Refining Ovarian Cancer Test Accuracy Scores:

A test accuracy study to validate new risk scores in women with symptoms of suspected ovarian cancer protocol

ROCkeTS Study containing the ROCkeTS-GEN Arm

Study Protocol Version 16.0 19th June 2023

REC Reference: 14/WM/1241

Sponsor Reference: RG_14-196

NIHR HTA Reference: 13/13/01

IRAS Reference: 168143

ISRCTN17160843

This protocol has regard for HRA guidance

ROCkeTS Project Management Group

Chief Investigator: Prof Sudha Sundar[1]

Statistics: Prof Jon Deeks[1]

Systematic Reviewers: Dr Clare Davenport[1], Dr Nirmala Rai[3], Prof Susan Mallett[11] and Dr Pawana Sharma[1]

Health Economist: Prof Tracy Roberts[1]

Study Management: Lauren Sturdy[1], Ryan Ottridge[1]

Patient representative: Hilary Stobart[6]

Primary Care: Prof Richard Neal[7]

GyneOncology: Prof Sean Kehoe[1, 8]

IOTA team: Prof Dirk Timmerman[9], Prof Tom Bourne[9,10] and Prof Ben VanCalster[9]

UCL: Prof Usha Menon[11], Dr Alex Gentry-Maharaj[11] and Dr Mathew Burnell[11]

1 – University of Birmingham, 6 – Lay member of NCRI Gynae CSG, 7 – Bangor University, 8 – St. Peter's College Oxford, 9 – KU Leuven, Belgium, 10 – Imperial College London, 11 – Centre for Medical Imaging, University College London.

ROCkeTS-GEN Project Management Group

Chief Investigator: Prof Sudha Sundar[1]

Statistics: Prof Susan Mallett[45]

Study Management: Lauren Sturdy[1], Ryan Ottridge[1]

Prof Paul Pharoah[2], Dr Caroline Rick[3], Dr Nitzan Rozenfield[2], Prof James Brenton[2] and Prof Usha Menon[4]

1 – University of Birmingham, 2 – University of Cambridge, 3 – University of Nottingham, 4 – University College London, 5 - Centre for Medical Imaging, University College London.

AOA-ROCkeTS-GEN Project Management Group

Chief Investigator: Prof Sudha Sundar[1]

Statistics: Yemisi Takwoinigi[1]

Study Management: Lauren Sturdy[1], Ryan Ottridge[1]

Dr Prasad Gawande[2,] Alex Fisher[2]

1 – University of Birmingham, 2 – AOADX

Project Oversight Group

Chair: Prof Peter Sasieini[1]

Independent (GyneOncology): Mr Andy Nordin[2]

Independent (Primary Care): Prof Peter Murchie[3]

Independent (Imaging): Dr Mike Weston[5]

Patient/charity representative: Annwen Jones, Target Ovarian Cancer

Study team representative: Prof Sudha Sundar

Study team representative: Prof Jon Deeks

Study team representative (non-voting): Prof Sean Kehoe

UKCTOCS/UKOPS representative (non-voting): Prof Usha Menon

IOTA representative (non-voting): Prof Timmerman

1 – Queen Mary University of London, 2 – Queen Elizabeth the Queen Mother Hospital, Kent, 3 – University of Aberdeen, 5 – Retired, formerly Leeds Teaching Hospitals Trust, Editor Clinical Radiology.

ROCkeTS and ROCkeTS-GEN Study Office
University of Birmingham Clinical Trials Unit (BCTU), Public Health building, Edgbaston, Birmingham B15 2TT.

Telephone: 0121 415 9127

Email: ROCkeTS@trials.bham.ac.uk

Clinical queries should be directed during office hours to an appropriate member of the Project Management Group.

Other queries should be directed to the ROCkeTS and ROCkeTS-GEN Study Office.

FOR STUDY REGISTRATION, visit **https://ROCkeTS.medscinet.com**

Funding and Support in Kind	
Funders	**Financial and non-financial support given:**
NIHR	£1,143,978.00
Funding Scheme	HTA
Funder's reference number	13/13/01
Cancer Research UK – For ROCkeTS-GEN	£376,210.58
Funding Scheme	Clinical Research Committee - Biomarker Project Award
Funder's reference number	C44294/A26962
AOA Dx Inc	£137,388.96

The Funders of the ROCkeTS and ROCkeTS GEN trials have had no role in the trial design, data collection, data analysis or data interpretation. The views expressed herein are those of the authors and not necessarily those of the NIHR, the Department of Health and Social Care, or Cancer Research UK.

The ROCkeTS AoA substudy is an industry collaboration that analyses samples and data collected in the ROCkeTS postmenopausal cohort. The funder has had no role in the sample collection or data collection, the funder will analyse the results and this will be crosschecked by the University of Birmingham. Results will be interpreted jointly with AoA Dx.

Compliance statement

This protocol describes the ROCkeTS study only (including the ROCkeTS-GEN arm and the AOA-ROCkeTS sub-study). The protocol should not be used as a guide for the treatment of participants not taking part in the ROCkeTS study.

The trial will be conducted in compliance with the approved protocol, UK Policy Framework for Health and Social Care Research 2017, the Data Protection Act 2018, and the principles of Good Clinical Practice as defined by the European Good Clinical Practice (GCP) Directive. Every care has been taken in the drafting of this protocol, but future amendments may be necessary, which will receive the required approvals prior to implementation.

Previous Protocol Versions

1.0, 2.0, 3.0, 4.0, 7.0, 8.0, 9.0, 10.0, 11.0, 13.0, 14.0, 15.0

Sponsor and Sponsor Roles

University of Birmingham is the sponsor. Prof Sudha Sundar is the Chief Investigator.

University of Birmingham is responsible for obtaining necessary approvals, the Project Management Group is jointly responsible for overseeing good clinical practice and the investigators are responsible for obtaining informed consent and care of the participants.

Signatures

The investigators and the sponsor have discussed this protocol. The investigators agree to perform the investigations and to abide by this protocol except in case of medical emergency or where departures from it are mutually agreed in writing.

Chief investigator		
Organisation	Signature	Date
Sponsor	For UoB sponsored trials, the sponsor will confirm approval of the protocol by signing the IRAS form and therefore a signature on the protocol is not required.	

Protocol amendments

Amendment number	Relevant Documents	Changes summary
Original application	Protocol, PIS, Consent v1.1 15.12.2014;	n/a
1	Protocol, PIS update to V2.0 01.04.2015; Consent to V2.1 06.05.2015; review of patient CRFs V1.0 01.04.2015 and poster (no version number)	Clarification on target population, comparator, outcome measures, eligibility criteria, collection of USG variables and storage of confidential data.
3	Protocol update to V3.0 05.10.15, PIS and Consent to V3.0 23.11.15	Protocol amended to include addition of PMG member, clarification on biomarker assessments, eligibility criteria. Updated schedule of events. Addition of ultrasound image collection requirements and their uses. Consent amended to include new PIS version, addition of NIHR disclaimer, collection of scan images. PIS amended to clarify blood volume collection, protocol version, NIHR disclaimer, ultrasound collection and transfer, cancer registry statement.
8*	Protocol v4.0 05.04.2017	Increase of study recruitment duration.
NB: protocols v5.0 and 6.0 were internally reviewed development versions only		
11	Protocol v7.0 14.03.2018 PIS Premenopausal v6.0 02.02.2018 PIS Postmenopausal v6.0 02.02.2018	Protocol change to design, target population, sample size, source of potential participants, eligibility criteria, Protocol clarification on schedule of events & CRF completion, approaching patients for consent, timeline of study procedures/tests, IOTA certification requirement of scanners, data collection, death, AE reporting, archiving PIS split into pre and postmenopausal (premenopausal now required to be scheduled for surgery), sample use, contact details, complaints, NIHR disclaimer and acknowledgement, Consent: wording clarified.
13*	Protocol v8.0 20.09.2018	Increase of study recruitment duration. Correction of CRF name used to record death
14*	Protocol v9.0	Amended sample size Increase of study recruitment duration
15*	Protocol v10.0	Increase of study recruitment duration
16	Protocol v11.0	Addition of the ROCkeTS-GEN Arm
18	Protocol v13.0	Amended sample size for ROCkeTS-GEN Increase study recruitment duration Addition of remote consent
19	Protocol v14.0	Addition of AOA-ROCkeTS sub-study

21*	Protocol v15.0	Correction of footer in PDF version of V14.0
24*	Protocol v16.0	Increase of study recruitment duration Removal of commercially sensitive data from AOA-ROCkeTS sub-study

* Non substantial amendment

Abbreviations

AE	Adverse Event
AUC	Area Under the Curve
BCTU	Birmingham Clinical Trials Unit at the University of Birmingham
CI	Chief Investigator
CMDL	Cancer Molecular Diagnostic Laboratory
CRF	Case Report Form
ctDNA	Circulating tumour DNA
DH	Department of Health
GCP	Good Clinical Practice
GP	General Practitioner
HGSOC	High-Grade Serous Ovarian Cancer
IOTA	International Ovarian Tumour Analysis
ISRCTN	International Standard Randomised Controlled Trial Number
NGS	Next Generation Sequencing techniques
NICE	National Institute for Health and Care Excellence
NPV	Negative Predictive Value
OC	Ovarian Cancer
PI	Principal Investigator
PIS	Participant Information Sheet
PMG	Project Management Group
POG	Project Oversight Group
PPV	Positive Predictive Value
RCOG	Royal College of Obstetricians and Gynaecologists
RMI	Risk of Malignancy Index
ROC	Receiver Operating Curve
ROCkeTS	Refining Ovarian Cancer Test Accuracy Scores
ROMA	Risk of Ovarian Malignancy Algorithm
RR	Relative Risk
SAE	Serious Adverse Event
sWGS	Shallow Whole Genome Sequencing
SOP	Standard Operating Procedure
UoB	University of Birmingham
UKCTOCS	UK Collaborative Trial of Ovarian Cancer Screening
UKOPS	UK Ovarian Cancer Population Study
USG	Ultrasound

CONTENTS

1 INTRODUCTION

1.1 Ovarian Cancer background

Ovarian cancer (OC) has an annual incidence of 6500 women and causes 4400 deaths annually in the UK; the lifetime risk of developing OC is 1 in 54.[1] 80% of patients will present at advanced stage and all stage, 5 year survival rate is 40%. OC is predominantly a disease of older, post-menopausal women, however 1000 women under 50 will be diagnosed with OC annually.[1] An international cancer bench marking project shows OC survival in the UK is significantly lower than other western countries; it is unclear as to whether this could be attributed to delay in diagnosis or differences in treatment received.[2]

1.2 Current diagnosis of Ovarian Cancer

National Institute for Health and Care Excellence (NICE) guidelines in 2011 recommended sequential testing using serum CA125 followed by pelvic ultrasound (USG) in women (particularly aged ≥50) presenting to primary care with symptoms on a persistent or frequent basis: persistent abdominal distension/'bloating', feeling full and/or loss of appetite, pelvic/abdominal pain, increased urinary urgency and/or frequency, unexplained weight loss, fatigue or changes in bowel habit. However these symptoms are very common[3,4] with abdominal bloating alone being documented in 16-30% of women presenting to General Practitioners (GPs).[5] Diagnostic challenges are considerable given (1) the low incidence of OC (a GP sees a woman with OC once in 3-5 years) (2) the low positive predictive value (PPV) of symptoms (only 1 in 400-600 symptomatic women have OC)[6,7] and (3) the lack of clear diagnostic pathways. Furthermore, NICE guidelines do not detail what USG abnormalities should prompt referral.

Use of this NICE guidance is extremely variable. A survey of 258 GPs report that the majority would refer patients on the basis of raised CA125 even if the USG was normal.[8] An unpublished audit at City Hospital, Birmingham of 448 referrals through the 2 week wait clinics reveals that the majority of referrals (90%) for suspected OC do not follow guidance. Referrals were heterogeneous in symptoms and in what the GPs considered were abnormal levels of CA125 or abnormal USG. Two thirds of women referred were premenopausal. Ovarian masses are common in premenopausal women and up to 10% of women will undergo surgery during their lifetime for the presence of an ovarian mass.[9] Women with complex masses considered benign can undergo laparoscopic or conservative management, whereas women with malignancy who undergo thorough surgery by gynaecological oncologist have the best outcomes.[10,11] Therefore, there are compelling health needs to improve early detection and reduce cancer mortality whilst minimising unnecessary interventions in women.

There is substantial literature on tests and scoring systems in women with ovarian masses presenting to secondary care. However, this literature is often derived in women with advanced stage cancer at secondary care. Literature is limited in studies of women with symptoms presenting to primary care, and no published studies of blood tests or USG in women with symptoms in primary care exist.

1.3 The need for a new algorithm to test for OC

Currently an average size NHS trust will receive about 100 urgent referrals a year for suspected OC. This translates to approximately 10,000 referrals each year in the UK through the rapid access referral system alone for suspected OC; approximately 2000 will be

diagnosed with OC. National Cancer intelligence network data shows that currently OC diagnosis is made in women presenting through diverse routes – 2 week referrals, routine GP referrals, cross specialty referrals, with a third of patients presenting as emergency presentations.[12] Thus risk prediction models must be assessed in a heterogeneous study population in all these settings.

Optimal diagnostic pathways and risk scores generated will be highly relevant in the NHS to streamline referrals for women with suspected OC. New scores may enable a stage shift in cancer presentations, helping the Department of Health (DH) aim for cancer mortality targets. Optimal diagnostic pathways for premenopausal women with suspected OC/complex ovarian mass and raised CA125 are not defined. In addition, a gynaecology consultant clinic will see 2 new referrals for premenopausal women with complex ovarian mass per week. Most will remain under follow-up for 12 months and undergo regular scans. ROCkeTS provides a unique opportunity to externally validate a clinical risk score that can reliably triage patients, save resource, build a dataset in this population and change practice. Providing USG training and regular quality assessments as part of study recruitment will also embed quality enhanced USG in the participating sites – this is likely to disseminate across the NHS.

It is also important to stratify patients into premenopausal and postmenopausal women. Whilst the risk of OC is higher in postmenopausal women, two thirds of patients referred through the rapid access pathway are premenopausal women. Current recommended best practice for premenopausal women (Royal College of Obstetricians and Gynaecologists (RCOG) guidance[9]) with a complex ovarian mass is to assess risk of malignancy using the Risk of Malignancy Index (RMI), 200 threshold, even though the score was derived for postmenopausal women and the use of logistic regression models in a different population is flawed.

