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BRIEF REPORT

Synchronous uterine and bladder cancers detected in urine and vaginal samples by cytology

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Abstract

Novel diagnostics for uterine cancer are urgently needed to reduce the burden of invasive testing for the majority of healthy women with postmenopausal bleeding. We have previously shown that uterine cancer cells can be detected by cytology in urine and vaginal samples with high diagnostic accuracy. Here, we demonstrate its potential to distinguish malignant cells of different aetiologies in the same urogenital biofluid sample according to their distinctive morphology and immunoprofiles. Synchronous tumours of the urogenital tract are uncommon but can cause diagnostic confusion, delays and poor outcomes. A 79-year-old woman presented to accident and emergency with postmenopausal bleeding. Voided urine and Delphi screener-collected vaginal samples were assessed by cytology and immunocytochemistry. Two malignant cell populations with distinct morphology and immunophenotypes consistent with synchronous uterine and urothelial tumours were identified. Subsequent routine diagnostics confirmed concurrent uterine carcinosarcoma and high-grade urothelial carcinoma of the bladder. This case demonstrates that cytology and adjunctive immunocytochemistry can simultaneously identify and phenotype cancers of different aetiologies from a single urogenital biofluid sample. This can help rationalise diagnostic pathways in complex, unusual cases of dual urogenital primaries.

KEYWORDS
cytology, endometrial cancer, immunocytochemistry, non-invasive diagnostics, urine, uterine cancer

1 | INTRODUCTION

Uterine cancer is the most common gynaecological malignancy in high-income countries.[1] Incidence rates are rising as a consequence of the obesity epidemic, an ageing population and non-surgical treatments for benign gynaecological disease.[2] Most women with uterine cancer present with postmenopausal bleeding, triggering sequential investigation by transvaginal ultrasound, hysteroscopy and endometrial biopsy. These tests provoke anxiety, can be unpleasant, painful and hazardous, and constitute an inefficient use of resource given that only 5%–10% women with postmenopausal women have sinister underlying pathology.[3] Considerable research effort is

therefore underway to develop a simple rule in/rule out test to efficiently triage high-risk women for diagnostic work up whilst low-risk women are safely reassured.[4]

We have previously shown that naturally shed uterine cancer cells can be identified by cytology in urine and vaginal samples with high diagnostic accuracy.[5] Typical cytological appearances enable confident classification by morphology alone but atypical appearances or mixed populations of malignant cells can be distinguished by adjunctive immunocytochemistry, since malignant cells maintain immunoprofiles consistent with their site of origin. Here we describe the identification and classification of two different malignant cell populations in urine and vaginal fluid from a woman subsequently diagnosed with synchronous uterine and bladder tumours.

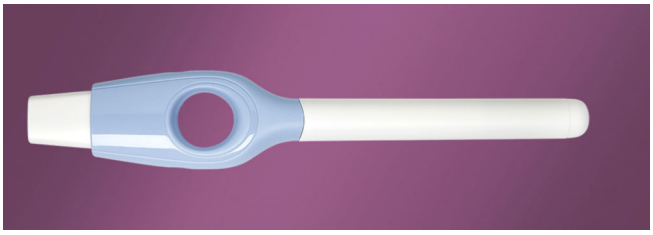

FIGURE 1 Delphi screener. The Delphi screener (Rovers Medical Devices) is a vaginal fluid collection device designed for self-sampling

2 | CASE REPORT

A 79-year-old woman presented to the Emergency Room with heavy postmenopausal bleeding. She was visually impaired from glaucoma and had a history of breast cancer. She lived with her son, who provided support with her activities of daily living. On examination, she was moderately frail with a clinical frailty score of 6. She mobilised with a zimmer frame. Vaginal examination revealed moderately heavy vaginal bleeding, a normal vulva and vagina, and a large, firm pelvi-abdominal mass. She was hemodynamically stable but with ongoing bleeding and a haemoglobin level of 114 g/L. She was therefore admitted to the gynaecology ward for tranexamic acid and further investigations.

