Collection of Cervical Cells at Colposcopy using a novel technique for analysis of high risk vs. low risk lesions

Ethics Ref: 17/SC/0203

Addenbrooke's R&D A094381

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Sponsor:	Cambridge University Hospitals NHS foundation Trust

AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	2	21/4/17	Dr. Mohamed Aslam Shiraz Dr. Robin Crawford Prof. John Doorbar	Added references to protocol
2	3	30/04/18	Dr. Mohamed Aslam Shiraz	Section on 10 double lifts
3	4	10/01/19	Dr. Mohamed Aslam Shiraz	Increased number of patients and duration of study
4	5	09/09/20	Richard Skells	Administrative corrections, details of funding, updated data protection wording and clarifications regarding the analysis of samples.
5	6	21/10/21	Dr. Mohamed Aslam Shiraz Dr. Robin Crawford Prof. John Doorbar Tulay Gulsen	Changes to the inclusion and exclusion criteria, increased number of patients and duration of the study. Addition of a research cervical screen. Testing of a prototype delivery device.
6	7	21/02/22	Dr. Mohamed Aslam Shiraz Dr. Robin Crawford Tulay Gulsen	The colposcopic imaging is being optional for the participants. The recruitment end date is extended to end of April and the study end date is clarified . Clarification of the sample labelling for research cervical screening samples.

List details of all protocol amendments here whenever a new version of the protocol is produced.

1. SYNOPSIS

It may be useful to include a synopsis of the study for quick reference. Delete or alter as appropriate/required.

Study Title	Collection of Cervical Cells at Colposcopy using a novel technique for analysis of high risk vs. low risk lesions
Internal ref. no.	R&D A094381
Study Design	Observational & Lab Based
Study Participants	Women referred to colposcopy with abnormal cytology or those requiring a cervical examination
Planned Sample Size	800 in total
Follow-up duration	Nil
Planned Study Period	5 years
Primary Objective	The aim of the project is to improve the collection and analysis of cells taken from the surface of the cervix in order to identify high risk or low risk cervical cancer precursor lesions
Secondary Objectives	None
Primary Endpoint	Demonstrating clear correlation of high/ low risk lesions with novel molecular techniques **or** achieving 800 samples (whichever is sooner)
Secondary Endpoints	
Intervention (s)	Removing a layer of cells from the cervix with no trauma and preserving the spatial distribution of cells for analysis

2. BACKGROUND AND RATIONALE

Outline the scientific justification for the research. Give an outline of the background to the study, with references to literature and other relevant research.

Give an outline of the main research questions. Give a brief outline of the intervention (if applicable) and summary of findings from previous studies (if relevant) that potentially have clinical significance.

Provide summary of the known and potential risks and benefits of any of the study procedures (where applicable)

Describe the population to be studied.

The aim of the project is to improve the collection and analysis of cells taken from the surface of the cervix in order to more accurately identify cervical disease when it is present. Cells taken from the surface of the cervix are currently sampled using a cytobrush or spatula as part of the cervical smear test. This approach scrapes and/or scarifies the surface of the cervix, and harvests a mixture of cells from the superficial to mid epithelial layers. Cytological abnormalities are subsequently identified from amongst these exfoliated cells by Papanicolau staining. The site and severity of cervical disease is then identified by colposcopy examination, and by pathology assessment of biopsies taken from areas where disease is suspected. In some instances, the areas of disease predicted by cytology can be difficult to identify at colposcopy, and in such instances, multiple biopsies may need to be taken.

Here we propose a modified approach in which cells are taken from the surface of the cervix using a 'filter-paper' disk of nylon (e.g. Sigma-Aldrich cat. Z134341) or cellulose (e.g. Sigma-Aldrich cat. Z612391 (nitrocellulose)/ cat Z61238/Z612359 (mixed cellulose esters)). In our modified approach, the disk of nylon or cellulose will be gently pressed onto the surface of the cervix in order to lift off a layer of exfoliating surface cells in their in situ positions. The morphology of the surface cells is indicative of underlying cervical disease, and this can then be assessed by cytological analysis. We have already demonstrated that this technique works in our original pilot study and our previous work has suggested that a small panel of biomarkers present at the surface of the cervix can identify both disease severity and (if used appropriately) HPV type. The two most significant of these markers are MCM, which is a marker of cells that are in cycle, and E4, which is an abundantly expressed viral protein. These two proteins change their distribution at the epithelial surface according to the severity of cervical neoplasia. MCM, and other similarly regulated proteins such as PCNA and Ki-67, are often regarded as surrogate markers of E7 expression when they are present in the supra-basal cell layers. E4 is a highly expressed viral protein, and represents a distinct class of biomarker, whose expression follows that of MCM during the productive HPV life cycle. A diagrammatic representation of how these proteins are expressed in cervical disease of different grades is shown in Fig. 1A. The figure also demonstrates