1.4 The ROCkeTS project

The ROCkeTS project aims to externally validate new tests/risk prediction models that estimate the probability of having OC in women with symptoms. **This protocol refers to the ROCkeTS phase 3 prospective study and the ROCkeTS-GEN sub-study only.**

This project will be conducted in four interlinked Phases:

1. Phase 1 will be to undertake systematic reviews of the accuracy of tests and risk prediction models used for identifying OC in women with suspected OC.
2. Simultaneously, in Phase 2 we will undertake refinement of an existing risk prediction model based on additional predictions within existing large datasets. For Phase 2, we have identified 3 datasets UKCTOCS, UKOPS and International Ovarian Tumour Analysis (IOTA) that are relevant to primary care and secondary care settings in post and premenopausal women.
3. **In Phase 3, Prospective study,** based on the evidence from Phases 1 and 2, the most promising tests and risk prediction models for post and premenopausal women will be externally validated, in a prospective study comprising newly presenting premenopausal and postmenopausal patients. In order to conduct this complex project as effectively as possible, we will start recruitment to the Phase 3 study and banking of samples from patients concomitant with Phases 1 and 2.
4. In Phase 4, we will develop models of pathways and cost comparisons of alternative testing. Pathways will incorporate the differences in patient management guided by

different thresholds of the risk prediction models, that inform the minimum predicted probability that flags a diagnosis of OC.

1.4.1 ROCkeTS prospective study

The ROCkeTS prospective study: patients entering the study will complete a symptom questionnaire, donate a sample of blood and undergo an USG scan. Patients who undergo surgery will have their histology details recorded for the study, for patients who do not undergo surgery wellbeing will be ascertained at 12 months follow-up after presentation by a clinic visit or a telephone call. These data will be used at the end of the study to externally validate the risk prediction models identified in Phases 1 and 2.

FOR THE PURPOSE OF THIS PROTOCOL, THE TERM OVARIAN CANCER INCLUDES FALLOPIAN TUBE CANCER AND PRIMARY PERITONEAL CANCER.

1.5 Sub-study: ROCkeTS-GEN

ROCkeTS-GEN is a sub-study of ROCkeTS that will recruit the same population of postmenopausal women as ROCkeTS and collect a subset of the data collected in ROCkeTS which will be held on a common study database and have the same reference standards.

1.5.1 Plasma circulating tumour DNA as a diagnostic biomarker for ovarian cancer

Using next generation sequencing (NGS) techniques it is now possible to amplify small amounts of free circulating DNA in the blood to identify molecular alterations observed in tumour DNA. Somatic mutations are highly specific biomarkers of cancer and if these could be used to detect circulating tumour DNA (ctDNA) this could provide a powerful non-invasive method for earlier cancer diagnosis [9].

The most common subtype of OC is high-grade serous ovarian cancer (HGSOC) which accounts for 70% of primary invasive epithelial ovarian cancers and the majority of mortality. To be useful as a diagnostic test a biomarker needs to be highly specific for the disease of interest and ubiquitous in the target population. The gene TP53 encodes the tumour suppressor protein p53, a transcription factor that regulates the expression of proteins involved in apoptosis and genomic integrity. We have previously shown that mutations in TP53 occur in at least 99% of HGSOC cases[10] making TP53 mutations ubiquitous in HGSOC. In HGSOC TP53 mutations are located throughout the gene in all 10 coding exons (exons 2 to 11) therefore a NGS method that is not limited to assaying hotspot mutations is required. HGSOC is also a tumour characterized by complex copy number changes[11] that can be detected by shallow whole genome sequencing (sWGS).

1.5.2 ROCkeTS-GEN Project

We propose to evaluate plasma ctDNA and collect additional samples suited for future evaluation in the ROCkeTS-GEN study by:

1) Collecting plasma samples and analysing ctDNA in a nested case control design within a prospective study (ROCkeTS-GEN) that will recruit **post**menopausal participants

2) Collecting representative tissue blocks from resected tissue from patients undergoing surgery or biopsy so that the mutational profiles of plasma ctDNA can be verified against the mutational profile of tumours.

3) Evaluating plasma samples collected from women in the ROCkeTS-GEN cohort who do not have a cancer diagnosis to clearly understand the true prevalence of TP53 mutations in women who do not have cancer and further refine our sWGS analysis algorithm. This collection is imperative for establishing the true specificity of ctDNA as a diagnostic biomarker and to further improve on sensitivity of detection.

1.6 Sub-study: AOA-ROCkeTS

AOA-ROCkeTS is a sub-study of ROCkeTS that will use blood samples previously collected in the ROCkeTS postmenopausal study. The purpose of this sub-study is to optimise a novel biomarker panel for the detection of OC in the symptomatic postmenopausal patient population.

1.7. Risks and Benefits

There are no vulnerable groups or risks associated with this project that would prolong/complicate the Ethics or R&D approval processes as there is no intervention and all participants follow their normal care pathway.

2 ROCKETS STUDY DESIGN

2.1 Aim of the study

- To externally validate risk prediction models that estimate the probability of having OC for women with symptoms suggestive of OC for postmenopausal and premenopausal women.
- To identify optimal risk thresholds for the models that can guide patient management.

2.2 Design

ROCkeTS study is a single arm prospective cohort diagnostic accuracy study to evaluate existing diagnostic tools (risk prediction models and scores) for diagnosing postmenopausal and premenopausal women with ovarian cancer.

As of Version 7.0 of the protocol, ROCkeTS has been amended with recruitment focused on premenopausal women undergoing surgery/biopsy for suspected ovarian cancer or adnexal mass only. This change is necessary due to the very low prevalence of ovarian cancer observed in premenopausal women.

A test accuracy study compares measurements obtained by index tests with those obtained by a reference standard. In this way the accuracy of index tests can be estimated. A reference standard is a test (or combination of tests) that confirms or refutes the presence or absence of disease beyond reasonable doubt.

Here, the reference standard will be histology of tissues taken from patients who proceed to surgery or biopsy or assessment of wellbeing using a structured questionnaire as follow-up at 12 months after presentation for patients who do not undergo surgery. This does not apply to premenopausal women recruited following amendment in protocol v7.0 as they will all undergo surgery/biopsy for suspected ovarian cancer or adnexal mass. The diagnostic performance of the index test will be compared against that of the comparator test – the existing standard risk prediction score RMI 1.

In ROCkeTS, the index tests (risk prediction models and risk scores) will be derived in phases 1 and 2 and externally validated at the end of the study. Therefore we will collect symptom questionnaires, blood and USG data in the study to be analysed and validated at the end of the study.

2.3 Components of the new risk prediction model/s

We identify biochemical markers, symptom indices and USG as likely components of a novel risk prediction model, as these may be implemented across primary and secondary care.

2.3.1 Symptoms

Case-control studies demonstrate that symptom questionnaires have good diagnostic accuracy; symptom scores need to be refined for use by patients in primary care.[13,14] However the duration of symptoms preceding diagnosis is uncertain.[14] Symptom questionnaires may help triage prior to referral and would also help standardise symptoms for any prediction model. This is particularly important, given the subjective nature of eliciting symptoms through unstructured clinical history taking and the existing audit evidence that they are interpreted variably. A robust symptom score that can triage referral based on a questionnaire may be very useful.

2.3.2 Biochemical markers

A number of serum biomarkers tests and multiple-marker based algorithms (ROMA, OVA1) have been identified in the last decade. Abnormal He4 biomarker levels is a novel test that may improve risk stratification for OC. A recent systematic review reports that He4 shows improved diagnostic performance to Ca125, however studies showed considerable heterogeneity.[15]

Study biomarker assessments will not be available to the participant's medical team. Study biomarker assessments will not be tested in real time with the participant's standard care.

2.3.3 Ultrasound based models – IOTA risk prediction models

DHSC has a policy of increasing access to USG in primary care.[15] Timmerman group suggest IOTA USG 'm' rules may be most accurate in triaging women.[16,17] However, 'm' rules are not in common practice and have not been extensively validated in non-specialist hands.

IOTA – The International Ovarian Tumour Analysis (IOTA) collaboration – have conducted 5 previous prospective studies to derive standardization of USG description of adnexal pathology. The group have developed USG based novel risk prediction models using prospectively collected large databases to determine the optimal approach to characterize adnexal pathology preoperatively. A two-step strategy using the IOTA simple rules (image below) supplemented with subjective assessment of USG findings when the rules do not apply also reached excellent diagnostic performance (sensitivity 90%, specificity 93%) and misclassified fewer malignancies than did the RMI. A pilot validation of a three step strategy (simple descriptors/simple rules/subjective assessment) to triage benign from malignant masses demonstrated improved diagnostic performance over the RMI, even with USG examiners of varying levels of experience and training as would be the case in routine NHS practice.[16-18]

Many adnexal masses have a typical USG appearance and can therefore be easily correctly classified even by relatively inexperienced USG examiners. IOTA group have established simple rules based on a number of clearly defined USG features that can guide examiners. Using these simple rules no risk estimates are produced, but tumours are classified as benign, malignant or unclassifiable. The simple rules consist of five USG features of malignancy (M-features) and five USG features suggestive of a benign mass (B-features) (see Figure 1). These features with corresponding USG images are shown in this image. A mass is classified as malignant if at least one M-feature and none of the B-features are present, and vice versa. If no B- or M-features are present, or if both B- and M-features are present, then the rules are considered inconclusive (unclassifiable mass) and a different diagnostic method should be used.

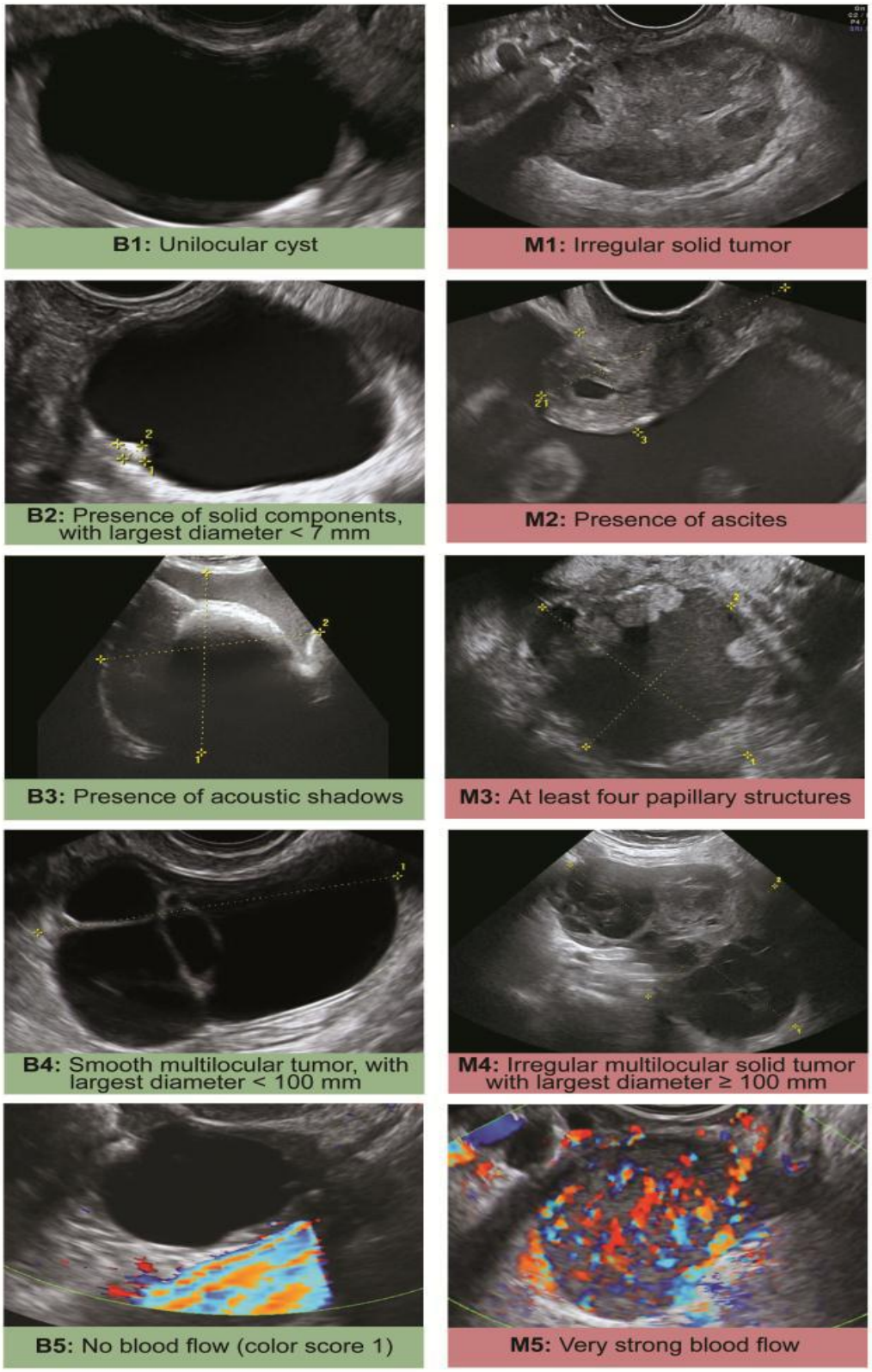


Figure 1. Illustrative examples of the use of IOTA 'm' malignancy rules are illustrated by USG images. B1–B5, benign features; M1–M5, malignant features.

2.4 Setting

Recruiting from NHS sites within the UK. Secondary care outpatients: 2 week referrals, USG clinics, routine GP referrals, cross specialty referrals. Inpatients: emergency presentations to secondary care.

2.5 Target Population

Women who have been referred to secondary care with symptoms of suspected OC.

As of Protocol v9.0: Only premenopausal women who have been referred to secondary care with symptoms of suspected OC undergoing surgery/biopsy for adnexal mass should be recruited.

Recruitment of postmenopausal women into ROCkeTS-GEN continues, see section 2.9 for further details.

Symptoms are as defined by NICE which include but are not restricted to persistent or frequent abdominal distension, feeling full (early satiety) and/or loss of appetite, pelvic or abdominal pain, increased urinary urgency and/or frequency. Symptoms listed here are not an exhaustive list.

2.6 Comparator for ROCkeTS

RMI at cut off 250.

2.7 Outcome measures and costs for ROCkeTS

External validation of diagnostic tools (risk prediction models and scores) for estimating the risk of OC in women with suspected OC.

2.7.1 Primary outcome measure

The accuracy of diagnostic tools in terms of their discrimination ability (e.g. sensitivity, specificity) and calibration (observed versus predicted probabilities) for diagnosing primary invasive ovarian malignant neoplasms versus benign, and the identification of thresholds to guide patient management decisions.

