed mucinous histology type will be analysed and a subset of participants with confirmed other tumour types will be analysed. This is to reduce the costs involved in the experimental work and to maximise our chances of discovering and validating new biomarkers. Participants will be asked to collect all of their urine in a 24-hour period in a single bottle to be brought to their next hospital visit, which will be collected by the research team. Sample collection instructions will be provided to sites to standardise collection and processing of samples.

5.3. Prospective study – Follow-Up Study 2

The second sub-study will recruit 50 individuals with confirmed histological biopsy diagnosis of primary mucinous ovarian cancer after completion of first-line treatment.

The urinary steroid metabolome in confirmed MOC will be profiled via GC-MS and other technologies to determine if steroid profiles change during follow-up or at recurrence. We anticipate that samples will be collected at three-month intervals to coincide with clinic visits over a two-year period, but some flexibility between two and six months is acceptable to undertake these visits.

Participants will be asked to collect all of their urine in a 24-hour period in a single bottle to be brought to their next hospital visit, which will be collected by the clinical care team or research nurses.

Specialist gynaecological pathologists will use their central pathology review facility to obtain accurate histology. Urinary steroid profiles and histologies will be compared by integrated analysis. The data will undergo statistical and computational analysis to include descriptive statistics and visualisations (SigmaPlot and Prism) and application of machine-based learning techniques (MatLab and other specialist software). If distinct steroid profiles are identified, a high-throughput urine LC-MS/MS assay will be developed and validated as a time-saving measure.

5.4. Prospective study – Appendiceal and Pseudomyxoma Peritonei Cancer Sub Study 3

The third sub-study will investigate urinary steroid profiling in mucinous appendiceal or pseudomyxoma peritonei cancer. 25 individuals will be recruited in total. This sub-study will only recruit at Christie Hospital, Manchester.

The urinary steroid metabolome will be profiled via GC-MS and other technologies to determine the “steroid fingerprint” in MOC, MBT, benign ovarian tumours, and mucinous appendiceal or pseudomyxoma peritonei cancer.

24-hour urine samples will be collected at a single time point from all recruited participants. Participants will be asked to collect all of their urine in a 24-hour period in a single bottle to be brought to their next hospital visit, which will be collected by the clinical care team or research nurses.

5.5. Statistical Data Analysis

The data analysis will include pathway analysis, univariate and multivariate statistical analysis and a machine-based learning approach in collaboration with academics at the University of Birmingham. This will be linked to the anticipated outcomes.

6. Project setting

This is a prospective study. We will collect urine from patients with confirmed or suspected ovarian cancer undergoing treatment as well as with confirmed mucinous appendiceal or pseudomyxoma peritonei cancer. Samples will be used as per the study design.

This is a multi-centre study. Participants will be recruited from multiple NHS sites. There are no site-specific requirements to run the study. The types of activity / procedures at each site will include:

Area of Activity	**Specific Activity**	**Approximate duration (min)**	**Performed by**
Consent Processes	Database search	10	Clinical care team, Research nurses
Consent Procedures	Approach potential participant to discuss study	10	Clinical care team or Research Nurses, with eligibility confirmed by clinical care team
Consent Procedures	Consent for Sample	15	
Research Interventions	Urinalysis - Urine collection only (at clinic)	10	Research nurses
Study Close Down	Archiving		Univ. of Birmingham

The laboratory work and data analysis will be performed at the University of Birmingham.

7. Participant recruitment

7.1. Eligibility criteria

The study population will involve patients with confirmed or suspected ovarian cancer undergoing treatment. These patients will be identified at the Gynaecology Oncology MDT meeting or clinic visits. Patients having oophorectomy for unrelated reasons will be invited to participate. These patients will also be identified at the General Gynaecology MDT meeting.

The study population will also involve patients who are undergoing surgery for specific types of appendiceal tumours, and a specific type of cancer called pseudomyxoma peritonei.

7.1.1. Inclusion criteria

Women over the age of 18 years with capacity to give informed consent and from the following patient groups:

- Sub-Study 1 - Diagnostic:
  - Participants undergoing surgery for adnexal masses.
  - Participants with confirmed biopsy of mucinous ovarian cancer prior to start of treatment.

- Sub-Study 2 – Follow-up:
  - Participants with a confirmed histological biopsy diagnosis of primary mucinous ovarian cancer after completion of first-line treatment.