the distribution of L1 which also represents a third discriminatory marker that is expressed in a sub-set of E4-positive cells in low-grade disease. Conversely, p16 is found in a subset of the MCM-positive cells in high-grade disease, and represents the fourth class of discriminatory marker that could be used in our system to identify potential pre-cancerous lesions as shown in Fig. 2A.

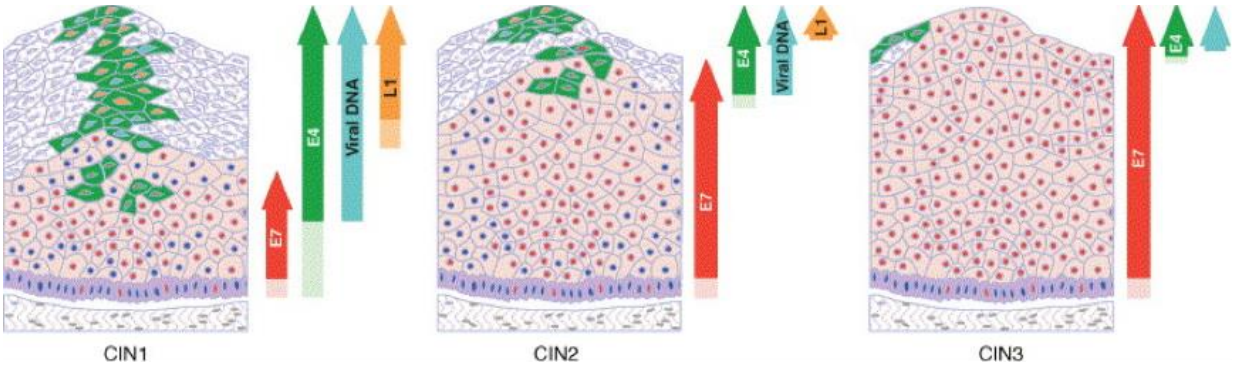

Fig 1A: Schematic representation of viral protein expression in CIN (Doorbar, John, et al. "Human papillomavirus molecular biology and disease association." *Reviews in medical virology* 25.S1 (2015): 2-23)

Using such an approach has a number of potential advantages over conventional methodologies. Firstly, it avoids scarification, which can act to spread virions present at the surface of LSIL and inoculate them at basal sites. In the Rhesus model of cervical disease, this scarification is a necessary event in the initiation of a new infection. Secondly, by keeping the exfoliated cells in their in situ positions will allow the position of cytological abnormalities, identified at the level of cytology or by immunostaining, to be localized to a particular cervical site. This should help to avoid some of the difficulties associated with the identification of disease areas at colposcopy, especially when lesion size is small. And thirdly, by combining this cell-collection approach with disease staging markers such as E4 and MCM, we will be able to more rapidly identify abnormal cells from the majority of cells with normal morphology, and provide a molecular assessment of disease severity at particular sites, which has the potential lead to more individualized treatment of these lesions.