2.7.2 Secondary outcome measures

The accuracy of the models as a binary outcome of:

- Primary invasive and secondary ovarian malignant neoplasms versus benign (borderline will be considered with benign).
- Primary invasive, secondary and borderline ovarian malignant neoplasms versus benign.
- Primary invasive, secondary and borderline ovarian malignant neoplasms versus benign in the subset of patients that were assessed in one-stop clinics.
- Primary invasive, secondary and borderline ovarian malignant neoplasms versus benign where ultrasound was performed by those who had passed IOTA Quality assurance.

Reference standard for the study will be histology of tissue taken at surgery or biopsy in women who are managed surgically following study enrolment or assessment of wellbeing using a structured questionnaire as follow-up at 12 months after presentation for patients who do not undergo surgery. This does not apply to premenopausal women recruited following amendment in protocol v7.0 as they will all undergo surgery/biopsy for suspected ovarian cancer or adnexal mass.

Study data collection will be undertaken prospectively for all participants in order to inform the costs for each pathway. Following giving consent at baseline, the participants and the local study team will complete a series of CRFs (see table 1) including:

1) Participant Baseline CRF
2) Registration form

A surgery CRF will be completed where histology is attempted from tissue taken at surgery or biopsy. Should histology from one of these procedures come back as unknown but is identified at a later date from a subsequent surgery/histology, the surgery CRF should be updated. The surgery CRF will completed by the clinical team.

If no surgery CRF has been completed by 12 months post study entry, a 12 month Clinical CRF will be completed by the clinical team after either a clinic visit or a telephone call to the participant or from medical records.

If no surgery CRF has been completed by 12 months post study entry, a 12 month Participant CRF will be sent directly to the participant from Birmingham Clinical Trials Unit (BCTU) with a freepost return envelope.

If insufficient information is available through the medical records and from the participant the GP will be contacted to ensure that all available data are collected.

Participation in the ROCkeTS study is completed on receipt of a completed surgery CRF. If the participant does not have surgery then completed 12 month Clinical and Participant CRFs will indicate completion.

2.8 ROCkeTS Sample size

Due to the expected difference in performance in pre and postmenopausal women, separate sample sizes have been computed.

2.8.1 For postmenopausal women

Performance of RMI 1 is assumed to be 70% sensitive and 90% specific.[37] A sample size of n=1299 will have 90% power to detect a 13% difference in sensitivity between the preferred ROCkeTS test (83% sensitivity) and current practice test RMI 1 at threshold 250 (70% sensitivity) at the 5% significance level, allowing for a moderate positive correlation between test errors of 65%.

As of Protocol v9.0, ROCkeTS is no longer open to recruitment for postmenopausal women.

2.8.2 For premenopausal women

Performance of RMI 1 is assumed to be sensitivity of 72% and a specificity of 46%. To compare the specificity of the index tests with RMI 1, a sample size of 575 (546 before 5% drop-outs) women will have 90% power to detect at the 5% significance level a difference of 10% from a baseline of 46% specificity using the Alonzo method, assuming uncorrelated test errors and prevalence of 2.5%.

To compare the sensitivity of the index tests with RMI 1, recruitment of premenopausal women will now focus on those more likely to have OC – i.e. those on the surgical pathway until we have 105 (accounting for 5% drop-outs) women identified as having OC (a minimum of 100 events has been suggested for external validation of prognostic models)[20]. At a prevalence of 14.9%, we predict that we will require 705 patients. However, we only expect 80% of recruits to undergo surgery, which reduces prevalence to 11.9%, meaning we will require a maximum of 880 patients.

The current prevalence was calculated from data extracted on 8th December 2021. On this date there were 1032 pre-menopausal recruitments meaning that the new sample size will be 1280-1455, or an additional 248-423 recruitments.

2.9 ROCkeTS-GEN

2.9.1 Primary aim

To evaluate plasma ctDNA as a diagnostic test with increased sensitivity and specificity compared to serum CA125 for earlier diagnosis of ovarian cancer.

2.9.2 Secondary aim

To collect representative blocks from resected tumour tissue to establish a translational resource for future early detection research.

2.9.3 ROCkeTS-GEN Design

This is a prospective single arm diagnostic accuracy study where all patients receive all tests and accuracy of tests is evaluated against a gold standard of histology or 12 month outcome.

Of note, both ROCkeTS and ROCkeTS-GEN recruit women at ‘low’ or unknown genetic risk of ovarian cancer i.e. not women known to have a BRCA or other germline mutation predisposing to ovarian cancer. Recruited women will have the following tests and follow up recorded:

- CA125 (if not performed already as part of standard care).
- IOTA transvaginal ultrasound and additional abdominal scan if performed as part of standard care
- Symptom questionnaire.
- Histology result where biopsy or surgery is clinically indicated
- 12 month follow up status through a questionnaire.
- Women entering ROCkeTS-GEN will donate a plasma sample at recruitment. Women undergoing surgery for ovarian pathology will also be consented to donate tissue block/s of representative tissue for research.

Plasma samples will be collected at the time of recruitment from approximately 732 postmenopausal women recruited to ROCkeTS-GEN, to enable recruitment of 55 women with early stage HGSOC (Stage I/II)

Representative tissue block/s and slide will be retrieved from pathology labs for women recruited into ROCkeTS-GEN and proceeding to undergo surgery as part of standard care. These blocks will first undergo specialist pathology review by an expert gynaecological pathologist. DNA will then be extracted from cancer tissues and undergo targeted exon sequencing using the same platforms and single nucleotide variants and indels analysed.

2.9.4 ROCkeTS-GEN Target population

ROCkeTS-GEN is open to **post**menopausal women only.

2.9.5 ROCkeTS-GEN Analysis

Plasma ctDNA analysis at Cancer Molecular Diagnostic Laboratory (CMDL) will be performed blind from tissue DNA analysis. Sensitivity and specificity of plasma ctDNA will be established by statistical analysis at UCL. ROCkeTS-GEN uses a rapidly changing technology for analysis and therefore exact detail on how the analysis will be performed will not be detailed in the protocol but will be detailed in full in the final report and any publications.

2.9.6 ROCkeTS-GEN - Definition of test results

- True positive: Reference test assigns OC (see study outcomes for OC definition) and blood ctDNA includes clonal p53 mutation which maps to a known pathogenic tumour p53 mutation based on IARC p53 pathogenic database (http://p53.iarc.fr/) and/or Curie database (http://p53.free.fr, database of p53 mutations). Where surgery takes place, confirmatory analysis of clonal pathogenic p53 mutations will use tumour p53 DNA analysis. (A sensitivity analysis for true positives will include only those where there is a match between blood and tumour p53 mutations).
- False negative: Reference test assigns OC (see study outcomes for OC definition) but no blood ctDNA clonal p53 mutation detected which maps to pathogenic p53 (see true positive definition). (A sensitivity analysis for true negatives will include only those where there is no match between blood and tumour p53 mutations).
- False positive: Where blood ctDNA clonal p53 mutation is detected but woman has no diagnosis of OC (see study outcomes for OC definition) within 12 months follow up.
- True negative: No blood ctDNA clonal p53 mutation detected which maps to pathogenic p53 (see true positive) AND no diagnosis of OC (see study outcomes for OC definition) within 12 months follow up AND any surgical mass does not have histological diagnosis of OC (see study outcomes for OC definition) AND any surgical mass has no clonal p53 pathology mutation matching to blood ctDNA.

2.9.7 ROCkeTS-GEN Sample size

Sample size for prospective cohort sample collection

- Following an interim prevalence analysis sample size is now 732 postmenopausal women blood samples at immediate referral, allowing 5% for loss to follow up.

- The number of women to be recruited will be reviewed annually to reflect any changes due to coronavirus effects on recruitment.

Within the 732 samples, ctDNA analysis will be performed only in:

- Based on current prevalence of ovarian cancer in postmenopausal women as identified in current recruitment from ROCkeTS trial, 157 blood samples will be from women diagnosed with ovarian cancer. The primary outcome will analyse 55 samples from women with early stage (HGSOC, stage I/II). The secondary outcome #1, analyses data from all 100 women with HGSOC and the secondary outcome #2 analysis will be on all 157 women with ovarian cancer.
- 200 controls from women who do not have ovarian cancer.

The team at CMDL Cambridge performing plasma ctDNA analysis will be blinded to the clinical dataset held at UoB and UCL sites. This is to eliminate bias in analysis of the samples and to test the validity of the biomarker. To ensure this the clinical dataset will be maintained in UoB and the team at UoB will supply CMDL with the samples anonymised with participant study numbers only. CMDL will send UoB the results of the analysis and the UoB and UCL team will then check that against outcome data to analyse trial outcomes..

We will use a nested case control design based on the ongoing ROCkeTS trial.

- Sample size method: Difference in paired proportions.
- Study is powered to show a 30% expected difference in sensitivity between ctDNA and CA125 at a threshold of 35U/ml across women with early stage disease (stage I & II) - 90% power type II error, type I error 5% ($p<0.05$)

2.9.8 ROCkeTS-GEN Outcome measures

Sensitivity and specificity of plasma ctDNA analysis in the diagnosis of OC. See 5.11 ROCkeTS GEN analysis.

2.10 Schedule of Events & CRF completion for ROCkeTS and ROCkeTS-GEN

Table 1: Schedule of events and CRF completion

	Screening		Baseline		≤ 12 months	
	ROCkeTS	ROCkeTS-GEN	**ROCkeTS**	ROCkeTS-GEN	**ROCkeTS**	ROCkeTS-GEN
Eligibility Check	x[1]	x[1]				
Valid Informed Consent	x[1]	x[1]				
Registration Form CRF			x	x		
Online registration			x[2]	x[2]		
GP Letter			x	x		
Blood sample			x[3]	x[4]		
IOTA USG			x[3] Optional	Optional		
Ultrasound CRF			Optional	Optional		
Participant Baseline CRF			x[3]	x[3]		
Surgery CRF					Post-surgery/biopsy	Post-surgery/biopsy
Representative tissue block/s						X[5]
Outcome CRF					12 months – *only required if histology not obtained*	12 months – *only required if histology not obtained*
Participant 12 month CRF [6]					12 months – *only required if histology not obtained[7]*	12 months – *only required if histology not obtained[7]*

1 See section 'Approaching Potential Participants to Consent'.

2 It is acceptable to delay registration until blood sample, IOTA USG & Participant Baseline CRF are complete.

3 Blood sample, IOTA USG and Participant Baseline CRF should all be captured within 3 months of whichever of the following occurred first: presentation, IOTA USG (see section 5.1).

4 Blood samples send immediately to CMDL

5 Tissue sample (if participant proceeds to surgery) or biopsy send to Central study lab.

6 Requested by and sent directly to BCTU.

7Note that length of follow up to be tapered towards the end of the study as per section 5.6

2.11 AOA-ROCkeTS substudy

2.11.1 Aim of sub-study

The AOA-ROCkeTS sub-study aims to evaluate the AKRIVIS GD™ test for the detection of OC in the symptomatic postmenopausal patient population using blood samples previously collected in the ROCkeTS postmenopausal cohort .

Blood samples collected in the ROCkeTS study from postmenopausal women will be used to assess a novel biomarker panel - a proprietary liquid biopsy panel for detection of OC. The panel involves a novel method of quantification of two tumour glycolipids GD2 and GD3 from blood samples. The samples collected in the ROCkeTS postmenopausal cohort will be evaluated for GD2 and GD3 expression. The dataset will be used to optimize the existing novel biomarker panel algorithm for the symptomatic population. The results of the tested cohort will also be compared to the clinical standard of care biomarker CA125 (cut-off 35 U/mL) as an exploratory analysis.

2.11.2 AOA-ROCkeTS Objectives

2.11.2.1 Primary objective

To evaluate the performance of the novel biomarker panel for the detection of OC in the symptomatic postmenopausal patient population. The performance will also be assessed in combination with CA125 and/or HE4 and clinical factors such as age in women with suspected OC. The dataset will be used to derive performance measures of the novel biomarker panel. Key outcome measures will be sensitivity and specificity for detection of OC.

The reference standard for the study will be histopathology of tissue taken at surgery or biopsy in women who are managed surgically following study enrolment or follow-up at a minimum of 12 months after presentation in patients who forgo surgery.

2.11.2.2 Secondary objective:

To compare the performance of the novel biomarker panel to CA125 for detection of OC.

2.11.3 Study Rationale

This is a retrospective study testing blood samples collected in the ROCkeTS prospective postmenopausal study to assess the AKRIVIS GD™ test for detection of OC. Using existing blood samples will make efficient and further use of this existing sample and dataset, thereby not requiring further patient involvement; participants in ROCkeTS consent at the time of recruitment to their samples and data being used in ethically approved studies both in the UK and abroad.

2.11.4 Target Population

The study population will be samples and data from postmenopausal women recruited into the ROCkETs study.

2.11.4.1 Inclusion Criteria

- Appropriate clinical data available

- Minimum 500 µL serum volume available

2.11.4.2 Exclusion Criteria

- Icteric, lipemic, haemolytic, substantial particulates
- More than 2 freeze thaw cycles

2.11.5 Primary aim

The following primary endpoints of novel biomarker panel performance will be assessed:

- Sensitivity of the novel biomarker panel for detection of OC
- Specificity of the novel biomarker panel for detection of OC

Sensitivity and specificity will be calculated with 95% confidence intervals for the novel biomarker panel test for detection of OC.

2.11.6 Secondary aim

- PPV of the novel biomarker panel for detection of OC
- Negative predictive value (NPV) of the novel biomarker panel for detection of OC
- Area Under the Curve (AUC) of the novel biomarker panel for detection of OC

PPV, NPV and AUC will be calculated with 95% confidence intervals for the novel biomarker panel for detection of OC.

2.11.7 Exploratory aims

- An exploratory analysis will be conducted to compare the performance of the novel biomarker panel to the CA125 test.
- PPV, NPV, sensitivity, specificity, and likelihood ratios for detection of OC will be calculated comparing the novel biomarker panel with CA125.

2.11.8 Sample Size

A sample size of 107 confirmed positive OC cases as determined by tissue histopathology achieves a minimum of 80% power to a sensitivity of 80% (i.e. lower bound of 95% CI >=80%) when the true underlying sensitivity is 90% and the study achieved sensitivity is at least 87.85%. When factoring in the prevalence of OC in the intended use population of 10%, the total number of samples needed for the study is increased to 1,070. The study overall sample is driven by the sensitivity performance target (including the desired threshold for the lower bound of the 95% CI) and the assumed prevalence of the intended use population. Approximately 1300 women were recruited as part of the ROCkeTS study.

