Application of advanced diagnostic tools in endometrial cancer

Version 13

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Protocol authorised by:

Name & Role	Date	Signature

Study Management Group

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Co-investigators: Mr Joseph Yazbek (gynaecological oncology consultant)
Prof Zoltan Takats (Analytical Chemist and creator of iKnife)
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Clinical Queries

Clinical queries should be directed to Dr Diana Marcus Clinical Research Fellow, who will direct the query to the appropriate person

Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office
Imperial College London & Imperial College Healthcare NHS Trust
2nd Floor Medical School Building
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Funder

This protocol describes the aforementioned study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Chief Investigator.

This study will adhere to the principles outlined in the NHS Research Governance Framework for Health and Social Care (2nd edition). It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

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GLOSSARY OF ABBREVIATIONS

PMB	Post menopausal Bleeding
IMB	Intermenstrual Bleeding
REIMS	Rapid Evaporative Ionisation Mass spectrometry
DESI	Desorption Electrospray Ionization
DWI	Diffusion weighted image

KEYWORDS

Innovations
Endometrial
Cancer
3D
Ultrasound
Radiomics

STUDY SUMMARY

TITLE Effectiveness of advanced diagnostic tools in women with endometrial cancer: A prospective cohort study

DESIGN Prospective

AIMS (1) Determine the performance characteristics of ultrasound-guided biopsy (using fine forceps e.g. Bettochi forceps) in the detection of endometrial malignancy and hyperplasia.
(2) Assess the diagnostic accuracy of 3D USS, compared to MRI and final histology with respect to myometrial invasion of endometrial cancer.
(3) Determine whether pelvic imaging (USS pelvis, MRI pelvis and MR spectroscopy) using radiomics can predict grade and type of tumour and prognosis.
(4) Determine if REIMS can diagnose cancer and normal tissue from womb biopsy samples and lymph glands

OUTCOME MEASURES Primary outcome measures:
(1) Histological diagnosis of ultrasound guided biopsy compared to routine care (hysteroscopy guided biopsy). Diagnostic ability (sensitivity, specificity, NPV, PPV) will be compared to gold standard.
(2) Accuracy of diagnosing myometrial invasion in 3D USS compared to MRI
(3) Comparison of radiomics model against standard histological diagnosis in the determination of grade and type of tumour.

Secondary outcome measures:
(1) Patient satisfaction (visual analogue score) of USS guided biopsy
(2) Comparison of accuracy of diagnosis of grade and type of tumour using MR spectroscopy compared to gold standard histopathology
(3) Diagnosis of endometrial cancer and hyperplasia (pre-cancer) in endometrial biopsy samples, and diagnose lymph node metastasis (spread of cancer to

	lymph glands), using REIMS, compared with standard histology. Comparison of mass spectroscopy findings of lymph nodes with the primary tumour (the womb cancer)
POPULATION	500 women referred to rapid access clinic with postmenopausal bleeding or intermenstrual bleeding. An additional 100 women will be recruited who were referred to Imperial college following a diagnosis of cancer in other hospitals.
ELIGIBILITY	All women presenting to rapid access gynaecology clinic with postmenopausal bleeding or intermenstrual bleeding
EXCLUSION	Anyone lacking capacity. <18years old. Pregnant. Any contra-indication carry out a biopsy
DURATION	8 years

1. INTRODUCTION

1.1 BACKGROUND

Endometrial cancer is a tumour originating in the endometrium (womb lining); it is the most common gynaecological cancer in the UK. In 2012, there were almost 100,000 new cases diagnosed in Europe. The lifetime risk of developing endometrial cancer is 1.7%-2.6%. Endometrial cancer classically presents with postmenopausal bleeding (bleeding after the menopause), or intermenstrual bleeding (bleeding imbetween periods).