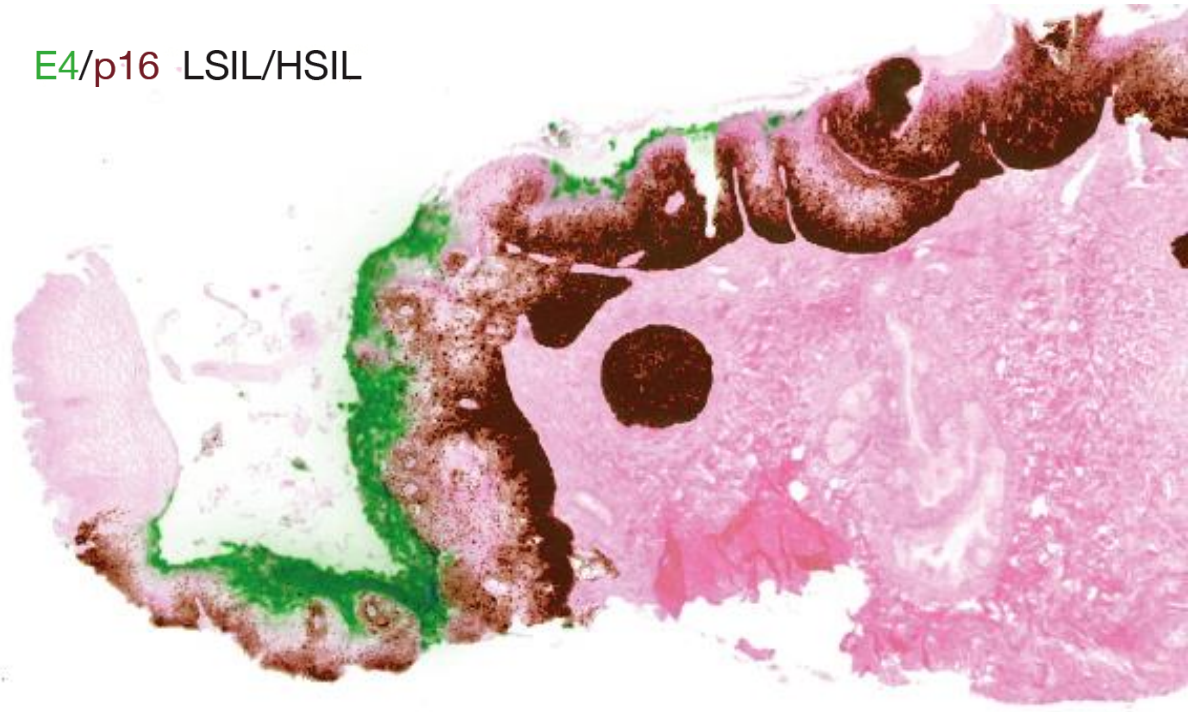

Fig 2A. Immunohistochemistry staining using p16 (red) and E4 (green). p16 is a surrogate marker of E7 expression. The above image demonstrates that as a low-grade lesion transforms into a high-grade lesion (CIN 1 to 3), p16 staining is dramatically increased while E4 staining (associated with a productive infection) diminishes in intensity (Image courtesy of Professor John Doorbar, HPV Lab, Department of Pathology, University of Cambridge)

The localization of cervical disease and the assessment of severity are key to accurate diagnosis and disease management. The approach suggested here is likely to be of value as an adjunct to colposcopy, and will also be useful in clinical trials such as those used to assess vaccine efficacy, where there is a need to monitor disease progression over time without taking biopsy specimens (which effectively remove the infected tissue, and prevents follow-up). This non-invasive sampling methodology should also provide new insight into aspects of papillomavirus disease, which are poorly understood, such as papillomavirus latency, re-activation and regression.

Alongside the assessment of this technique to better identify HPV positive women who truly have disease needing excision, another population of women that would perhaps benefit from this approach are those women who suffer with conditions such as post-coital bleeding or morphologically abnormal cervixes. These patients make up nearly 2/3rd of our colposcopy workload, the majority of whom go through the cancer 2 week wait pathway with considerable anxiety and out of these referrals approximately 1% of women would have clinically relevant disease[6,7] in contrast to nearly 20-30% of HPV positive women that have such disease. Thus the ability to perform a diagnostic test as proposed here that can identify lesions at the outset would

have a multitude of advantages including preventing unnecessary referrals to the already over-stretched colposcopy service and improve patient satisfaction by reassuring patients in a quicker and less intrusive manner.

There are no perceptible risks for the patient in this study. There are no direct benefits for the participant. Patients attending the colposcopy clinic will be asked to participate.

3. OBJECTIVES

There is usually only one primary objective, the rest are secondary objectives.
The wording of the objectives should be clear, unambiguous and as specific as possible.

3.1 Primary Objective

The aim of the project is to improve the collection and analysis of cells taken from the surface of the cervix in order to identify high risk or low risk cervical cancer precursor lesions

3.2 Secondary Objectives: None

4. STUDY DESIGN

4.1 Summary of Study Design

Observational, Basic Science Study
Duration: Up to 5 years

4.2 Primary and Secondary Endpoints/Outcome Measures

Demonstrating clear correlation of high/ low risk lesions with novel molecular techniques **or** achieving 800 samples (whichever is sooner)

4.3 Study Participants

4.3.1 Overall Description of Study Participants

Female participants with screen detected abnormal cervical cytology varying from mild dyskaryosis to severe dyskaryosis aged 25 to 65 years (with these ages corresponding to that of women that are eligible to be part of the cervical cancer screening programme.