2.11.9 Clinical data

Clinical data and samples supplied to AOA Dx Inc. will be anonymised.

2.11.10 Sample Testing at AOA Dx Inc

All blood samples will be tested at the AOA lab for biomarkers.

3 SELECTION OF PARTICIPANTS

3.1 Source of potential participants

Patients referred as Outpatients; either as 2 week or routine referrals, USG clinics, inpatient and emergency presentation to secondary care. The whole spectrum of the suspected OC population should be considered for ROCkeTS. Participants can also be recruited into the study on the morning of surgery if there has not been a previous opportunity to recruit.

These should be new patients only, i.e. first presentation to the service; patients who are on routine follow up in the secondary care service as part of standard practice should not be approached for recruitment.

ROCkeTS will only recruit premenopausal women proceeding to surgery/biopsy for suspected OC or adnexal mass.

ROCkeTS-GEN will only recruit postmenopausal women.

3.2 Inclusion and Exclusion Criteria

3.2.1 Inclusion criteria for ROCkeTS

- Women referred with symptoms of suspected OC (typical referral symptoms are defined in section 2.5 of the protocol).
- Aged between 16 and 55 years. Premenopausal women only (as of Protocol v9.0). Menopause is defined as >12 months without menstruation. Those no longer menstruating >12 months for reasons such as contraception or hysterectomy should have their menopausal status categorised according to age; <50 years premenopausal, 51+ years postmenopausal. Patients who are still menstruating at time of recruitment and past the age of 50 will be considered premenopausal.
- In addition women must have test results from one of the following:
  1) A raised Ca125 test result (even if imaging has not been done yet)
  2) Abnormal imaging result showing a lesion (even if CA125 test is not raised).
  3) Both a raised CA125 test and an abnormal imaging result showing a lesion
- Patients able to provide informed consent.

<u>Additional inclusion criteria for premenopausal women participating in ROCkeTS:</u>

- All above inclusion criteria AND must be scheduled to undergo surgery/biopsy for suspected OC or adnexal mass.

FOR THIS STUDY WE FOLLOW THE IOTA DEFINITION OF A LESION: A LESION IS PART OF AN OVARY OR AN ADNEXAL MASS THAT IS JUDGED TO BE INCONSISTENT WITH NORMAL PHYSIOLOGICAL FUNCTION.

3.2.2 Exclusion criteria for ROCkeTS

- USG reveals simple ovarian cysts <5cm in size (very low risk of malignancy) and patient does not have a raised CA125.
- Patients who are pregnant.
- Previous ovarian malignancy.

- Active non ovarian malignancy – Women with a past history of cancer are only eligible if there are no documented persistent or recurrent disease and they have not received treatment for this in the last 12 months. This exclusion does not apply to patients with premalignant disease e.g. cervical intra-epithelial neoplasia or patients receiving Tamoxifen/other drugs to prevent breast cancer recurrence.

3.2.3 Inclusion criteria for ROCkeTS-GEN

- Women referred with symptoms of suspected OC (typical referral symptoms are defined in section 2.5 of the protocol).
- Aged between 51 and 90 years. Only postmenopausal women are included. Menopause is defined as >12 months without menstruation. Those no longer menstruating >12 months for reasons such as contraception or hysterectomy should have their menopausal status categorised according to age; <50 years premenopausal, 51+ years postmenopausal.
- In addition women must have test results from one of the following:
  1) A raised Ca125 test result (even if imaging has not been done yet)
  2) Abnormal imaging result showing a lesion (even if CA125 test is not raised).
  3) Both a raised CA125 test and an abnormal imaging result showing a lesion
- Patients able to provide informed consent.

3.2.4 Exclusion criteria for ROCkeTS-GEN

- USG reveals simple ovarian cysts <5cm in size (very low risk of malignancy) and patient does not have a raised CA125.
- Previous ovarian malignancy.
- Active non ovarian malignancy – Women with a past history of cancer are only eligible if there are no documented persistent or recurrent disease and have not received treatment for this in the last 12 months. This exclusion does not apply to patients with premalignant disease e.g. cervical intra-epithelial neoplasia or patients receiving Tamoxifen/other drugs to prevent breast cancer recurrence.

3.3 Approaching potential participants for consent

Potential participants will be approached at their clinic appointment or whilst they are inpatients in hospital. Eligible patients may also be identified at scan departments. If potential participants cannot be approached at this time then remote or virtual consent can be obtained as documented in 3.4. A member of the usual care team will approach patients via phone or remotely. The approach process will parallel the in-person process.

If they are interested they will be supplied with the participant information sheet (PIS) and given the opportunity to ask any questions.

Patients who are admitted into hospital as emergencies and undergoing investigations for OC will be approached to give informed consent. In our experience, these patients are unwell enough to need hospital stay but are not critically unwell to the extent that they cannot fully understand the implications of consent.

Participants who potentially fulfil the inclusion criteria must have their eligibility confirmed by a medically qualified doctor.

Consent should be sought under unhurried circumstances – however consent can be obtained on the same day the potential participant is approached (i.e. the potential participant may choose to consent on the day that they receive their PIS or take time to reflect and consent at a later date) when entry criteria are fulfilled. Consent will be sought as follows:

- A PIS will be given to all women referred through rapid access 2 week wait clinics, USG and outpatient clinics for suspected OC. This PIS and posters on the study will also be made generally available and prominently displayed in various areas within the participating hospitals and their primary care practices – including clinics, corridors, MDT meeting rooms, ultrasound rooms, offices. PIS are also available via the trial website. All women presenting as acute admissions to hospital will be offered the PIS and the option of study participation, unless deemed inappropriate by the attending clinical team for clinical reasons. However wherever possible the patient should make the decision on whether to receive the study information or not.
- A potential participant can also be approached over the telephone/video call. Once the potential participant has shown interest in the study the PIS, Informed Consent Form (ICF) and baseline participant booklet can also be sent out for them to read at home. The site staff will then follow this up with remote consent as documented in 3.4.
- A potential participant can also be approached on the morning of surgery if it is not possible to approach in clinic or by remote consent before this time. They will be given the PIS and enough time to read the information before consent takes place.
- Where necessary, appropriate trust interpreters will be asked to aid discussion relating to study participation. Patients who do not understand English are eligible to enter the study provided an interpreter can fully explain the ICF, PIS and symptom questionnaire to them.
- The initial approach to the potential participant will be through their clinician or appropriately trained person delegated the responsibility to approach patients to discuss the study. The consent form will be signed by the patient prior to any blood samples being taken and counter signed by the person delegated the responsibility of taking consent.
- If the scan is part of the standard care pathway, participants may receive an USG prior to consent as part of usual care. Data from these scans can be collected retrospectively into the study if the site already follows the IOTA models/rules for scanning following a participant consenting to join the trial. As the Royal College of Obstetricians and Gynaecologists has recommended IOTA rules in their guidance on management of women with cysts, it is likely that at some sites patients will have the scan performed prior to study entry. Prospective collection of USG data will be used at centres that do not use the IOTA rules as part of the trust's standard practice where no prior scan has been performed. This flexibility is important as it will reduce additional burden on both participant and the trial centre.

  For ROCkeTS-GEN and for ROCkeTS (as of protocol v9.0), the IOTA scan is optional. Those collected from ROCkeTS participants must have been conducted by an IOTA certified scanner. Scans for ROCkeTS-GEN participants do not need to be conducted by an IOTA certified scanner.
- For patients who have already attended a hospital visit and who have not been given a PIS, provided a clinician has confirmed eligibility for inclusion into the study the research nurse can approach them by phone to provide information about the study.

3.4 Obtaining consent

The participant's written informed consent to participate in the study will be obtained before entry and after a full explanation has been given of the study. PIS and ICF will be provided so that patients can find out more about the study before deciding whether or not to participate.

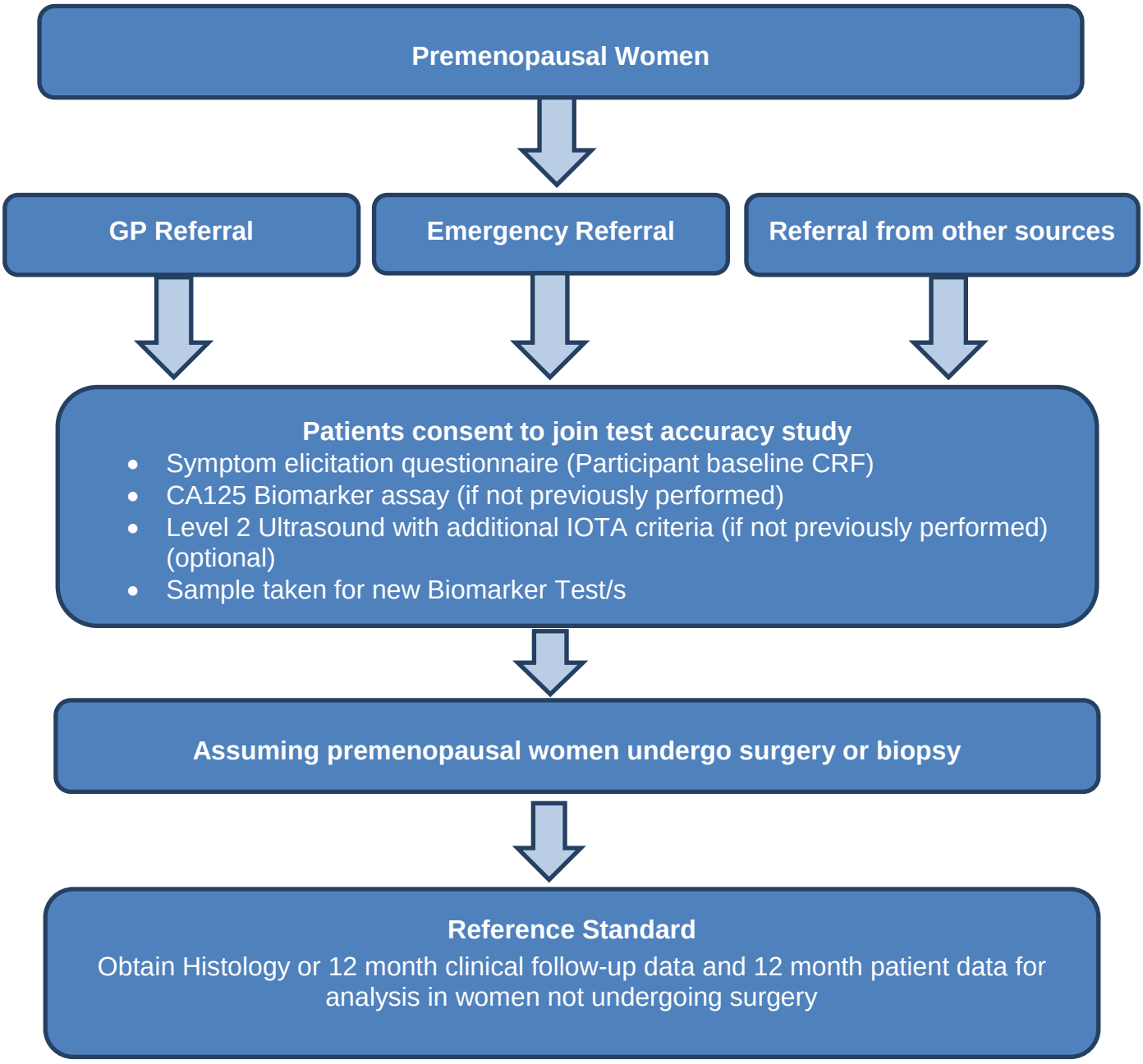
- Visits throughout the informed consent process will take place in person at site or by telephone/video call as per local practice where patient and/or public health circumstances dictate. Signed informed consent forms will be on paper, completed in person or remotely if the circumstances dictate.
- If consent is not being taken in person, then the patient should be sent a PIS and ICF if not already supplied; then the clinical staff or delegated person will talk through each statement of the ICF with the patient, who will be asked to initial the boxes on the ICF and sign and date the form whilst on the call. This will be documented in the clinical notes. The ICF will then be returned to the clinical site for countersignature by the person who took consent remotely and the date the ICF was countersigned will be documented. Once a fully signed ICF has been completed it will be stored in the Investigator Site File and a copy will be sent to BCTU and to the participant for their records. The participant baseline booklet can also be returned with the ICF.
- Participants will be assigned a study trial number after registration.

3.4.1 Informing the participant's GP

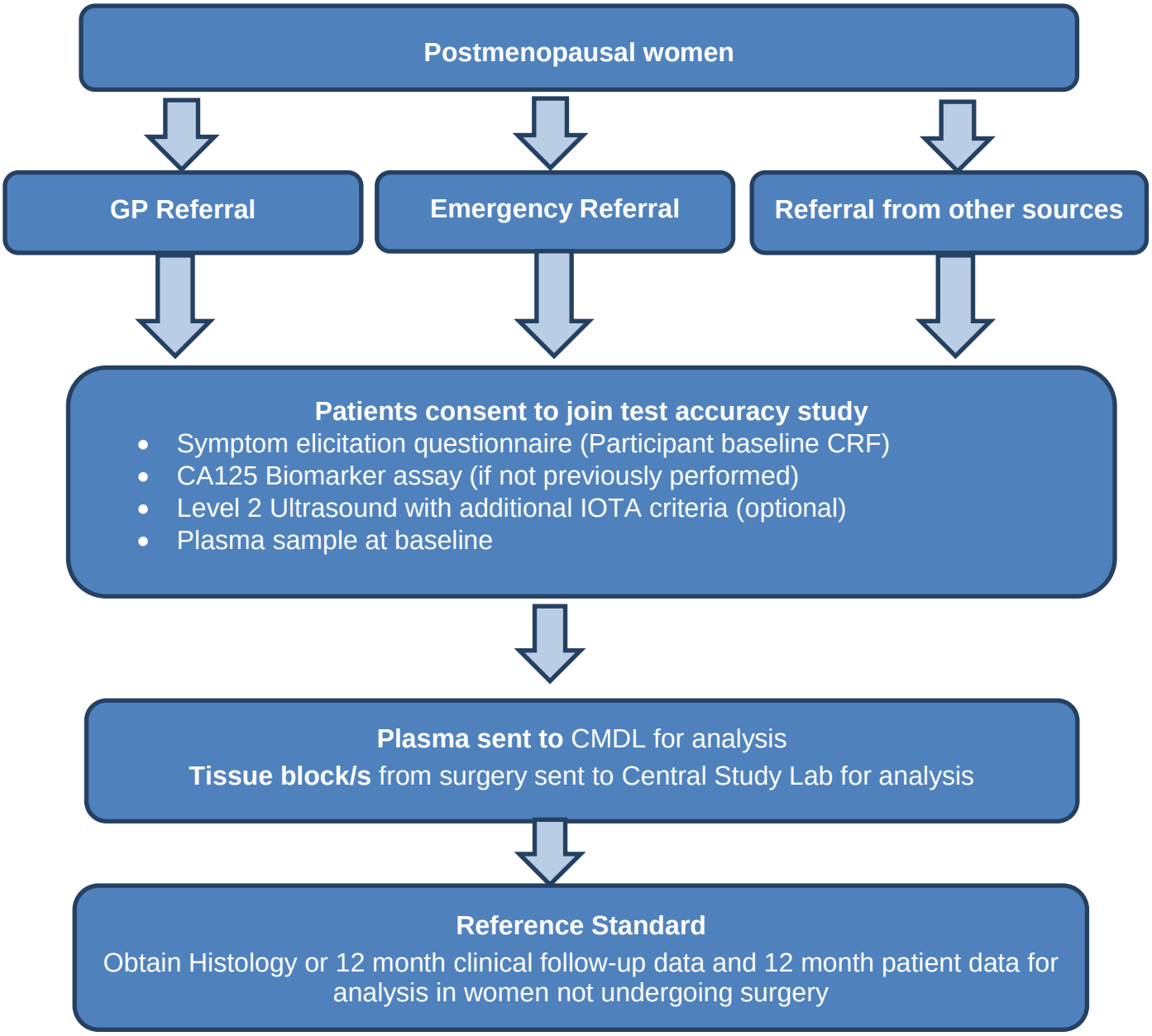
The participant's GP should be notified with the participant's consent and a specimen "GP Letter" is supplied.