The patient was recruited to the developing tests for endometrial cancer detection (DETECT) study,[5] a prospective observational study of urine and vaginal cytology for the detection of endometrial cancer in women with postmenopausal bleeding. Study participation involved the provision of a urine sample self-collected in a sterile pot and a

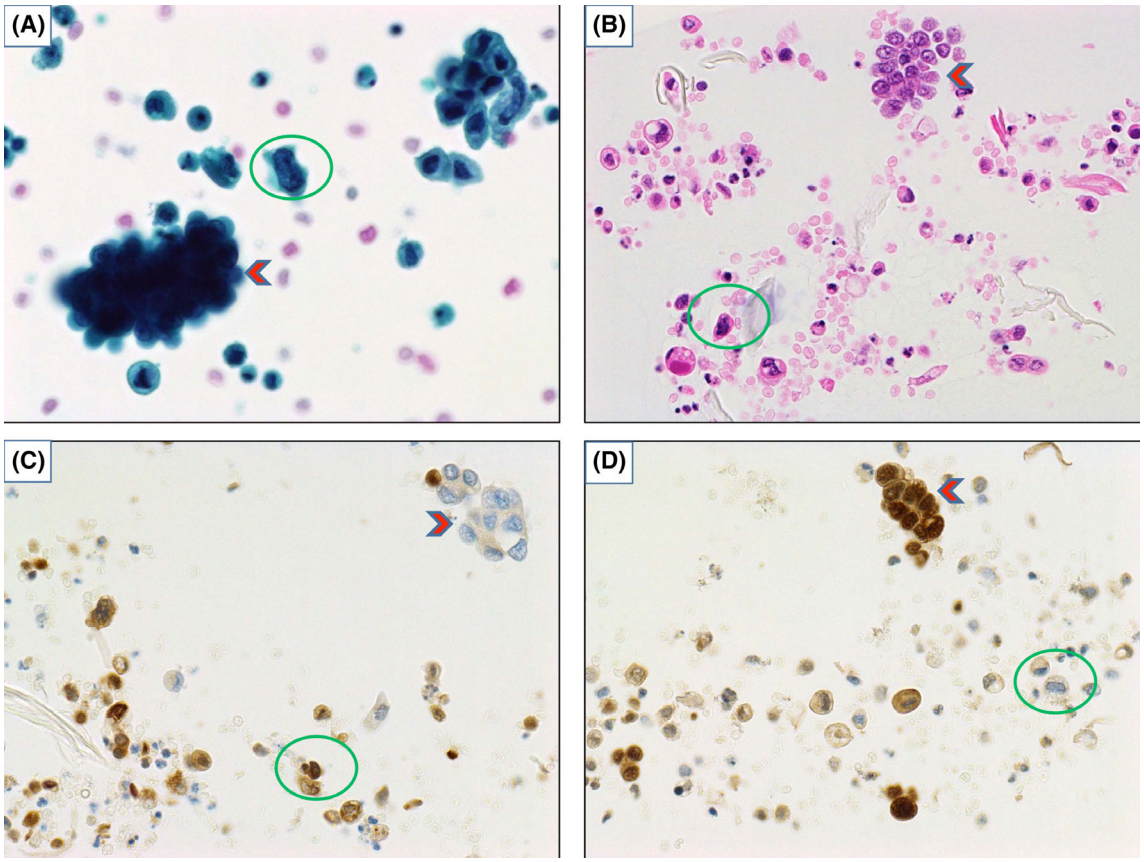

FIGURE 2 Microphotographs of voided urine sample. Single malignant high-grade urothelial carcinoma cells (green circle) and clusters of adenocarcinoma cells (red arrowhead). (A) Papanicolaou slide section stained with haematoxylin & eosin ×600; (B) Agar cell block section stained with haematoxylin & eosin ×400; (C) Immunocytochemistry for GATA3 using agar cell block section ×400; and (D) Immunocytochemistry for PAX8 using agar cell block section ×400