Alongside this we will also aim to recruit all women referred to colposcopy with post-coital or abnormal bleeding that requires a cervical evaluation or a visually abnormal looking cervix

4.3.2 Inclusion Criteria

- Participant is willing and able to give informed consent for participation in the study.
- Female, aged between 25 to 65 years.

- Diagnosed with abnormal cervical cytology by routine screening or having been referred for a clinical indication by the GP/Clinical Practitioner for an abnormal looking cervix or post-coital/inter-menstrual bleeding concerns

4.3.3 Exclusion Criteria

The participant may not enter the study if ANY of the following apply:

- Participant is unable to give consent.
- Pregnant
- HIV/Systemic Immuno-suppression
- Previous contraindication or allergy nail varnish/ nitrocellulose

4.4 Study Procedures

There are no additional visits required for this study.

1. During the colposcopy examination, a number of digital images of the cervix will be taken according to standard practice. Images are non-mandatory for the purpose of the research and will not result as a non-compliance if not taken.

2. A filter disk (of up to 25mm diameter and marked to indicate the 12 O'clock position) will then be gently pressed onto the surface of the cervix for 10 to 15 seconds prior to it being peeled off and removed for storage. The filter with adherent exfoliated cells will then be fixed (in acetone/methanol, ethanol, or 5% formaldehyde) prior to analysis at the level of cytology or immunostaining or DNA/ RNA analysis as part of the research collaboration between the University of Cambridge and Addenbrookes Hospital.

3. In the majority of cases, the remainder of the colposcopy examination will then follow its standard course, without further modification. Colposcopic photographs, if taken, will be compared as well as the colposcopist' view of the cervix.

   For a number of cases (10 high grade and 20 low grade) we shall take a conventional cervical screen after the cell lift has been taken. This will be similar in discomfort to a normal cervical screen and add approximately 30 seconds to the overall procedure. This will permit the potential impact of the cell lift procedure on the routine cervical screen to be evaluated. It has already been established that a routine cervical screen disrupts the cell lift procedure. However, it is hypothesised that performing the cell lift procedure before a routine screen will permit both sampling procedures to be performed without issue.

During the final phase of the cell lift project we shall test variations of a prototype device designed to deliver the filter disk to the cervix. In the prototype testing we shall use 10 samples of each prototype and evaluate for purposes of clinician user and patient experience. Subsequently, the prototype will be evaluated against the laboratory process.

The standard operating procedure for use of device follows as:

- Insert a speculum and visualise the cervix.
- Select device (standard for normal or larger speculum; small when using a small speculum). Orientate with fin on handle at 12 o'clock and apply to cervix.
- Maintain light pressure for approximately 15 seconds and then withdraw.
- Avoid agitating the head when in contact with the cervix.
- Remove from vagina and hand to research nurse.
- Research nurse places in formalin fixative for 10-15 seconds and then detaches device head into transport medium.
- The wand is disposed of in clinical waste.

4.4.1 Informed Consent

Informed consent will be taken prior to the examination. The participant will be sent an invitation to take part in the study. When they attend the clinic, the research nurse will discuss the consent and obtain this prior to the participant seeing the colposcopist.

4.4.2 Study Assessments

For the study, we envisage that 800 cell-lifts will be sufficient.

These will be double stained with a pan E4 antibody to identify areas of virus infection (LSIL), along with MCM to identify cells that are progressing through the cell cycle (HSIL). Images of the stained cells will be captured using a digital imaging system prior to H+E staining or staining with the Pap stain. The immunostaining and histology images will be compared against the colposcopy findings and with the results of the biopsy analysis.. In some instances, we will request sections from the biopsy material in order to better understand how biomarker expression at the epithelial surface (in the cell-lifts) relates to expression of the same biomarkers in the underlying disease. Once able to define consistently areas where productive infection and high rates of cell turnover is occurring we aim to use P16INK4A (surrogate marker of E7) and P53 (decline of P53 expression as surrogate marker of E6 expression) to further delineate the regions of the lift from high risk or low risk lesions.