4 RECRUITMENT

4.1 Flow chart for ROCkeTS



4.2 Flow chart for ROCkeTS-GEN



4.3 Recruitment for ROCkeTS and ROCkeTS-GEN

In order to obtain the large number of participants necessary for the reliable evaluation of the index tests, the study will need the participation of more than one centre. Study procedures therefore need to be kept simple, with minimal extra workload placed on participating clinicians, beyond that required to manage their patients. This will be achieved by simple entry procedures, early consent of women, the use of standard local testing regimens, minimising documentation and streamlining data collected procedures. Regular newsletters will keep collaborators informed of study progress, and regular meetings will be held to report progress of the study and to address any problems encountered in the conduct of the study.

4.4 Organisation of recruitment

Recruitment will be organised and supported by dedicated research nurses who will work with local lead investigators and lead sonographer/radiologists. We believe that that the following strategy is likely to be successful in achieving maximum recruitment.

- Each participating centre has nominated an interested local lead clinician and imaging lead who will take responsibility for ensuring IOTA implementation, training and quality assurance is organised at the site. Sites recruiting to ROCkeTS will demonstrate competency in IOTA rules/models of ultrasound. Training may be delivered online or face to face. Where appropriate, sites will receive a site visit from a person nominated by the IOTA team.
- **Whilst some participants in ROCkeTS-GEN will receive an IOTA ultrasound, the relevant scanner does not need to be IOTA certified, as they do in ROCkeTS.**
- Identification of appropriate research staff (doctors, clinical nurse specialists, research nurses, sonographers).
- A nurse at each site with responsibility for consent and study specific procedures.
- As part of the standard care pathway participants may receive an USG prior to consent and the data from these scans can be collected retrospectively if the scan centre already follows the IOTA rules which is likely at some centres as the use of IOTA rules is recommended by the RCOG. Prospective collection of ultrasound scan data will be used at centres that do not use the IOTA rules in the trust's standard practice. This flexibility is important as it will help reduce additional burden on both participant and the trial centre.
- For participants in ROCkeTS as of protocol v9.0 and ROCkeTS-GEN the IOTA scan is desirable, but not mandatory.
- Appointment of a trial manager at BCTU, who will liaise with all the coordinating research nurses at each site and coordinate the screening at this hospital, provide training and trouble-shoot recruitment and follow-up problems.
- Provision of simple written study information, supported by face to face discussion with clinical staff.
- Provision of regular feedback on progress in study recruitment, including individual hospital teams' performance and progress against targets.
- Regular newsletters to all relevant staff involved in the study.

4.5 Other management at discretion of local doctors

Apart from the study tests, all other aspects of participant management are entirely at the discretion of the local doctors and as per the RCOG guidelines for management of these

participants.[9,19] Treating clinicians will be asked to record their treatment recommendations as per standard care and after any additional USG information in order to assess the impact of this test on care pathways.

4.6 Withdrawal

Participants will be free to withdraw from the study at any time without any effect on standard of care; data and samples provided up to the point a participant withdraws will be retained.

5 STUDY PROCEDURES AND TESTS

5.1 Assessments for ROCkeTS

All assessments should be done within 3 months of one another – the clock starting at either presentation or at the point the first assessment is done – whichever occurs first.

1. Whilst symptoms are routinely elicited and recorded as part of a clinical assessment at presentation to secondary care, this is not standardised and involves the doctor transcribing elicited symptoms from the participant. Study participants entering the study will complete a symptom elicitation questionnaire, anxiety questionnaire and a general CRF including resource usage

2. A transabdominal and transvaginal USG is always performed for all patients suspected of OC as part of NICE guidelines. This is usually performed by trained ultrasonographers who report the scan as per routine.

   Ultrasonographers will record the USG variables and score the USG using a scoring system called the IOTA 'm' rules.[16,18] The IOTA rules have been recommended by the RCOG in women with ovarian cysts in their guidelines.[9] For most women in the study this will only mean some additional data being collected during their USG appointment; for sites that use these rules in standard practice already it may mean data collection only with no additional work for the sonographer. For a small number of women, this may mean an additional USG scan after consent. Some sites will have already used IOTA 'm' rules as part of standard care; at these sites it may be possible to collect retrospective USG data after consent. Surgery should be planned within 120 days after USG.

   For ROCkeTS as of protocol v9.0 an additional transvaginal scan recording IOTA variables is desirable but not mandatory. For ROCkeTS, this is because the study team recognise that in women on the surgical pathway, timelines may not permit scheduling of an additional transvaginal scan. If a transvaginal scan recording IOTA variables has been performed within 120 days prior to the surgery then that scan can be used to provide information for ROCkeTS.

3. Participants will have an additional blood sample taken at baseline for biomarker assessment at the end of the study. Details of blood sample collection will be provided in the lab manual.

5.2 Index tests for ROCkeTS

The diagnostic tools are split into two lists of clinical interest and academic interest.

Clinical list:

- RMI 1 (Thresholds: 200, 250)
- ROMA (Thresholds: 13.1% for premenopausal and 27.7% for postmenopausal, 12.5% for premenopausal and 14.4% for postmenopausal, 7.4% for premenopausal and 25.3% for postmenopausal, 11.4% for premenopausal and 29.9% for postmenopausal)
- UCL model
- ADNEX (Primary threshold: 10%, secondary threshold: 3%)
- IOTA simple rules (see Appendix C for more details)

Academic list:

- IOTA sRisk model (Primary threshold: 10%, secondary threshold: 3%)
- CA125 (Thresholds: 87 IU/ml for premenopausal and 35 IU/ml for postmenopausal)

5.3 Assessments for ROCkeTS-GEN

1. Whilst symptoms are routinely elicited and recorded as part of a clinical assessment at presentation to secondary care, this is not standardised and involves the doctor transcribing elicited symptoms from the participant. Study participants entering the study will complete a symptom elicitation questionnaire, anxiety questionnaire and a general CRF including resource usage.

2. A transabdominal and transvaginal USG is always performed for all patients suspected of OC as part of NICE guidelines. This is usually performed by trained ultrasonographers who report the scan as per routine. **For ROCkeTS-GEN an additional transvaginal scan recording IOTA variables is desirable but not mandatory.**

3. Participants will have an additional blood sample taken at baseline for ctDNA analysis.

5.4 Quality assurance of index tests

The study team recognise that USG in particular is subjective and operator dependent. Therefore only those sonographers/doctors who are IOTA certified may perform USG as part of the ROCkeTS study. Sites will commit to undergoing quality assurance as part of this.

Quality assurance of testing will begin with a clearly documented staff training programme. A register of staff who have been trained and had their competence assessed will be maintained, and only staff whose names appear on this list will be permitted to undertake testing. Staff will also receive a site visit and assessment of their competence. Competence will be assessed by those authorised by the IOTA team.

As part of ROCkeTS, sites will be required to upload ultrasound images to the ROCkeTS study database for each participant enrolled in the study. We require at least 5 images per participant (but more can be supplied), but it is expected that the images should show all aspects of the IOTA standards reported.

For ROCkeTS-GEN, scanners are not required to be IOTA certified in order to perform the IOTA ultrasound. Currently, no quality assurance for these images is planned.

5.5 Reference standard/Follow-up schedule

Reference standard for the study will be histology of tissue taken at surgery or biopsy in women who are managed surgically following study enrolment. Outcome of participants referred for suspected OC that do not undergo surgery will be assessed by a follow-up visit at 12 months or by a telephone call or a questionnaire from the research nurse at 12 months, as per the local investigators' discretion and clinical assessment. Wellbeing will be ascertained at this follow-up. This does not apply to premenopausal women recruitment following amendment in protocol v7.0 as they will all undergo surgery/biopsy for suspected ovarian cancer or adnexal mass.

5.6 Study duration

For **ROCkeTS post-menopausal participants** we anticipate recruitment of 1299 participants. This arm of the trial has completed recruitment and follow up.

For **ROCkeTS pre-menopausal participants** we anticipate recruitment of 1280-1455 participants. The current recruitment end date for ROCkeTS is 31st March 2023. The follow-up end date is 30th June 2023.

As per protocol, where histology is unavailable, participants should be followed up for the full 12 months, however the study recognises that for participants recruited within the last 12 months of the recruitment period (i.e. after the 1st July 2022) the full 12 month follow-up will not be possible; instead these participants should only be followed up until 30th June 2023.

For **ROCkeTS-GEN participants**, we anticipate recruitment of 732 participants. The current recruitment end date for ROCkeTS-GEN is 31st October 2023. The follow up end date is 31st January 2024.

As per protocol, where histology is unavailable, participants should be followed up for the full 12 months, however the study recognises that for participants recruited within the last 12 months of the recruitment period (i.e. after 1st February 2023) the full 12 month follow-up will not be possible; instead these participants should only be followed up until 31st January 2024.

5.7 Sample acquisition, storage and transport

Please refer to the laboratory manual.

5.7.1 ROCkeTS-GEN Sample Acquisition

Blood collection and initial processing (labelling, handling) will be performed at trial sites and will then be transferred for further processing at CMDL.

5.8 Data collection

All information will be collected on standard proformas (Table 1) and identified by study number, initials and date of birth. Registration Form, Participant Baseline CRF, Ultrasound CRF, Surgery CRF, and Outcome CRF will be entered by the relevant site directly into the study database via a web interface. For ROCkeTS, images obtained from ultrasounds will

be anonymised and uploaded by the relevant site directly into the study database via a web interface.

The coordinating centre (BCTU) will send the Participant 12 month CRF directly to the participant's home, requesting the completed form is returned directly to it. Upon receipt BCTU will enter data directly into the study database via a web interface.

We aim to collect a minimal demographic dataset including age, ethnicity, parity, GP details and significant medical/surgical history. We aim to use the NHS number as the primary identifier when linking to national registries and to track individuals throughout the NHS. Some additional data will be collected at follow-up.

Data will be collected on relevant medical, obstetric and gynaecological, surgical history, emotional impact as well as information on the symptoms that prompted GP referral or investigation. USG information will be collected. Data on the reference diagnosis will be obtained from the histopathology form and a structured template to assess wellbeing for participants who do not undergo surgery will be developed in association with the participating sites.

5.9 Death

If a participant dies prior to histology data being provided to BCTU (if available), inform BCTU immediately via a Change of Status form. This is to ensure that the Participant 12 months CRF is not sent to the home of the deceased.

5.10 Analysis - ROCkeTS

5.10.1 Test accuracy

We will report estimates of sensitivity, specificity, c-statistic (area under Receiver operating curve (ROC) curve), PPV and NPV and for models a calibration plot.

The risk prediction models identified in the ROCkeTS project phases 1 and 2 will each produce a predicted risk of OC by 12 months for all the individuals in our study. Therefore, we will compare the observed outcome at 12 months with this predicted risk. The calibration (in terms of calibration slope) and discrimination (e.g. c-statistic) will be evaluated for the models identified in Phases 1 and 2, and their performance compared to the existing RMI 1 model. The calibration will be shown visually by grouping women into deciles ordered by predicted risk and considering the agreement between the mean predicted risk and the observed events in each decile.

5.10.2 Cost consequence analysis ROCkeTS

Resource usage for each of the diagnostics will be broken down and displayed along with their unit costs alongside the outcomes for each pathway. The resource usage will include the different types of tests administered, the number of inpatient and outpatient consultations, and any operative procedures undertaken. This approach will help to show which are the major cost drivers for each of the diagnostic pathways and will be collected as part of the clinical CRF.

5.11 Analysis - ROCkeTS-GEN

UCL will compare ctDNA to CA125 35U/ml as an alternative test used at this point in the patient pathway in clinical practice as specified in primary and secondary outcomes. All test

comparisons will be in relation to the reference test results, so the comparative accuracy of tests to detect ovarian cancers can be calculated. We will report estimates of sensitivity, specificity, PPV and NPV. Imputation will be used to account for missing data and imperfect reference data.

5.11.1 Primary outcome

Comparison of sensitivity and specificity of ctDNA to CA125 at threshold of 35U/ml for early stage (stage I/II) high grade serous ovarian cancer (HGSOC) in postmenopausal women.