- Sub-Study 3 – Appendiceal/ pseudomyxoma peritonei Cancer
  - Participants with a pre-operative clinical radiological diagnosis of pseudomyxoma peritonei.
  - Participants with a confirmed histological diagnosis of low grade appendiceal mucinous neoplasm (LAMN) and appendiceal carcinomas (excluding non-mucin-producing and neuroendocrine tumours).

7.1.2. Exclusion criteria

- Sub-Study 1 – Diagnostic:
  - Participants lacking capacity to consent.
  - Using hormonal contraception / implant within last 3 months.
  - Used Hormone Replacement Therapy or GnRH analogues in last 3 months.
  - Used steroid treatment in last 3 months including inhalers.
  - Commenced chemotherapy treatment for ovarian carcinoma.
  - Undergoing delayed debulking surgery for ovarian cancer after chemotherapy.

- Sub-Study 2 – Follow-up:
  - Participants lacking capacity to consent.
  - Using hormonal contraception / implant in last 3 months.
  - Used HRT or GnRH analogues in last 3 months.
  - Used steroid treatment in last 3 months including inhalers.

- Sub-Study 3 – Appendiceal / pseudomyxoma peritonei Cancer:
  - Participants lacking capacity to consent.

7.2. Sampling

See 7.2.2 Sampling Technique

7.2.1. Size of sample

As a minimum to power the study we wish to recruit: 265 participants to be assessed for primary mucinous ovarian cancer for the eventual identification of ~25 participants. 50 participants identified to have mucinous ovarian cancer, to be followed for two years. 25 participants identified to have mucinous appendiceal cancer or pseudomyxoma peritonei .

Sample size estimates for the clinical study are challenging given the rarity of mucinous cancer. Nevertheless, our data from the Pan Birmingham gynaecological cancer centre, City Hospital, Birmingham shows we have treated 122 confirmed primary mucinous ovarian cancer patients in the last 16 years (average of 7/year).

Sub-Study 1 – Diagnostic:

We will aim to recruit 25 participants with primary mucinous ovarian cancer. CA125 has sensitivity of 50% for early-stage ovarian cancer. We hypothesise that urinary steroid analysis will have a better sensitivity than CA125. With 25 participants with histology identified mucinous cancer, assuming a sensitivity of 80%, a 95% confidence interval around this estimate would range from 59.3% to 93.2%. With 250 participants without histology identified mucinous cancer assuming specificity of 80% a 95% confidence interval would range from 74.5% to 84.8%. With a loss to follow-up of 5% we will need to recruit about 265 patients.

Sub-Study 2 – Follow-up:

In the absence of steroid data, it is difficult to accurately sample size this. Given our local data, we are confident to recruit 50 mucinous cancer patients in study 2 to donate samples quarterly over 2 years as previously described (allowing a year of follow-up for the last patient recruited and assuming that samples will be stored for analysis at last 6 months of project). This sample size is adequate to provide proof of principle that these metabolites are consistently elevated in mucinous cancer and to generate evidence to design a larger study.

Sub-Study 3 – Appendiceal or Pseudomyxoma Peritonei Cancer:

We will recruit patients with mucinous appendiceal or pseudomyxoma peritonei cancer aiming for 25 patients to be recruited within the first 2.5 years.

7.2.2. Sampling technique

Potential participants will be identified from the Gynaecological Multi-Disciplinary Team (MDT) meetings or at clinic visits by the clinical care team. Consent, providing participant information, and collection and transport of

urine samples can be delegated to research nurses at site; however eligibility must be confirmed by a member of the clinical care team.

Patients will be asked to collect all their urine in a 24-hour period in a single bottle and bring this with them to their next hospital visit, which will be collected by the research nurses. This is not invasive. The collection bottle is supplied to the patients, and they should complete the collection within 72 hours of next attending the hospital.

Samples will be first catalogued and anonymised and then processed in accordance with the Sample Collection Instructions provided to the study teams. The sample will then be stored in a locked -20 freezer within the hospital. Access to this will only be for the clinical care team or research nurses at site. At periodic intervals (when the research team at the University are ready) samples will be transported by courier . There will be a log of samples collected at the site and a log of samples received at the University. Any mislabelled samples or damaged samples will be destroyed and recorded. Samples will be transported on dry ice and kept in primary and secondary containment to the Institute of Biomedical Research, University of Birmingham. No personal or clinical data will be transported with the samples. At the end of the trial, further ethical approval will be sought to utilise any remaining urine samples, otherwise they will be destroyed. This study will comply with the University's Quality Manual and SOP on Research using human biomaterials which concerns the collection, storage and use of human tissue.