As part of the validation of this biomarker approach, we would also like to perform a DNA validation. Here we would like to obtain an additional lift on 10 patients and by performing an in-situ localization of DNA we aim to overlay the biomarker map over the DNA map so as to demonstrate co-localisation.

4.5 Definition of End of Study

The end of study is when we have either met our proposed primary objective or if we reach our maximum number of samples.

5. INTERVENTIONS

No interventions beyond those seen in colposcopy

6. SAFETY REPORTING (IF APPLICABLE)

6.1 Definition of Serious Adverse Events

Not applicable

6.2 Reporting Procedures for Serious Adverse Events

Not relevant

7. STATISITICS

7.1 The Number of Participants

For the study, we envisage that 800 cell-lifts will be sufficient (with the majority being smear abnormal patients and approximately 50 patients being referred for clinical indications). Though this number has not been formulated by a formal power calculation, we envisage that this would certainly be an adequate number so as to get over certain hurdles such as obtaining a monolayer of cells on the membrane, staining with low level of background noise and demonstrating lesions.

During the first year of study we have recruited 105 patients. The majority of these patient samples have been used to create a standardized, reproducible protocol. This protocol has then been tested on 31 patients successfully with a corresponding sensitivity of 87% and positive-predictive value of 87%. We have now commenced a collaboration with Prof. Peter Sasieni who has calculated that a sample size of 800 patients would give us a robust analysis of the true sensitivity, specificity, PPV and NPV of our diagnostic approach with narrow confidence intervals. As we have already recruited 105 patients, we would like to therefore increase the total number of patient during the study time to 800 in total. To effectively do this we would also require an increase in the duration of the study period to the end of April 2022. The end of April is referring to end of study recruitment and the sample analysis will be completed within a year after the recruitment is closed.

7.2 Analysis of Endpoints

An analytical plan will be developed by the Translational Lead, Prof. John Doorbar. This will include triple staining and analysis of collected samples, against sensitivity, specificity, PPV/NPV corrected for population prevalence.

8. ETHICS

The procedure will take a few minutes longer than the standard procedure and this will fall within the normal range of times for this type of clinical investigation. The application of the template is not felt to cause discomfort.

The study has been reviewed and approved by the South Central – Oxford B Research Ethics Committee.

8.1 Participant Confidentiality

The study staff will ensure that the participants' anonymity is maintained. The participants will be identified only by date of birth and a participants ID number on the CRF and any electronic database and where required on sample labels. All documents will be stored securely and only accessible by study staff and authorised personnel. The study will comply with the General Data Protection Regulation (GDPR) 2018, which requires data to be anonymised as soon as it is practical to do so.

8.2 Other Ethical Considerations

Only participants able to understand the study will be involved

9. DATA HANDLING AND RECORD KEEPING

All study data will be entered on an MS Excel spreadsheet by the research nurse. The participants will be identified by a study specific participants number and/or code in any database. The name and any other identifying detail will NOT be included in any study data electronic file.

10. FINANCING AND INSURANCE

Finance:

The cost of staining and handling of the templates will be borne by the programme grant and CRUK Early Detection Award, from Professor John Doorbar (University of Cambridge). Incidental expenses required at Addenbrookes will be borne by ACT (Gynaecological Cancer Fund). Research Costs incurred by Addenbrookes, including Trial Management and Research Nurse time, will be covered by the CRUK Early Detection Award.

Insurance: Cambridge University Hospitals NHS Foundation Trust

11. REFERENCES

1) Griffin, Heather, and John Doorbar. "Detection of papillomavirus gene expression patterns in tissue sections." *Current protocols in microbiology*(2016): 14B-7.
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4) Griffin, Heather, et al. "Stratification of HPV-induced cervical pathology using the virally encoded molecular marker E4 in combination with p16 or MCM." *Modern Pathology* 28.7 (2015): 977-993.
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6) Cohen O, Schejter E, Agizim R, et al. Postcoital bleeding is a predictor for cervical dysplasia. *PLoS One*. 2019;14(5):e0217396. Published 2019 May 23. doi:10.1371/journal.pone.0217396

7) Shapley M, Blagojevic-Bucknall M, Jordan KP, Croft PR. The epidemiology of self-reported intermenstrual and postcoital bleeding in the perimenopausal years. BJOG. 2013 Oct;120(11):1348-55. doi: 10.1111/1471-0528.12218. Epub 2013 Mar 26. PMID: 23530690.