- >90% power to detect a 30% difference in sensitivity (estimated sensitivity ctDNA 80% for early stage (stage I/II) HGS ovarian cancer, compared to 50% sensitivity CA125 at threshold of 35U/ml. This power calculation assumes a conservative estimate of complete independence of CA125 and ctDNA test results.
- With 200 blood samples from women without disease, the specificity can be estimated to a precision of 95% CI range of 10% (5% above and 5% below the estimated specificity).
- 80% power to detect a 11% difference in specificity (estimated 75% for ctDNA and 82% for CA125 at 35U/ml) based on all 200 women without cancer

5.11.2 Secondary outcomes

- **Secondary outcome #1:** Diagnostic accuracy for all stages of HGSOC. Comparison ctDNA sensitivity and specificity to tests suitable for primary care (CA125 at 35U/ml) 80% power to detect an expected 20% difference in sensitivity (82% ctDNA, 68% CA125 35U/ml). under conservative assumption that tests are independent. With only 200 controls from women without cancer, there is 90% power to detect a 11% difference in specificity (estimated 93% for ctDNA and 82% for CA125 at 35U/ml) based on all 200 women without cancer.
- **Secondary outcome #2:** Diagnostic accuracy for all ovarian cancers. Greater power for this outcome than for both primary and secondary outcomes, as expect 157 women with cancer based on a prevalence of 8% for all cancers. Assuming ctDNA all stages all types: 82% sensitivity, 93% specificity all stages, there is 80% power to detect 18% difference in sensitivity under conservative assumption that tests are independent. Also 90% power to detect a 11% difference in specificity (estimated 93% for ctDNA) based on all 200 women without cancer.

5.11.3 Reduction of bias: blinding of test interpretations

- Index test interpretations (ctDNA) will be blinded from other index tests (RMI, CA125, USG). Some of the index tests in current pathways will be interpreted as part of current practice and blinding between these test interpretations will be maximized whilst maintaining patient best care.
- Index test interpretations will be blinded where possible from rest of diagnostic pathway – i.e. only using relevant clinical information known at time in clinical pathway.
- Index test interpretations will be blinded from reference standard interpretation (histology and clinical follow up).
- As part of the blinding, the analysis algorithm for ctDNA from blood will be analysed using auditable analysis code which will be locked down prior to comparison to surgical tumour mutation comparison. The ctDNA mutation algorithm includes the ratio of core p53 mutations [polymerase error rate propagated on PCR] to pathogenic p53 [identified from p53 cancer DNA mutations] threshold for a positive test result.

5.12 Analysis – AOA-ROCkeTS

An analysis of sensitivity and specificity for detection of OC will be conducted with 95% confidence intervals calculated per section 2.11. Analysis will be performed by AOA Dx and crosschecked University of Birmingham.

6 ADVERSE EVENT REPORTING FOR ROCKETS AND ROCKETS-GEN

There are no foreseeable risks of mortality or significant morbidity associated with testing. Every effort will be made to minimise any risk through training. **Therefore only serious adverse events[*] (SAEs) believed to be associated with any study procedures should be reported**. SAEs should be reported via email to the study email address.

The collection and reporting Serious Adverse Events (SAEs) will be in accordance with Good Clinical Practice (GCP) and the Research Governance Framework 2005.

Safety will be assessed continuously throughout the study. Safety monitoring has been delegated by the Sponsor (University of Birmingham) to BCTU. There are no Investigational Medicinal Products being used as part of the ROCkeTS study or ROCkeTS-GEN sub-study and the tests evaluated in the study are not being used to determine patient management. A risk assessment of the ROCkeTS study and ROCkeTS-GEN sub-study has been performed with all testing considered to be of low risk.

6.1 Definition of a Serious Adverse Event

The definition of an SAE is an untoward event that:

- results in death
- is life-threatening*
- requires hospitalisation** or prolongation of existing hospitalisation
- results in persistent or significant disability or incapacity
- Consists of a congenital anomaly/ birth defect
- or, is otherwise considered medically significant by the Investigator

*The term “life-threatening” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Patients must be formally admitted – waiting in out-patients or A&E does not constitute an SAE (even though this can sometimes be overnight). Similarly, planned hospitalisations that clearly are not related to the condition under investigation or hospitalisations/prolongation of hospitalisation due to social reasons should not be considered as SAEs.

[*]For the purposes of this study, “**serious**” adverse events are those occurring in participants which are fatal, life-threatening, disabling or require some form of medical or surgical treatment.

6.2 Reporting period

The main theoretically possible recognised reportable SAEs associated with this study relate to the blood sample being taken, USG conducted or distress following completion of baseline questionnaire. SAEs occurring within 24 hours of one of these events should be reported immediately upon awareness to BCTU on an SAE form. The assessment of relatedness and expectedness is a clinical decision based on all available information at the time.

SAEs outside of this timeframe can also be reported if it is the opinion of the Investigator that there is a possible causal relationship to another aspect of the study. An assessment of relatedness and expectedness will also be undertaken by the Chief Investigator (or designee).

6.3 Reporting procedure – at Site

SAEs believed associated with any study procedures will be notifiable to BCTU **immediately and within 24 hours of becoming aware of the event**. On becoming aware that a participant has experienced said SAE, the Investigator (or delegate) must complete, date and sign an SAE Form. The form should be sent to BCTU using the study email address. The Investigator will also be asked to provide a categorisation of seriousness and causality (continue reading for further details).

For contact details, refer to the 'ROCkeTS Study Office' section at the front of this protocol.

For SAE Forms completed by a member of the site trial team other than the Principal Investigator (PI), the PI will be required to countersign the original SAE Form to confirm agreement with the causality and seriousness/severity assessments. The form should then be returned to BCTU and a copy kept in the Site File.

Investigators should also report SAEs to their own Trust in accordance with local practice.

6.4 Causality assessment

AEs defined as serious and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the PI (or delegate) will be asked to define the causality and the severity of the AE.

Causality (relatedness) will be categorised according to the following Table 2.

Table 2: Definitions of relatedness

Category	Definition	Causality
Definitely	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out	Related
Probably	There is evidence to suggest a causal relationship, and the influence of other factors is unlikely	
Possibly	There is some evidence to suggest a causal relationship. However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant events or medication)	
Unlikely	There is little evidence to suggest there is a causal relationship. There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant events or medication)	Unrelated
Unrelated	There is no evidence of any causal relationship	

6.5 Assessment of Expectedness

Expectedness will be assessed by the CI or designee using this study protocol as the reference document. Table 3 gives definitions of expectedness with respect to SAEs.

Table 3: Definitions of expectedness

Category	Definition
Expected	An adverse event that is consistent with known information about the study related procedures.
Unexpected	An adverse event that is not consistent with known information about the study related procedures

6.6 Provision of follow-up information

Participants should be followed up until resolution or stabilisation of the event. Follow-up information should be provided on a new SAE Form, making sure to include the SAE reference number, provided by the Trials Unit upon receipt of the initial SAE.

6.7 Reporting procedure – ROCkeTS Study Office

On receipt the Study Office will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt within 1 working day. The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE.

On receipt of an SAE Form, seriousness and causality (relatedness to the study intervention) will be assessed independently by the CI. Further information may be immediately requested from the clinical team at site. The CI will not overrule the causality or seriousness assessment given by the site PI, but may add additional comment on these.

An SAE judged to have a reasonable causal relationship with study processes will be regarded as a related SAE. The CI or delegate will assess all related SAEs for expectedness. If the event is assessed as unexpected it will be classified as **an unexpected and related SAE**.

6.8 Reporting procedure to Research Ethics Committee (REC)

SAEs categorised by the CI as unexpected and related will be subject to expedited reporting to the REC by the Study Office within 15 days after the Study Office has been notified. A copy will also be sent to the University of Birmingham Research Governance Team at the same time.

The Study Office (on behalf of the CI) will inform all PIs of relevant information about SAEs that could adversely affect the safety of participants.

The REC will be notified immediately if a significant safety issue is identified during the course of the study. The University of Birmingham Research Governance Team will also be informed at the time that the REC is informed.

7 DATA ACCESS AND QUALITY ASSURANCE

7.1 Confidentiality of personal data

Individual participant information obtained as a result of this study is considered confidential. Each participant will be allocated a unique study number at recruitment.

Personal data and sensitive information required for ROCkeTS and ROCkeTS-GEN will be collected directly from study participants and hospital notes. Participants will be informed about the transfer of this information to BCTU and asked for their consent. The data will be entered onto a secure computer database, either directly via the internet using secure socket layer encryption technology or indirectly from paper by BCTU staff. Study database will be held in a secure internet facility, through an ISO 9001 accredited and FDA compliant Integrated Trial Management System (MedSciNet). Data will be stored on a secure server under the provisions of the Data Protection Act and/or applicable laws and regulations. Access to data will be restricted by usernames and passwords. The necessary study data will be encrypted. No study data will be held in handheld media, laptops, personal computers, or other similar media.

The online database will be maintained according to prescribed security policies of BCTU or MedSciNet. These cover assignment of passwords, encryption, database immediate back-up, offsite back-up and disaster recovery processes. Electronic data will be backed up every 24 hours to both local and remote media in encrypted format. Paper-based data (e.g. signed consent forms) will be kept in locked filing cabinets.

Participants will also be informed that, and consent to, their samples, being transferred from local centres to the central laboratory. Samples will only be identified by study number. Central laboratory staff will not have access to personal data.

All personal information received in paper format for the study will be held securely and treated as strictly confidential according to BCTU policies. All staff involved in the study

(clinical, academic, BCTU) share the same duty of care to prevent unauthorised disclosure of personal information. No data that could be used to identify an individual will be published.

7.2 Monitoring and audit

Investigators and their host Trusts will be required to permit study-related monitoring and audits to take place by the Study Office or Sponsor, providing direct access to source data and documents as requested. Trusts may also be subject to inspection by the Research and Development Manager of their own Trust and should do everything requested by the CI to prepare and contribute to any inspection or audit. Study participants will be made aware of the possibility of external audit of data they provide in the PIS.

7.3 Statistical monitoring throughout the study

The prevalence of OC in the study will be constantly monitored and sample size calculations will be reviewed to check if the study has accrued enough samples and data to report.

7.4 Project Oversight Group

The Project Oversight Group (POG) provides independent supervision for the study, providing advice to the CI and Co-Investigators and the Sponsor on all aspects of the study and affording protection for participants by ensuring the study is conducted according to the Research Governance Framework for Health and Social Care and/or the Research Governance Framework for Health and Community Care.

If the CI and Co-Investigators are unable to resolve any concern satisfactorily, Principal Investigators (PI) and all others associated with the study may write through the Study Office to the chairman of the POG, drawing attention to any concerns they may have or about any other matters thought relevant.

7.5 Long-term storage of data

After the end of the study, the site files from each centre should be archived by the NHS Trust as per regulations for a non-CTIMP.

All data will be stored for at least 10 years. Any queries or concerns about the data, conduct or conclusions of the study can also be resolved in this time.

Study data will be stored within BCTU under controlled conditions for at least 3 years after closure. Long-term offsite data archiving facilities will be considered for storage after this time. The BCTU has standard processes for both hard copy and computer database legacy archiving.

7.5.1 Data sharing for ROCkeTS only

There are data sharing agreements in place between the IOTA, UKCTOCS groups and AOA Dx, Inc. with the ROCkeTS Trial Team. Over the duration of the study data, will be shared between these groups. All data shared between groups will be fully anonymised and has been clearly explained in the PIS and consent form.

8 ORGANISATION AND RESPONSIBILITIES

To ensure the smooth running of the study and to minimise the overall procedural workload, it is proposed that each participating centre should designate individuals who would be chiefly responsible for local co-ordination of clinical and administrative aspects of the study.

All investigators are responsible for ensuring that any research they undertake follows the agreed protocol, for helping care professionals to ensure that participants receive appropriate care while involved in research, for protecting the integrity and confidentiality of clinical and other records and data generated by the research, and for reporting any failures in these respects, adverse drug reactions and other events or suspected misconduct through the appropriate systems.

8.1 Local Co-ordinator at each centre

Each Centre should nominate a clinical lead Doctor and an USG lead; one of whom will act as the local PI and bear responsibility for the conduct of research at their centre. The responsibilities of the local PI will be to ensure that all medical and nursing staff involved in the care of participants are well informed about the study and trained in study procedures, including obtaining informed consent. The local PI should liaise with the Trial Manager on logistic and administrative matters connected with the study. The USG lead will take responsibility for co-ordinating IOTA USG delivery within the research study.

8.2 Nursing Co-ordinator at each centre

Each participating centre should also designate one nurse as local Nursing Coordinator. This person would be responsible for ensuring that all eligible participants are considered for the study, that patients are provided with study information sheets, and have an opportunity to discuss the study if required. The nurse may be responsible for collecting the baseline participant data and for administering the follow-up evaluations. Again, this person would be sent updates and newsletters, and would be invited to training and progress meetings.

8.3 The Study Office

The BCTU Study Office is responsible for providing all study materials, including the study folders containing printed materials and the update slides. These will be supplied to each collaborating centre, after relevant ethics committee approval has been obtained. Additional supplies of any printed material can be obtained on request. The Study Office is responsible for collection and checking of data (including reports of SAEs thought to be due to study investigations), for reporting of serious and unexpected AEs to the sponsor and/ or regulatory authorities and for analyses. The Study Office will help resolve any local problems that may be encountered in study participation.

8.4 AOA-ROCkeTS

8.4.1 University of Birmingham

University of Birmingham will provide anonymised blood samples of participants enrolled into the ROCkeTS study along with matched anonymised clinical data.

8.4.2 AOA Dx Inc.

AOA Dx Inc. will conduct testing of the blood samples and novel biomarker panel optimization. The named statistical team will then undertake the statistical analysis as per section 2.10. Upon undertaking the analyses, the results will be shared with the University of Birmingham team for review and crosschecking.

8.5 Research Governance

The conduct of the study will be according to the Research Governance Framework for Health and Social Care and/or the Research Governance Framework for Health and Community Care.

All centres will be required to sign an Investigator's Agreement, detailing their commitment to accrual, compliance, Good Clinical Practice (GCP), confidentiality and publication. Deviations from the agreement will be monitored and the POG will decide whether any action needs to be taken, e.g. withdrawal of funding, suspension of centre.

The Study Office will ensure researchers not employed by an NHS organisation hold an NHS honorary contract for that organisation.

8.6 Ethical and Trust management approval

Trust R&D departments will conduct local governance checks and assess the facilities and resources needed to run the study, in order to give host site permission. The Study Office is able to help the local PI in the process of the site specific assessment by completing much of Site Specific Information section of the standard IRAS form as possible. The local PI will be responsible for liaison with the Trust management with respect to locality issues and obtaining the necessary signatures at their Trust.

As soon as Trust approval has been obtained, the Study Office will send a folder containing all study materials to the local PI. Potential study participants can then start to be approached.

8.7 Funding and cost implications – ROCkeTS

The research costs of the study are funded by a grant from the National Institute of Health research awarded to the University of Birmingham.

The study has been designed to minimise extra 'service support' costs for participating hospitals, with no extra visits to hospital and no extra tests. Additional NHS service support costs associated with the study, e.g. gaining consent, aliquoting extra blood samples etc, are estimated in the Site Specific Information section of the standard IRAS form.