7.3. Recruitment

Potential participants for studies 1 and 2 will be identified from the clinic visits or at the Gynaecological MDT meeting by the clinical care team.

Eligible participants will be approached at the pre-admission/new patient appointments by members of the clinical care team, the research nurses, or delegated team members. When needed, eligible participants will be contacted by telephone/video conference by members of the clinical care team, the research nurses, or delegated team members. They will be provided with a participant information sheet by the research nurses or delegated team members. Written informed consent will be obtained by the clinical care team, the research nurses, or delegated team members at these pre-assessment appointments/calls. If consent is obtained, members of the research team will record a signed consent form in the patient's notes and provide the patient with a 24hr urine collection bottle and instruction sheet.

If patients do not require urgent treatment, participant information sheets will be given to eligible patients at their outpatient clinical appointments. Members of the clinical care team, the research nurses, or delegated team members will discuss the study with the patient, and the patient will be given time to consider their enrolment. A member of the research team will then contact the patient later to determine interest in participation. If consent is acquired, a urine collection bottle and instruction sheet will be provided at their next

medical visit. On the day of surgery, the participant would then sign the informed consent form and provide their 24hr urine sample.

7.3.1. Sample identification

Potential participants for studies 1 and 2 will be identified from the weekly Gynaecological Cancer MDT meeting, gynaecology and oncology clinics and surgical lists. Potential participants for study 3 will be identified from oncology clinics and surgical lists. Participants will be approached at the pre-admission appointments or clinic appointments by members of the clinical care team. In cases where potential participants have been given time for consideration, they may then be contacted via telephone/video conference for the consenting process.

7.3.2. Consent

Eligible participants will be approached at the pre-admission/new patient appointments by members of the clinical care team, research nurse, or delegated team member. They will be provided with a participant information sheet appropriate for their sub study.

Written informed consent will be obtained by the clinical care team, research nurses, or delegated team members at these appointments. Study procedures can be performed by research nurses, or delegated team members, however eligibility must be confirmed by clinical care team.

Most patients will require urgent treatment so there are no further opportunities to consent later in those cases. However, when urgent treatment is not required, patients will be given time to consider their participation in the study and contacted by telephone/video conference later for the consenting process.

If consent is obtained on the first clinical visit, the research team will record a copy of the signed consent form in the patient's notes and provide the patient with a 24hr urine collection bottle and instruction sheet. If consent is acquired via telephone/video conference following a clinical visit, the research team will record a copy of the signed consent form on the day of surgery. The original consent form will be kept in a site file by the research nurses or delegated team members

7.4. Participant withdrawal

There will be a contact email address on the participation information leaflet which participants can use to withdraw from the project. Participants can withdraw their samples from analysis at any time, However, once a sample has been analysed withdrawal is no longer possible as samples will be fully anonymised and cannot be traced back to individual participants.. Their samples and data will be discarded and not included in the project and there will be no consequences for the participant. Confirmation will be sent to the participant that their sample has been excluded. To maintain anonymity, participants will be able to contact the direct care team, the research nurses, or delegated team members to ask for their sample to be withdrawn.

7.5. Definition of end of trial

The end of the trial will be 30 June 2029. This is a laboratory-based project using human samples. Some samples will be frozen initially so that samples can be processed in batches thus reducing costs and improving data quality. At the end of the trial, further ethical approval will be sought to utilise any remaining urine samples, otherwise they will be destroyed.

8. Storage and analysis of human tissue

This study will adhere to the Human Tissue Act, Good Clinical Practice and the Data Protection Act 2018.

Participants must complete a 24-hour urine collection at home, which requires them to collect all urine over a continuous 24-hour period, store it in a refrigerator, and transport it to their next hospital appointment. While not invasive, this represents a logistical burden and may impact their daily activities. The procurement of all samples will be conducted in accordance with the Human Tissue Act and will observe the principles of Good Clinical Practice, with careful attention to minimizing participant burden while maintaining research quality.

Clinical data from the patients' records will be accessed and used to inform and support the researcher during the analysis of the results. This is clear in the patient information leaflet and the Consent Form. Data will be anonymised and securely to ensure confidentiality and data protection. Any identifiable personal data will be stored on encrypted directories on NHS computers. This is in accordance with the Data Protection Act 2018.

8.1. Quality Management Procedures

There are no significant risks to the participants. Urine collection is non-invasive and is surplus material which is collected in the patient's own home. The safety reporting process will follow the measures set by the NHS Trusts and an Annual Safety Report will be produced each year for the REC.