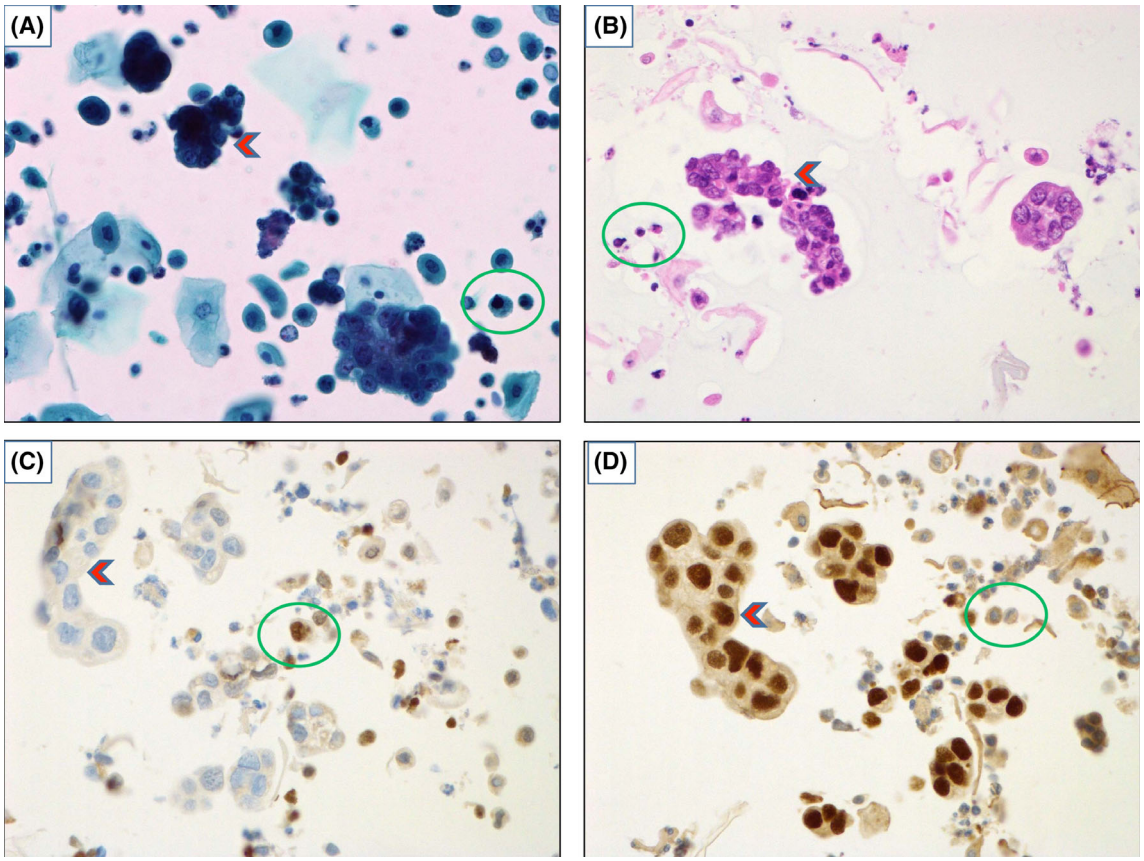

FIGURE 3 Microphotographs of vaginal sample. Single malignant high-grade urothelial carcinoma cells (green circle) and clusters of adenocarcinoma cells (red arrowhead). All images at ×400 magnification. (A) Papanicolaou slide section stained with haematoxylin & eosin; (B) Agar cell block section stained with haematoxylin & eosin; (C) Immunocytochemistry for GATA3 using agar cell block section; and (D) Immunocytochemistry for PAX8 using agar cell block section