8.8 Funding and cost implications – ROCkeTS-GEN

The ROCkeTS-GEN sub-study was funded by Cancer Research UK through money raised in the Stand Up To Cancer Campaign awarded to the University of Birmingham.

8.9 Funding and cost implications – AOA-OVC-ROCkeTS

The AOA-ROCkeTS sub-study was funded by a grant from AOA Dx Inc. awarded to the University of Birmingham.

8.10 Indemnity

There are no special arrangements for compensation for non-negligent harm suffered by participants as a result of participating in the study. The study is not an industry-sponsored study and so ABPI/ABHI guidelines on indemnity do not apply. The normal NHS indemnity liability arrangements for research detailed in HSG96(48) will operate in this case.

However, it should be stressed that in terms of negligent liability, NHS Trust hospitals have a duty of care to a patient being treated within their hospital, whether or not that patient is participating in a clinical study. Apart from defective products, legal liability does not arise where there is non-negligent harm. NHS Trusts may not offer advance indemnities or take out commercial insurance for non-negligent harm.

As Sponsor, the University is responsible for the general conduct of the study and shall indemnify the Clinical Centre against any claims arising from any negligent act or omission by the University in fulfilling the Sponsor role in respect of the Study.

8.11 Publication

A meeting will be held after the end of the study to allow discussion of the main results among the collaborators prior to publication. The success of the study depends entirely on the wholehearted collaboration of a large number of doctors, nurses and others. For this reason, chief credit for the main results will be given not to the committees or central organisers but to all those who have collaborated in the study.

8.12 Ancillary studies

It is requested that any proposals for formal additional studies of the effects of the study treatments on some participants (e.g. special investigations in selected hospitals) be referred to the Project Management Group for consideration. In general, it would be preferable for the study to be kept as simple as possible, and add-on studies will need to be fully justified.

9 REFERENCES

1.CRUK.http://www.cancerresearchuk.org/cancer-info/cancerstats/types/ovary/incidence/uk-ovarian-cancer-incidence-statistics, 2013.

2. MARINGE C, WALTERS S, BUTLER J, COLEMAN MP, HACKER N, HANNA L, ET AL. STAGE AT DIAGNOSIS AND OVARIAN CANCER SURVIVAL: EVIDENCE FROM THE INTERNATIONAL CANCER BENCHMARKING PARTNERSHIP. *GYNECOL ONCOL* 2012;127(1):75-82.

3. WHEATLEY G. OVARIAN CANCER. USEFULNESS OF ABDOMINAL SYMPTOMS IN EARLY DIAGNOSIS. *BMJ* 2009;339:B3954.

4. PITTS MK, HEYWOOD W, RYALL R, SMITH AM, SHELLEY JM, RICHTERS J, ET AL. HIGH PREVALENCE OF SYMPTOMS ASSOCIATED WITH OVARIAN CANCER AMONG AUSTRALIAN WOMEN. *AUST N Z J OBSTET GYNAECOL.2011*;51(1):71-8.

5. AGRAWAL A, WHORWELL PJ. REVIEW ARTICLE: ABDOMINAL BLOATING AND DISTENSION IN FUNCTIONAL GASTROINTESTINAL DISORDERS--EPIDEMIOLOGY AND EXPLORATION OF POSSIBLE MECHANISMS. *ALIMENT PHARMACOL THER* 2007;27(1):2-10.

6. SHARMA A, MENON,U., JACOBS, IJ. SYMPTOMS IN THE DIAGNOSIS OF OVARIAN CANCER IN: HILLARD T, EDITOR. *THE YEARBOOK OF OBSTETRICS AND GYNAECOLOGY*, 2007:159-70

7. HAMILTON W, PETERS TJ, BANKHEAD C, SHARP D. RISK OF OVARIAN CANCER IN WOMEN WITH SYMPTOMS IN PRIMARY CARE: POPULATION BASED CASE-CONTROL STUDY. *BMJ* 2009;339:B2998.

8. MOSS EL, MORAN A, REYNOLDS TM, STOKES-LAMPARD H. VIEWS OF GENERAL PRACTITIONERS ON THE ROLE OF CA125 IN PRIMARY CARE TO DIAGNOSE OVARIAN CANCER. *BMC WOMENS HEALTH* 2013;13(1):8.

9. RCOG/BSGE. GREEN TOP GUIDELINE NO: 62, MANAGEMENT OF SUSPECTED OVARIAN MASSES IN PREMENOPAUSAL WOMEN. LONDON: RCOG, NOVEMBER 2011.

10. VERNOOIJ F, HEINTZ AP, WITTEVEEN PO, VAN DER HEIDEN-VAN DER LOO M, COEBERGH JW, VAN DER GRAAF Y. SPECIALIZED CARE AND SURVIVAL OF OVARIAN CANCER PATIENTS IN THE NETHERLANDS: NATIONWIDE COHORT STUDY. *J NATL CANCER INST* 2008;100(6):399-406.

11. VERNOOIJ F, HEINTZ P, WITTEVEEN E, VAN DER GRAAF Y. THE OUTCOMES OF OVARIAN CANCER TREATMENT ARE BETTER WHEN PROVIDED BY GYNECOLOGIC ONCOLOGISTS AND IN SPECIALIZED HOSPITALS: A SYSTEMATIC REVIEW. *GYNECOL ONCOL* 2007;105(3):801-12.

12. ELLISS-BROOKES L, MCPHAIL S, IVES A, GREENSLADE M, SHELTON J, HIOM S, ET AL. ROUTES TO DIAGNOSIS FOR CANCER - DETERMINING THE PATIENT JOURNEY USING MULTIPLE ROUTINE DATA SETS. *BR J CANCER* 2012;107(8):1220-6.

13. RUFFORD BD, JACOBS IJ, MENON U. FEASIBILITY OF SCREENING FOR OVARIAN CANCER USING SYMPTOMS AS SELECTION CRITERIA. *BJOG* 2007;114(1):59-64.

14. LIM AW, MESHER D, GENTRY-MAHARAJ A, BALOGUN N, JACOBS I, MENON U, ET AL. PREDICTIVE VALUE OF SYMPTOMS FOR OVARIAN CANCER: COMPARISON OF SYMPTOMS REPORTED BY QUESTIONNAIRE, INTERVIEW, AND GENERAL PRACTITIONER NOTES. *J NATL CANCER INST* 2012;104(2):114-24.

15. WU L, DAI ZY, QIAN YH, SHI Y, LIU FJ, YANG C. DIAGNOSTIC VALUE OF SERUM HUMAN EPIDIDYMIS PROTEIN 4 (HE4) IN OVARIAN CARCINOMA: A SYSTEMATIC REVIEW AND META-ANALYSIS. *INT J GYNECOL CANCER* 2012;22(7):1106-12.

16. TIMMERMAN D, AMEYE L, FISCHEROVA D, EPSTEIN E, MELIS GB, GUERRIERO S, ET AL. SIMPLE ULTRASOUND RULES TO DISTINGUISH BETWEEN BENIGN AND MALIGNANT ADNEXAL MASSES BEFORE SURGERY: PROSPECTIVE VALIDATION BY IOTA GROUP. *BMJ* 2010;341:C6839.

17. SAYASNEH A, KAIJSER J, PREISLER J, JOHNSON S, STALDER C, HUSICKA R, ET AL. A MULTICENTER PROSPECTIVE EXTERNAL VALIDATION OF THE DIAGNOSTIC

PERFORMANCE OF IOTA SIMPLE DESCRIPTORS AND RULES TO CHARACTERIZE OVARIAN MASSES. *GYNECOL ONCOL* 2013.

18. TIMMERMAN D, VAN CALSTER B, TESTA AC, GUERRIERO S, FISCHEROVA D, LISSONI AA, ET AL. OVARIAN CANCER PREDICTION IN ADNEXAL MASSES USING ULTRASOUND-BASED LOGISTIC REGRESSION MODELS: A TEMPORAL AND EXTERNAL VALIDATION STUDY BY THE IOTA GROUP. *ULTRASOUND OBSTET GYNECOL* 2010;36(2):226-34.
19. RCOG GN. OVARIAN CYSTS IN POSTMENOPAUSAL WOMEN, OCTOBER 2003 REVIEWED 2010.
20. COLLINS GS, OGUNDIMU EO, ALTMAN DG. SAMPLE SIZE CONSIDERATIONS FOR THE EXTERNAL VALIDATION OF A MULTIVARIABLE PROGNOSTIC MODEL: A RESAMPLING STUDY. *STAT MED*. 2016;35:214–226
21. SUNG H, FERLAY J SIEGEL RL ET.AL. GLOBAL CANCER STATISTICS 2020: GLOBOCAN ESTIMATES OF INCIDENCE AND MORTALITY WORLDWIDE FOR 36 CANCERS IN 185 COUNTRIES. CA CANCER J CLIN. 2021;71(3):209-249.
22. REID F, BHATLA N, JONES, A. THE WORLD OVARIAN CANCER COALITION ATLAS: GLOBAL TRENDS IN INCIDENCE, MORTALITY AND SURVIVIAL. THE WORLD OVARIAN CANCER COALITION; 2018;39. ACCESSED FEBRUARY 4, 2021. https://worldovariancancercoalition.org/wp-content/uploads/2018/10/THE-WORLD-OVARIAN-CANCER-COALITION-ATLAS-2018.pdf
23. SIEGEL RL, MILLER KD, FUCHS HE, JEMAL A, CANCER STATISTICS, 2021. CAN CANCER J CLIN. 2021;71(1);7-33. doi:10.3322/caac.21654
24. OVARIAN CANCER STATISTICS. CANCER RESEARCH UK. PUBLISHED MAY 14, 2015. ACCESSED JANUARY 5, 2022. https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/ovarian-cancer
25. BRETT M.R., JENNIFER B. P., THOMAS A. S. EPIDEMIOLOGY OF OVARIAN CANCER: A REVIEW. CANCER BIOL MED. 2017; 14(1):9-32. doi:10.20892/j.issn.2095-3941.2016.0084
26. MONTANARO L, TRERÉ D., DERENZINI M. NUCLEOLUS, RIBOSOMES AND CANCER AM J PATHOL. 2008; 173(2):301-310. doi:10.2353/ajpath.2008.070752
27. KIM J, PARK E, KIM O, ET. AL. CELL ORIGINS OF HIGH-GRADE SEROUS OVARIAN CANCER. CANCERS. 2018;10(11):433. doi:10.3390/cancers10110433
28. ACOG COMMITTEE OPINION NUMBER 280: THE ROLE OF THE GENERALIST OBSTETRICIAN-GYNECOLOGIST IN THE EARLY DETECTION OF OVARIAN CANCER. OBSTET GYNECOL. 2022;100(6):1413-1416. doi:10.1016/S0029-7844(02)02630-3
29. GOFF BA, MANDEL L, MUNTZ HB, MELANCON CH. OVARIAN CARCINOMA DIAGNOSIS: RESULTS OF A NATIONAL OVARIAN CANCER SURVEY. CANCER. 2000;89(10):2068-2075. doi:10.1002/1097-0142(20001115)89:10<2068::AID-CNCR6>3.0.CO;2-Z
30. GOFF BA. FREQUENCY OF SYMPTOMS OF OVARIAN CANCER IN WOMEN PRESENTING TO PRIMARY CARE CLINICS. JAMA. 2004;291(22):2705. doi:10.1001/jama.291.22.2705
31. EBELL MH, CULP MB, RADKE TJ. A SYSTEMATIC REVIEW OF SYMPTOMS FOR THE DIAGNOSIS OF OVARIAN CANCER. AM J PREV MED. 2016;50(3):384-394. doi:10.1016/j.amepre.2015.09.023
32. BANKHEAD CR, KEHOE ST, AUSTOKER J. SYMPTOMS ASSOCIATED WITH DIAGNOSIS OF OVARIAN CANCER: A SYSTEMATIC REVIEW. BJOG INT J OBSTET GNAECOL. 2005;112(7):857-865. doi:10.1111/j.1471-0528.2005.00572.x
33. LIM A, MESHER D, GENTRY-MAHARAJ A, ET AL. TIME TO DIAGNOSIS OF TYPE I OR II INVASIVE EPITHELIAL OVARIAN CANCERS: A MULTICENTRE OBSERVATIONAL STUDY USING PAITNET QUESTIONNAIRE AND PRIMARY CARE RECORDS. BJOG INT J OBSTET GYNAECOL. 2016;123(6):1012-1020. doi:10.1111/1471-0528.13447

34. STEWART C, RALYEA C, LOCKWOOD S. OVARIAN CANCER: AN INTEGRATED REVIEW. SEMIN ONCOL NURS. 2019;35(2):151-156. doi:10.1016/j.soncn.2019.02.001

35. DOCHEZ V, CAILLON H, VAUCEL E, DIMET J, WINER N, DUCARME G. BIOMARKERS AND ALGORITHMS FOR DIAGNOSIS OF OVARIAN CANCER. CA125, HE4, RMI AND ROMA, A REVIEW. J OVARIAN RES. 2019;12(1):28. doi:10.1186/s13048-019-0503-7

36. ESSELEN KM, CRONIN AM, BIXEL K, ET AL. USE OF CA-125 TESTS AND COMPUTED TOMOGRAPHIC SCANS FOR SURVEILLANCE IN OVARIAN CANCER. JAMA ONCOL. 2016;2(11):1427. doi:10.1001/jamaoncol.2016.1842

37. PEPIN K, CARMEN M DEL BROWN A, DIZON DS. CA 125 AND EPITHELIAL OVARIAN CANCER: ROLE IN SCREENING, DIAGNOSIS AND SURVEILLANCE. AM J HEMATOL ONCOL. 2014;10(6). ACCESSED FEBRUARY 26, 2021. https://www.gotoper.com/publications/ajho/2014/2014dec/ca-125-and-epithelial-ovarian-cancer-role-in-screening-diagnosis-and-surveillance

38. COMMITTEE OPINION NO. 477: THE ROLE OF THE OBSTETRICIAN-GYNECOLOGIST IN THE EARLY DETECTION OF EPITHELIAL OVARIAN CANCER. OBSTET GYNECOL. 2011;1117(3):742-746. doi:10.1097/AOG.0b013e31821477db

39. ROBINSON KM, OTTESE B, CHRISTENSEN KB, KRASNIK A. DIAGNOSTIC DELAY EXPERIENCED AMONG GYNECOLOGICAL CANCER PATIENTS: A NATIONWIDE SURVEY IN DENMARK. ACTA OBSTET GYNECOL SCAND. 2009;88(6):685-692. doi:10.1080/00016340902971482