9. Safety reporting

The safety of participants is paramount in this study. A comprehensive system for recording, reporting, and following up on Adverse Events (AEs) will be implemented to ensure participant well-being and study integrity. This comprehensive safety reporting system will ensure that any potential risks to participants are promptly identified, thoroughly evaluated, and appropriately managed throughout the duration of the study.

9.1. Definition of Adverse Events:

An Adverse Event (AE) is defined as any untoward medical occurrence in a study participant, which does not necessarily have a causal relationship with the study. This includes any unfavourable and unintended sign, symptom, or disease temporally associated with the study.

For 24-hour urine collection, adverse events might include musculoskeletal strain from handling collection containers, psychological impact related to the collection process, or logistical complications affecting daily activities. Additional adverse events could include accidental exposure to biological samples, compromise of participant privacy, or any unexpected physiological or psychological symptoms during the study period.

9.2. Recording of Adverse Events:

All AEs will be recorded in the participant's Case Report Form (CRF), regardless of severity or perceived association with the study. The following information will be documented for each AE: description, date of onset, severity, duration, relationship to study, action taken, and outcome.

9.3. Severity Grading:

AEs will be graded for severity using a standardized scale:

- Mild: Awareness of sign or symptom, but easily tolerate
- Moderate: Discomfort enough to cause interference with usual activity
- Severe: Incapacitating with inability to work or perform usual activities

9.4. Causality Assessment:

The relationship between each AE and the study will be assessed as:

- Definitely related
- Probably related
- Possibly related
- Unlikely related
- Not related

9.5. Reporting Procedures:

All AEs will be reported to the principal investigator within 24 hours of awareness.

Serious Adverse Events (SAEs) will be reported to the Research Ethics Committee and sponsor within 24 hours of the research team becoming aware of the event. A summary of all AEs will be submitted to the sponsor and ethics committee in annual safety reports.

9.6. Follow-up of Adverse Events:

All AEs will be followed until resolution or stabilization. Participants experiencing AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the investigator.

9.7. Serious Adverse Events (SAEs):

SAEs are defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect

All SAEs will be reported immediately and investigated thoroughly.

9.8. Training:

All study personnel will receive training on the identification, recording, and reporting of AEs before the study commences.

9.9. Documentation:

A safety reporting log will be maintained, documenting all AEs, their management, and outcomes.

10. Ethical and regulatory considerations

The proposed research aims to use gas chromatography-mass spectrometry (GC-MS) and other technologies to identify biomarkers that can distinguish between early ovarian cancer, healthy conditions, and benign diseases. To do this, participants with ovarian and appendiceal and pseudomyxoma peritonei tumours will be recruited and urine samples will be obtained. A subset of participants with confirmed mucinous histology as well as those with other histology types will be analysed to identify novel markers.

This research design fits within the ethical and regulatory framework by directly addressing an important clinical need - the early detection of ovarian cancer, which can significantly improve patient outcomes. The use of biological samples from participants is necessary to achieve the research aims.

The key risks to participants include the potential for incidental findings related to their health status, as well as concerns around data privacy and confidentiality. To mitigate these risks, robust data protection protocols, establish clear pathways for reporting any safeguarding concerns that arise, and provide comprehensive training

to the research team on maintaining appropriate boundaries will be implemented. Participants will also be fully informed about the nature of the research and the potential risks, and their informed consent will be obtained prior to sample collection.

The potential benefits to participants include the possibility of earlier cancer detection, which could lead to more timely and effective treatment. For those with confirmed mucinous ovarian cancer, the opportunity to provide serial urine samples may also contribute to the identification of markers for early cancer recurrence. Additionally, there is a broader societal benefit of advancing understanding of ovarian cancer and improving diagnostic capabilities.

Overall, the research question, design, and methods are well-aligned with relevant ethical and regulatory frameworks. The risks and potential benefits to participants have been thoroughly considered, and appropriate safeguards to protect participant dignity and well-being throughout the study have been outlined.

While this study involves minimal physical risk to participants, it requires the collection of samples beyond standard clinical care which places additional burden on participants. The 24-hour urine collections, though non-invasive, require significant participant commitment. Participants must collect all urine over a full day, properly store collection bottles, and transport samples to hospital appointments within a specified timeframe. For participants in the follow-up study, this burden is repeated quarterly every two - six months over a two-year period.