Although routine management for these women does vary, in general a screening test is performed, typically a pelvic (internal) ultrasound to assess the endometrium (womb lining). A thin and regular endometrial line is associated with a very low risk of endometrial cancer. The pre-test probability of endometrial cancer in women presenting with postmenopausal bleeding is approximately 10%, but this increases with age and other risk factors [Gredmark]. In cases where the endometrial thickness is above the threshold for investigation an endometrial biopsy (sampling cells from the womb lining) is indicated. The biopsy can be taken blindly or under scan or hysteroscopic guidance. Hysteroscopy, the insertion of a small camera into the womb to visualise the womb lining allows direct visualization of the cavity.

In theory an ultrasound-guided biopsy using a very fine forceps (such as Bettocchi) could be used instead of hysteroscopy- guided biopsies of the endometrium. In the literature, only case reports exist; showing its use in the context of investigations for postmenopausal bleeding in women with cervical stenosis [Hammoud]. The technique of ultrasound-guided biopsy is ubiquitous when used elsewhere in the body. It is frequently used to provide histological diagnosis of pelvic masses, (presumed ovarian origin), both abdominally (scanning of the tummy) and transvaginally (internally). Potentially ultrasound guided biopsies could be used as a cheaper, faster and less painful alternative to a hysteroscopy directed biopsies. This technique has not been evaluated before in endometrial cancer.

Once the endometrial biopsy is performed, it is sent to the histologist for further examination. Histological analysis may take several days and patients are either brought back to clinic for discussion of the results or are given a telephone appointment. The patient often will not know the result of the test for up to 2 weeks; thus causing a lot of unnecessary anxiety and distress. Novel diagnostic tests, such as rapid evaporative ionization mass spectrometry (REIMS) may enable real time point of care diagnosis for the first time.

REIMS technique uses standard electrosurgical methods to convert tissue constituents into gas-phase ionic species amenable to mass spectroscopy [Balog, Schafer]. The coupling of electrosurgical dissection with REIMS technology has been coined the iKnife or ‘intelligent surgical device’; and it enables near-real-time characterisation of human tissues in vivo. It uses a combination of principal component analysis (PCA) and linear discriminant analysis (LDA) to translate spectral data into more relevant clinical information. Studies have shown that all human tissues have distinctive phospholipid characteristics; and it is these that are interrogated by mass spectroscopy (MS) to provide a tissue signature [Balog]. The surgical aerosol produced by tissue ablation harbours a plethora of information that is not currently used, but could be used to help intra-operative decision-making and diagnoses. Ex-vivo studies have demonstrated clear identification of major tissue types; lung, colon and liver [Balog]. Furthermore, it has been used in vivo to identify intestinal cancer, polyps and the origin of metastatic lesions [Balog]. Unpublished data by Takats and Ghaem-Maghami’s

group have shown that REIMS technology can distinguish between normal, benign, borderline and malignant ovarian masses with nearly 100% diagnostic accuracy [Phelps]. Part of this research project is to evaluate this new technology in endometrial cancer; in particular to assess if REIMS can diagnose cancer and pre-cancer from biopsies taken in clinic to allow a true one stop diagnosis.

In desorptive electrospray ionisation (DESI) samples of histological slides are taken and imaged pixel-by-pixel; producing mass spectra for each pixel (Takats). It can therefore provide topographically localized biochemical information to supplement standard histopathological classification systems.

Once a diagnosis of cancer is confirmed on biopsy, women get staged to assess the level of spread. Current gold standard is an MRI. A large proportion of women, however, are unable to undergo or complete an MRI scan due to their morbid obesity or claustrophobia. To this end, many have assessed the role of ultrasound in staging endometrial cancer as a cheaper and easier alternative to an MRI. In the last 20 years there has been a rise in the use of 3D ultrasound as it has become commercially available. There is, however a paucity of data on the role of 3D ultrasound in the assessment of myometrial invasion. This research project will assess whether 3D ultrasound is a safe, effective alternative to MRI to assess the depth of spread of womb cancer.

Lastly, the use of radiomics, which is the analysis of vast amounts of quantitative information from imaging, is an emerging technology, used to provide predictive models. The idea being that images of MRIs and Ultrasound from women with known endometrial cancer can be used to develop models which can accurately predict type of tumour and prognosis. This has been applied in a number of cancers such as prostate and lung but not for endometrial cancer.