40. ALTMAN AD, LAMBERT P, LOVE AJ ET AL. EXAMINING THE EFFECTS OF TIME TO DIAGNOSIS, INCOME SYMPTOMS, AND INCIDENTAL DETECTION ON OVERALL SURVIVAL INN EPITHELIAL OVARIAN CANCER: MANITOBA OVARIAN CANCER OUTCOMES (MOCO) STUDY GROUP. INT J GYNECOL CANCER. 2017;27(8):1637-1644. doi:10.1097/IGC.0000000000001074

41. SUD A, JONES ME, BROGGIO J, ET AL. COLLATERAL DAMAGE: THE IMPAACT ON OUTCOMES FROM CANCER SURGERY OF THE COVID-19 PANDEMIC. ANN ONCOL. 2020;31(8):1065-1074. doi:10.1016/j.annonc.2020.05.009

42. SUD A, TORR B, JONES ME, ET AL. EFFECT OF DELAYS IN THE 2-WEEK-WAIT CANCER REFERRAL PATHWAY DURING THE COVID-19 PANDEMIC ON CANCER SURVIVAL IN THE UK: A MODELLING STUDY. LANCET ONCOL. 2020;21(8):1035-1044. doi:10.1016/S1470-2045(20)30392-2

43. TRINIDAD CV, TETLOW AL, BANTIS LE, GODWIN AK. REDUCING OVARIAN CANCER MORTALITY THROUGH EARLY DETECTION: APPROACHES USING CIRCULATING BIOMARKERS. CANCER PREV RES (PHILA, PA). 2020;13(3):241-252. doi:10.1158/1940-6207.CAPR-19-0184

44. CHARKHCHI P, CYBULSKI C, GRONWALD J, WONG FO, NAROD SA, AKBARI MR. CA125 AND OVARIAN CANCER: A COMPREHENSIVE REVIEW. CANCERS. 2020;12(12):3730. doi:10.3390/cancers12123730

45. VAUGHAN S, COWARD JI, BAST RC, ET AL. RETHINKING OVARIAN CANCER; RECOMMENDATIONS FOR IMPROVING OUTCOMES. NAT REV CANCER. 2011;11(10):719-725. doi:10.1038/nrc3144

46. LISIO MA, FU L, GOYENECHE A, GAO Z HUA, TELLERIA C HIGH=GRADE SEROUS OVARIAN CANCER: BASIC SCIENCES, CLINICAL AND THERAPEUTIC STANDPOINTS. INT J MOL SCI. 2019;20(4):952. doi:10.3390/ijms20040952

47. JACOBS IJ, MENON U, RYAN A, ET AL. OVARIAN CANCER SCREENING AND MORTALITY IN THE UK COLLABORATIVE TRIAL OF OVARIAN CANCER SCREENING (UKCTOCS): A RANDOMISED CONTROLLED TRIAL. THE LANCET. 2016;387(10022):945-956. doi:10.1016/S0140-6736(15)01224-6

48. BUYS SS, PARTRIDGE E, GREENE MH, ET AL. OVARIAN CANCER SCREENING IN THE PROSTATE, LUNG, COLORECTAL AND OVARIAN (PLCO) CANCER SCREENING TRIAL: FINDINGS FROM THE INITIAL SCREEN OF A RANDOMISED TRIAL. AM J OBSTET GYNECOL. 2005;193(5):1630-1639. doi:10.1016/j.ajog.2005.05.005

49. STATEMENT ON THE RELEASE OF UKCTOCS STUDY RESULTS. WORLD OVARIAN CANCER COALITION. PUBLISHED MAY 13, 2021. ACCESSED OCTOBER 27, 2021. https://worldovariancancercoalition.org/statement-on-the-release-of-ukctocs-study-results/

50. CONSENSUS STATEMENT RELEASED ON OVARIAN CANCER SYMPTOMS|OVARIAN CANCER NEWS | IMAGINIS-THE WOMEN'S HEALTH & WELLNESS RESOURCE NETWORK. ACCESSED JANUARY 8, 2022. https://www.imaginis.com/ovarian-cancer-news/consensus-statement-released-on-ovarian-cancer-symptoms?r

51. BALDWIN LM, TRIVERS KF, MATTHEWS B, ET AL. VIGNETTE-BASED STUDY OF OVARIAN CANCER SCREENING: DO U.S. PHYSICIANS REPORT ADHERING TO EVIDENCE-BASED RECOMMENDATIONS? ANN INTERN MED. 2012; 156(3):182. doi:10.7326/0003-4819-156-3-201202070-00006

52. GOFF BA, MATTHEWS B, ANDRILLA CHA, ET AL. HOW ARE SYMPTOMS OF OVARIAN CANCER MANAGED?: A STUDY OF PRIMARY CARE PHYSICIANS. CANCER. 2011;117(19):4414-4423. doi:10.1002/cncr.26035

53. BROWN PO, PALMER C. THE PRECLINICAL NATURAL HISTORY OF SEROUS OVARIAN CANCER: DEFINING THE TARGET FOR EARLY DETECTION. PHAROAH PDP, ED. PLOS MED. 2009;6(7):E1000114. doi:10.1371/journal.pmed.1000114

54. CAN OVARIAN CANCER BE MISSED ON AN ULTRASOUND? THE FACTS. HEALTHLINE. PUBLISHED JULY 13, 2021. ACCESSED OCTOBER 31, 2021. https://www.healthline.com/health/ovarian-cancer/can-ovarian-cancer-be-missed-on-ultrasound

55. HAMILTON W, PETERS TJ, BANKHEAD C, SHARP D. RISK OF OVARIAN CANCER IN WOMEN WITH SYMPTOMS IN PRIMARY CARE: POPULATION BASED CASE-CONTROL STUDY. BMJ. 2009;339:B2998. doi:10.1136/bmj.b2998

56. KALOO P, LODEN K, KHAZALI S, HOY D. RCOG/BSGE NATIONAL GREEN TOP GUIDELINES NO: 62 "MANAGEMENT OF SUSPECTED OVARIAN MASSES IN PREMENOPAUSAL WOMEN, PUBLISHED ONLINE NOVEMBER 1, 2011.

57. CANCER OF THE OVARY - CANCER STAT FACTS. SEER. ACCESSED FEBRUARY 9, 2021. https://seer.cancer.gov/statfacts/html/ovary.html

58. WHAT IS OVARIAN CANCER; SYMPTOMS, DETECTION AND TREATMENT | THE UNIVERSITY OF KANSAS CANCER CENTER. ACCESSED NOVEMBER 10, 2021. https://www.kucancercenter.org/news-room/blog/2020/08/what-is-ovarian-cancer-symptoms-treatment

59. FUJIWARA H, SUZUKI M, TAKESHIMA N, ET AL. EVALUATION OF HUMAN EPIDIDYMIS PROTEIN 4 (HE4) AND RISK OF OVARIAN MALIGNANCY ALGORITHM (ROMA) AS DIAGNOSTIC TOLLS OF TYPE I AND TYPE II EPITHELIAL OVARIAN CANCER IN JAPANESE WOMEN. TIMOR BIIOL. 2015;36(2):1045-1053. doi:10.1007/s13277-014-2738-7

60. GUPTA V, BERNARDINI MQ. ALGORITHMS USED IN OVARIAN CANCER DETECTION: A MINIREVIEW ON CURRENT AND FUTURE APPLICATIONS. J APPL LAB MED. 2018;3(2):290-299. doi:10.1373/jalm.2017.025817

61. U.S. DEPRTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION. K143534 - ELECSYS CA 125//ASSAY -510(K) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION MEMORANDUM. ACCESSED NOVEMBER 10, 2021. https://www.accessdata.fda.gov/cdrh_docs/reviews/K143534.pdf

62. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION. K112624 -ELECSYS HE4 ASSAY - 510(K) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY. ACCESSED NOVEMBER 10, 2021. https://www.accessdata.fda.gov/cdrh_docs/reviews/K112624.pdf

63. HERTLEIN L, STIEBER P, KIRSCHENHOFER A, ET AL. HUMAN EPIDIDYMIS PROTEIN 4 (HE4) IN BENIGN AND MALIGNANT DISEASES. CLIN CHEM LAB MED. 2012;50(12). doi:10.1515/cclm-2012-0097
64. DRAPKINR, VON HORSTEN HH, LIN Y, ET AL. HMAN EPIDIDYMIS PROTEIN 4 (HE4) IS A SECRETED GLYCOPROTEIN THAT IS OVEREXPRESSED BY SEROUS AND ENDOMETRIOID OVARIAN CARCINOMAS. CANCER RES. 2005;65(6):2162-2169. doi:10.1158/0008-5472.CAN-04-3924
65. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES FOOD AND DRUG ADMINISTRATION. K153607 - ROMA CALCULATION TOOL USING ELECSYS ASSAYS (RCTUEA) - 510(K) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY. ACCESSED NOVEMBER 10, 2021. https://www.accessdata.fda.gov/cdrh_docs/reviews/K153607.pdf
66. PHINYO P, PATUMANOUD J, SAENRUNGMUAENG P, ET AL. EARLY-STAGE OVARIAN MALIGNANCY SCORE VERSUS RISK OF MALIGNANCY INDICES: ACCURACY AND CLINICAL UTILITY FOR PREOPERATIVE DIAGNOSIS OF WOMEN WITH ADNEXAL MASSES. MEDICINA (MEX). 2020;56(12):702. doi:10.3390/medicina56120702
67. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES, FOOD AND DRUG ADMINISTRATION. K081754 -OVA1™ TEST - 510(K) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY. ACCESSED NOVEMBER 10, 2021. https://www.accessdata.fda.gov/cdrh_docs/reviews/K081754.pdf
68. COLEMAN RL, HERZOG TJ, CHAN DW, ET AL. VALIDATION OF A SECOND-GENERATION MULTIVARIATE INDEX ASSAY FOR MALIGNANCY RISK OF ADNEXAL MASSES. AM J OBSTET GYNECOL. 2016;215(1):82.E1-82.E11. doi:10.1016/j.ajog.2016.03.003
69. BRISTOW RE, SMITH A, ZHANG Z, ET AL. OVARIAN MALIGNANCY RISK STRATIFICATION OF THE ADNEXAL MASS USING A MULTIVARIATE INDEX ASSAY. GYNECOL ONCOL. 2013;128(2):252-259. doi:10.1016/j.ygyno.2012.11.022
70. UELAND F. PERSPECTIVE ON OVARIAN CANCER BIOMARKERS: PAST, PRESENT AND YET-TO-COME. DIAGNOSTICS. 2017;7(1):14. doi:10.3390/diagnostics7010014
71. LABIDI-GALY SI, PAPP E, HALLBERG D, ET AL. HIGH GRADE SEROUS OVARIAN CARCINOMAS ORIGINATE IN THE FALLOPIAN TUBE. NAT COMMUN. 2017;8(1):1093. doi:10.1038/s41467-017-00962-1
72. SON H, KHAN SM, RAHAMAN J, ET AL. ROLE OF FDG PET/CT IN STAGING OF RECURRENT OVARIAN CANCER. RADIOGRAPHICS. 2011;31(2):569-583. doi:10.1148/rg.312105713
73. DAKHLY DMR, GAAFAR HM, SEDIEK MM, IBRAHIM MF, MOMTAZ M. DIAGNOSTIC VALUE OF THE INTERNATIONAL OVARIAN TUMOR ANALYSIS (IOTA) SIMPLE RULES VERSUS PATTERN RECOGNITION TO DIFFERENTIATE BETWEEN MALIGNANT AND BENIGN OVARIAN MASSES. INT J GYNECOL OBSTET. 2019;147(3):344-349. doi:10.1002/ijgo.12970
74. TIMMERMAN D, VAN CALSTER B, TESTA A, ET AL. PREDICTING THE RISK OF MALIGNANCY IN ADNEXAL MASSES BASED ON THE SIMPLE RULES FROM THE INTERNATIONAL OVARIAN TUMOR ANALYSIS GROUP. AM J OBSTET GYNECOL. 2016;214(4):424-437. doi:10.1016/j.ajog.2016.01.007
75. BRODSKY BS, OWENS GM, SCOTTI DJ, NEEDHAM KA, COOL CL. ECONOMIC IMPACT OF INCREASED UTILISATION OF MULTIVARIATE ASSAY TESTING TO GUIDE THE TREATMENT OF OVARIAN CANCER: IMPLICATIONS FOR PAYERS. AM HEALTH DRUG BENEFITS. 2017;10(7):351-359.
76. VAN GORP T, CADRON I, DESPIERRE E, ET AL. HE4 AND CA125 AS A DIAGNOSTIC TEST IN OVARIAN CANCER: PROSPECTIVE VALIDATION OF THE RISK OF OVARIAN MALIGNANCY ALGORITHM. BR J CANCER. 2011;104(5):863-870. doi:10.1038/sj.bjc.6606092

77. HELLSTRÖM I, RAYCRAFT J, HAYDEN-LEDBETTER M, ET AL. THE HE4 (WFDC2) PROTEIN IS A BIOMARKER FOR OVARIAN CARCINOMA. CANCER RES. 2003;63(13):3695-3700.
78. BUAMAH P. BENIGN CONDITIONS ASOCIATED WITH RAISED SERUM CA-125 CONCENTRATION. J SURG ONCOL. 2000;75(4):264-265. doi:10.1002/1096-9098(200012)75:4<264::AID-JSO7>3.0.CO;2-Q
79. MULDER EE, GELDERBLOM ME, SCHOOL D, VERGELDT TF, NIJSSEN DL. PIEK JM. EXTERNAL VALIDATION OF RISK OF MALIGNANCY INDEX COMPARED TO IOTA SIMPLE RULES. ACTA RADIOL. 2021;62(5):673-678. doi:10.1177/0284185120933990
80. UELAND FR, DESIMONE CP, SEAMON LG, ET AL. EFFECTIVENESS OF A MULTIVARIATE INDEX ASSAY IN THE PREOPERATIVE ASSESSMENT OF OVARIAN TUMORS. OBSTET GYNECOL. 2011;117(6):1289-1297. doi:10.1097/AOG.0b013e31821b5118
81. SRAGOVI HU. NOVEL BIOMARKER PANEL QUANTIFYING TUMOR GANGLIOSIDES DEMONSTRATES IMPROVED DIAGNOSTIC PERFORMANCE FOR DETECTION OF EARLY OVARIAN CANCER COMPARED TO STANDARD OF CARE CA125. *Unpublished*. Published online 2022.