We believe these demands on participants are justified by the potential benefits of the research - including improved early detection of ovarian cancer, better understanding of mucinous ovarian cancer biology, and development of new biomarkers for diagnosis and recurrence monitoring. However, we acknowledge the impact on participants' time and daily activities. To minimize burden, the study team will:

- Provide detailed collection instructions and materials
- Time collections to align with planned hospital visits where possible
- Maintain regular communication with follow-up study participants
- Be available to address any practical difficulties with sample collection
- Regularly review the sampling frequency to ensure it remains proportionate to study needs

This additional participant burden has been carefully considered in our risk assessment and informed consent processes. The participant information sheet clearly outlines these requirements to ensure potential participants can make an informed decision about study participation.

10.1. Assessment and management of risk

The research involves obtaining biological samples (urine) from participants with ovarian, appendiceal and pseudomyxoma peritonei tumours, including those with confirmed mucinous histology and other histology types. This raises potential safeguarding implications, as the researchers may come across information that has sensitive or confidential implications for the participants. To address these concerns, the University of Birmingham's Quality Management System (QMS) will be implemented to ensure robust data protection and confidentiality measures in the secure storage and handling of all participant information and biological samples. The Institute of Biomedical Research laboratory is GCP accredited and works alongside the clinical compliance team at the University.

Clear protocols will be established for the identification and reporting of any safeguarding concerns that may arise, including procedures for escalating issues to the appropriate authorities or support services. Comprehensive training for the research team on recognizing and responding to potential safeguarding issues, including respecting participant privacy and maintaining appropriate boundaries, will be crucial.

Ensuring that all participants are fully informed about the nature of the research and the potential risks, and that they provide informed consent before taking part, is also essential. By proactively addressing these potential risks, the safety and well-being of the participants will be ensured while also advancing the understanding of ovarian cancer.

10.2. Research ethics committee (REC) and other regulatory review & reports

Prior to study initiation, ethical approval will be obtained from the relevant Institutional Review Board (IRB) or Ethics Committee. Any amendments to the protocol will be submitted for review and approval before implementation.

Following Sponsor approval, the protocol, informed consent form, participant information sheet will be submitted to an appropriate Research Ethics Committee (REC), and HRA (where required) and host institutions for written approval.

The CIs shall submit once a year throughout the study, or on request, an Annual Progress report to the HRA (where required) host organisation, Sponsor and funder (where required). In addition, an End of Study notification and final report will be submitted to the same parties.

The study will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimised by making use of a unique participant study number only on all study documents and any electronic database(s). All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data.

10.2.1. Regulatory review & compliance

Before any site can enrol patients into the study, the Chief Investigator will ensure that appropriate approvals from participating organisations are in place. Specific arrangements on how to gain approval from participating organisations are in place and comply with the relevant guidance. All investigators have GCP and Human Tissue Act training. The University of Birmingham runs an oversight program and may monitor the study at periodic intervals.

10.2.2. Amendments

For any amendment to the study, the Chief Investigator or designee, in agreement with the sponsor will submit information to the appropriate body for approval of the amendment to be issued. The Chief Investigator or designee will work with sites (R&D departments at NHS sites as well as the study delivery team) so they can put the necessary arrangements in place to implement the amendment to confirm their support for the study as amended.

10.3. Peer review

This study has been subjected to rigorous peer review process by the Scientific Advisory Panels at the Eve Appeal (co-funder), the Birmingham Women's and Children's Hospital Global Research Fund (co-funder), and the clinical leads for the Pan-Birmingham Gynaecological Cancer Centre.

10.4. Patient & public involvement

The research process has been presented to a focused patient group of three individuals with Mucinous Ovarian Cancer (MOC) and to a larger audience at Eve Appeal cancer charity events. There will be no patient and public involvement throughout the duration of the study.

10.5. Protocol compliance

Protocol compliance will be managed by the Chief Investigator and the Clinical Research Compliance Team. Protocol deviations, non-compliances, or breaches are departures from the approved protocol. Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and

reported to the Chief Investigator and Sponsor immediately. Deviations from the protocol which are found to frequently recur are not acceptable, will require immediate action and could potentially be classified as a serious breach (refer to University of Birmingham Serious Breach Protocol). In this scenario, the University's SOP for a serious breach will be followed.