1.2 RATIONALE FOR CURRENT STUDY

This study will assess the application of new technologies such as ultrasound-guided biopsies, 3D ultrasound and Rapid evaporative ionization mass spectrometry (REIMS) in women with suspected endometrial cancer. These technologies may offer more effective and/or cheaper alternative methods to assess women with suspected cancer. If successful, these technologies can be used in the rapid access gynaecology clinic to provide point of care diagnosis of endometrial cancer from an endometrial biopsy (using REIMS) and accurate assessment of lymph node involvement to help intra-operative decision-making.

2. STUDY OBJECTIVES

Objectives:
(1) Determine the performance characteristics of ultrasound-guided biopsy (using fine forceps e.g. Bettochi forceps) in the detection of endometrial malignancy and hyperplasia.
(2) Assess the diagnostic accuracy of 3D USS, compared to MRI and final histology with respect to myometrial invasion of endometrial cancer.
(3) Determine whether pelvic imaging (USS pelvis, MRI pelvis and MR spectroscopy) using radiomics can predict grade and type of tumour and prognosis.
(4) Determine if REIMS can diagnose cancer and normal tissue from womb biopsy samples and lymph glands

3. STUDY DESIGN

Prospective cohort study
Setting: Imperial College NHS Trust Hospitals, London
Participants: 500 women referred to rapid access clinic with postmenopausal bleeding or intermenstrual bleeding. An additional 100 women will be recruited who were referred to Imperial college following a diagnosis of cancer in other hospitals.
Duration: 3 years

3.1 STUDY OUTCOME MEASURES

Primary outcome measures:
(1) Histological diagnosis of ultrasound guided biopsy compared to routine care (hysteroscopy guided biopsy). Diagnostic ability (sensitivity, specificity, NPV, PPV) will be compared to gold standard.
(2) Accuracy of diagnosing myometrial invasion in 3D USS compared to MRI
(3) Comparison of radiomics model against standard histological diagnosis in the determination of grade and type of tumour.

Secondary outcome measures:
(1) Patient satisfaction (visual analogue score) of USS guided biopsy
(2) Comparison of accuracy of diagnosis of grade and type of tumour using MR spectroscopy compared to gold standard histopathology
(3) Diagnosis of endometrial cancer and hyperplasia (pre-cancer) in endometrial biopsy samples, and diagnose lymph node metastasis (spread of cancer to lymph glands) using REIMS, compared with standard histology. Comparison of mass spectroscopy findings of lymph nodes with the primary tumour (the womb cancer)

3.1 MAIN STUDY

PART 1: PRE-DIAGNOSIS OF CANCER

All women presenting to the rapid access clinic with postmenopausal bleeding (PMB) and intermenstrual bleeding (IMB) will be approached for consent for inclusion into this study (group A). When women normally attend this clinic the doctor will discuss what will happen - this includes an internal (pelvic scan) and an examination. If the womb lining is thickened on the scan the patient will need a tissue biopsy (this is not that common, most women only need a scan), this will be taken at the same time as the examination in clinic. The device used to take the tissue biopsy is called a pipelle. Only if the patient still needs a biopsy but the patient can't tolerate the examination, or the sample is insufficient or focal (in a small area of the womb as opposed to generalised) then the patient will need to have a hysteroscopy. Hysteroscopy is a procedure in which a hysteroscope; a small camera, is inserted into the womb under local or general anaesthetic. If a patient needs hysteroscopy this will occur at a different appointment.

In this study, all women in group A will be approached for enrolment into the study. Patients will be identified in the gynaecology clinic in Imperial NHS trust (where patients with post menopausal bleeding and suspected cancer get referred). Patients will be identified by the clinical consultants (who are also research supervisors) and clinical research fellow. Patient medical notes will not be used prior to clinic to identify patients, instead the patients will be selected as they attend clinic. At the point of consent, patients will be de-identified given a trial number and anonymised.

Prior to any imaging or examination women will be told what the research involves and if they are happy to proceed. They can consent to all or part of the study. The extra elements of the study include: a 3D ultrasound (not just standard 2D), a second pipelle biopsy sample (only if patients actually would have needed a pipelle anyway) and ultrasound guided biopsy (again only if patients actually would have needed a hysteroscopy guided biopsy). Each will now be discussed in more detail.

All women (group A) will receive a pelvic (internal) ultrasound as per routine care. In addition to the normal 2D ultrasound, women will receive a 3D ultrasound and images will be saved. This adds an additional 1-2 minutes scan time. Depending on the clinician it is sometimes performed as part of routine care.

If the endometrium (womb lining) is thickened >4mm on ultrasound they will require an endometrial biopsy. Usually this will be a pipelle. If the patient is agreeable a second endometrial sample will be taken specifically for research. The histology of first sample will be analysed by conventional pathology and the second sample will be analysed by the new technology REIMS/DESI. REIMS is Rapid Evaporative Ionization Mass Spectrometry and DESI is Desorption Electrospray Ionization. Both are new technologies that use mass spectrometry to analyse chemical composition, and in this case tissue composition. REIMS has been dubbed the 'iKnife' or 'intelligent knife' colloquially as when coupled with surgical diathermy the gas pattern produced is unique to the tissue and helps surgeons know where to cut. The diagnostic ability of this new technology will be compared against the gold standard, which is histological examination.

In cases where the pipelle biopsy cannot be performed in clinic due to poor tolerance, insufficient material or focal lesions a hysteroscopy guided biopsy will be required (local or GA). In this case, the patients will be offered an ultrasound (USS) guided biopsy in the first instance, this will be performed at the same time in clinic. The biopsy will be taken under scan guidance using a very fine forceps such as Bettochi forceps. These forceps are used routinely during hysteroscopy so whilst the instruments are common the application is novel. The biopsy results of this will be sent for standard histology. If the results are inadequate patients will be sent for standard hysteroscopy and biopsy. Patients will also be asked to assess tolerability of the procedure using a visual analogue score.

The tissue generated from the study will be stored in a tissue bank subcollection in a locked and secure freezer in the Institute of Reproductive and developmental biology building on the Hammersmith Hospital site. The sample labels will be coded in order to maintain confidentiality and only authorized members of the research team will have access to the samples. At the end of the research period the tissue samples will be kept for up to 10 years. They would remain securely stored and may be used in subsequent research. It is hoped that the tissue could be used to validate any biomarkers that arise from the study using a combination of immunohistochemical, epigenetics, protein and gene expression analysis. Any data generated from tissue analysis will be stored on Imperial College computers and will be accessed only by members of the research team directly involve in the project.

PART 2: AFTER DIAGNOSIS OF CANCER

In those women with confirmed cancer on histology (subgroup B) - an MRI is performed to assess stage of cancer (current routine standard of care). This will include T1, T2, DWI and contrast enhanced sequences. Patients referred to Hammersmith Hospital from other trusts who are diagnosed with endometrial cancer will be approached for consent and enrolment into latter part of study. These patients will have had an MRI but may not have had all the sequences needed and may therefore require a further MRI scan with additional sequences. MR spectroscopy will be performed on a subset of patients with confirmed cancer (20 patients).

Definitive management of endometrial cancer includes a hysterectomy and the surgical specimen will be examined for depth of myometrial invasion. In the meantime, the saved 3D images from the rapid access clinic will be analysed and rendered to predict the stage of the endometrial cancer. The results of 3D ultrasound, and MRI will be compared against the stage found on the gold standard test (on histological examination).

In women with confirmed endometrial cancer who later go on to require lymphadenectomy as part of standard care (e.g. advanced stage, type 2 tumours or high grade) following MDT discussion will have their lymph nodes examined ex-vivo using REIMS/iKnife/DESI in addition to the gold standard histopathological examination. The diagnostic capabilities of this new technology to identify cancerous lymph nodes will be compared to standard histopathological diagnosis. If this technology is accurate at diagnosing involved lymph nodes it could be used in vivo to enhance intra- operative decision-making. In addition to build the data for endometrial tissue, women receiving surgery will also be consented for an additional endometrial biopsy (under GA or as an ex-vivo sample).