The use of REDCap serves as an important tool in preventing protocol deviations and enhancing protocol compliance. Customisable data entry forms with built-in validation rules ensure that data is collected accurately and consistently according to the study protocol (e.g. informed consent process, cooling-off period, and research personnel training logs). Real-time monitoring via REDCap enables prompt identification and correction of any deviations from the protocol, maintaining data integrity throughout the study. Additionally, with role-based access control, only authorised research staff that have completed the document set training will have access to study data, reducing the risk of unauthorised modifications, and improving protocol compliance. Automated reminders and notifications within REDCap will also keep research staff informed of upcoming tasks, the approved schedule of events, and protocol requirements.

10.6. Data protection and confidentiality

All data will be anonymised by an unrelated sequence of characters. No identifiable data will be stored on the University of Birmingham secured network in keeping with the University's Data Protection and Information Security policies, but data associated with the laboratory analysis of the samples will be stored for up to 10 years. Personal information of the participants will be retained only on NHS encrypted directories on the NHS network at the recruiting sites

The data custodians will be Prof Sudha Sundar (CI) and the local site PIs. The study is compliant with the requirements of the Data Protection Act 2018. All investigators and study site staff must comply with the requirements of the Data Protection Act 2018 with regards to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Secure maintenance of the data and the linking code will be held on NHS encrypted directories within password protected folders and storage media.

We will limit access to the minimum number of individuals necessary for quality control, audit, and analysis. Access will also be given to Sponsor Representatives and regulatory authorities as required. Confidentiality of data will be preserved when the data are transmitted to co-investigators using NHS servers only. The University of Birmingham runs an oversight program which will intermittently check the study is compliant with data protection requirements.

10.7. Indemnity

The Sponsor (University of Birmingham) will be responsible for indemnity to meet the potential legal liability for harm to participants arising from the management of the research and for harm to participants arising from the design of the research.

The NHS sites will be responsible for potential legal liability of investigators/collaborators arising from harm to participants in the conduct of the research.

10.8. End of study and archiving

The end of the study will be defined as the completion of data collection and analysis for all participant groups, including a planned sample size of 265 participants with ovarian tumours, 50 participants with confirmed mucinous ovarian cancer for follow-up analysis, and 25 participants with mucinous appendiceal or pseudomyxoma peritonei cancer.

We anticipate that the full scope of data collection and analysis for these 340 participants will take approximately 4.5 years to complete, at which point the study will be considered concluded and the researchers will begin the process of archiving the data. All data collected during the study will be securely archived for 10 years, in alignment with common guidelines and regulations for the retention of research data and materials. Further ethical approval will be sought to utilise and store samples collected in this study, otherwise they will be destroyed.

The specific archiving arrangements will include secure, long-term storage of all participant data in an encrypted, access-controlled database, as well as appropriate storage and cataloguing of all biological samples in a dedicated biorepository with strict temperature and security controls. Detailed documentation of the archiving process, including information on data formats, storage locations, access protocols, and anticipated retention timelines, will also be maintained to ensure the responsible stewardship of the valuable information collected during this important ovarian cancer research.

10.9. Access to the final dataset

All data will be anonymised. No identifiable data will be stored on the University of Birmingham secured network in keeping with the University's Data Protection and Information Security policies, but data associated with the laboratory analysis of the samples will be stored for up to 10 years. Prof Sudha Sundar (Chief Investigator), regulatory authorities and sponsor representatives will have access to the full dataset. All patient documentation will reflect the future use of these data in research.

11. Dissemination policy

11.1. Dissemination policy

The data will be owned by the University of Birmingham (Sponsor). On completion of the study, the data will be analysed and tabulated and a Final Study Report prepared which will be accessible on request. All participating investigators will have rights to publish any of the study data. There are no time limits or review requirements on the publications.

The funding body will be acknowledged within the publications, but they do not own publication rights of the data from the study. None of the participants will be contacted regarding the outcome of the study. Participants will be able to contact the PI for information about the Final Study Report, but no individual specific results will be released.

The study protocol, full study report, anonymised participant level dataset, and statistical code for generating the results will be made publicly available at the end of the study period.

11.2. Authorship eligibility guidelines and any intended use of professional writers

The final study report will aim to grant authorship to all members of the study group. Individually named authors or group authorship will follow the criteria defined by The International Committee of Medical Journal Editors.

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13. Appendices

13.1. Appendix 1 – schedule of procedures

Procedures	Screening	Baseline	Follow Up Study 2 Only Every 3 Months for two years*
Consent processes	x		
Demographics		x	
Medical history		x	
Urine collection		x	x

* We anticipate that samples will be collected at three-month intervals to coincide with clinic visits over a two-year period, but some flexibility between two and six months is acceptable to undertake these visits.