FOLLOW UP:

Following surgery patients will continue with routine standard of care and follow up. Patients will be followed up for 5 years to provide 5-year survival data. Images from ultrasound, MRI pelvis and MR spectroscopy will be analysed to see if they can predict the 5-year survival.

4. PARTICIPANT ENTRY

4.1 PRE-REGISTRATION EVALUATIONS

No need for any specific screening tests prior to participant entry into the study.

4.2 INCLUSION CRITERIA

All women presenting to rapid access gynaecology clinic with postmenopausal bleeding or intermenstrual bleeding

4.3 EXCLUSION CRITERIA

Anyone lacking capacity. <18years old. Pregnant. Any contra-indication carry out a biopsy

4.4 WITHDRAWAL CRITERIA

Withdrawal procedure occurs if patients choose to exit the study or if they loose capacity. After withdrawal from the study the anonymised tissue already obtained with consent will remain stored for the duration of the study. In addition, the clinical details up till the point of withdrawal of the patient from the study will remain on file.

5. ADVERSE EVENTS

5.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical study subject.

Serious Adverse Event (SAE): any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject 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*

- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

5.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

5.3.1 Non serious AEs

All such events, whether expected or not, should be recorded.

5.3.2 Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However, relapse and death due to endometrial cancer and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the JCRO at Imperial where in the opinion of the Chief Investigator, the event was:

- 'related', ie resulted from the administration of any of the research procedures; and
- 'unexpected', ie an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

Contact details for reporting SAEs
Attention Miss D Marcus

6. ASSESSMENT AND FOLLOW-UP

Following surgery patients will continue with routine standard of care and follow up. Patients will be followed up for 5 years to provide 5-year survival data. Images from ultrasound, MRI pelvis and MR spectroscopy will be analysed to see if they can predict the 5-year survival.

End of trial will be defined as when the last patient for recruitment received her operative surgery (and final histology was obtained) and has been followed up for 5 years.

7. STATISTICS AND DATA ANALYSIS

Statistical analysis: Sample size was calculated on the basis of recently published work and anticipated ease of recruitment. It is estimated that approximately 10% of women attending rapid access clinic will have endometrial cancer. In previous studies assessing new endometrial sampling devices a population of 50 women was used. In this population it is expected that 80 women will require a hysteroscopy and could be offered an ultrasound guided biopsy.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

8. REGULATORY ISSUES

8.1 ETHICS APPROVAL

The Chief Investigator has obtained approval from the Research Ethics Committee. The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Chief Investigator will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

8.2 CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the study the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

8.3 CONFIDENTIALITY

The Chief Investigator will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

8.4 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study/ Imperial College Healthcare NHS Trust holds standard NHS Hospital Indemnity and insurance cover with NHS Litigation Authority for NHS Trusts in England, which apply to this study (delete as applicable)

8.5 SPONSOR

Imperial College London/Imperial College Healthcare NHS Trust (delete as applicable) will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

8.6 FUNDING

We have applied for funding from the wellbeing of women charity.

8.7 AUDITS

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies to ensure adherence to GCP and the NHS Research Governance Framework for Health and Social Care (2nd edition).

9. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Diana Marcus, clinical research fellow.

10. PUBLICATION POLICY

The data obtained from this study will be anonymised and analysed. The anonymised data will be published in peer-reviewed journals and presented in relevant conferences and at patient/public engagement events.

10. REFERENCES

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EXAMPLE APPENDICES

Appendices should be additional information to the protocol and can consist of:

- Common Terminology Criteria for Adverse Events (NCI CTC)
- RECIST criteria
- WHO / ECOG Performance status
- PIS, Consent form, GP letter (although may be more practical to have them separate)
- Expected side effects
- Schedule of events table

DIAGNOSIS

Appendix 1. Summary of investigations & treatment of patient (blue routine, red trial)