TABLE 1 List of antibodies and dilutions used for immunocytochemical analysis

Antibody	Clone	Dilution	Malignant urothelial cells	Adenocarcinoma cells
CK7	OV-TL 12/30	1:250	Positive	Positive
CK20	KS20.8	1:100	Positive	Negative
GATA3	L50-823	1:100	Positive	Negative
PAX 8	Polyclonal	1:200	Negative	Positive

vaginal sample collected with a Delphi screener (Rovers Medical Devices), prior to routine investigations for postmenopausal bleeding. A member of the research team collected the vaginal sample with the patient in the supine position, legs bent at the knee and abducted at the hips. The Delphi screener (Figure 1) was inserted into the posterior fornix of the vagina and 3 ml saline expelled from the reservoir by depressing the plunger for 3 s. The fluid was then re-aspirated by releasing the plunger whilst slowly withdrawing and rotating the device. A sterile pot at the introitus collected residual fluid. The process was repeated twice by re-filling the reservoir with saline and re-aspirations performed until clear fluid was obtained.

The urine sample was positive for blood, protein, ketones and bilirubin by dipstick (blood-3+, protein-2+, ketones-2+, bilirubin-1+). Both urine and vaginal samples were fixed immediately after acquisition with equal volumes of BD CytoRich™ Red Preservative (CRR) (Becton, Dickinson and Company), labelled with a unique study identifier and transported to the Manchester Cytology Centre. Here, they were centrifuged at 3000 RPM for 5 min, supernatant decanted and the pellet re-suspended in 6 ml CRR for an hour to complete fixation. Further centrifugation was carried out at 1500 RPM for 10 min, the supernatant decanted and the remaining pellets prepared into liquid based cytology (LBC) Papanicolaou stained slides using the BD Prepstain (Becton, Dickinson and Company). The paired urine and vaginal Pap slides were then screened and reported independently by a consultant Biomedical Scientist and two consultant cytopathologists.

Both urine and vaginal cytology preparations contained two morphologically distinct populations of malignant epithelial cells. The first population comprised single cells with a small rim of cytoplasm

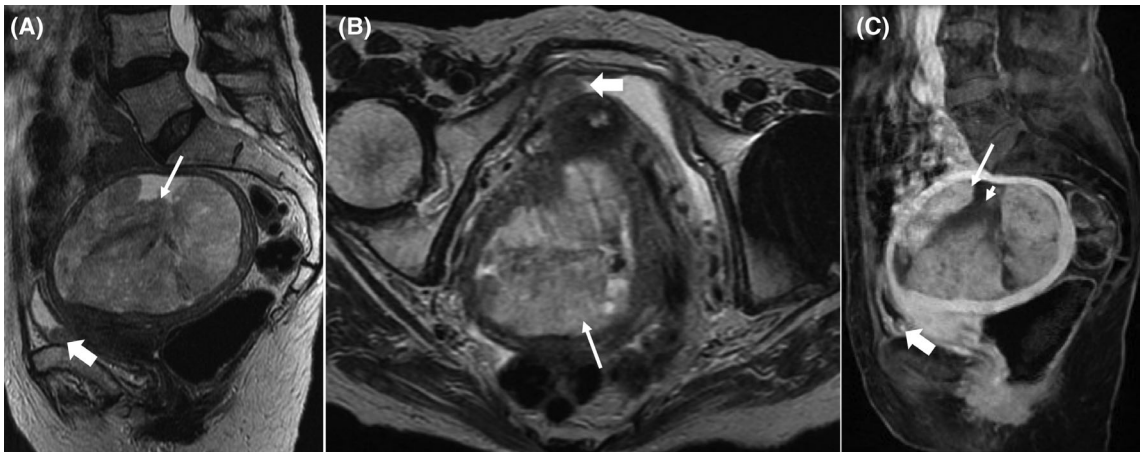

FIGURE 4 Magnetic resonance imaging scan of pelvis. Sagittal (A) and axial (B) T2 weighted sequences through the pelvis showing a large, heterogeneous, solid mass distending the endometrial cavity (thin arrow) and a small synchronous lesion within the minimally distended urinary bladder (block arrow). Sagittal T1 weighted sequence acquired 5 min post intravenous Gadolinium administration (C) shows heterogeneous progressive enhancement of the endometrial mass with areas of hyperenhancement (thin arrow) anteriorly and non-enhancement/necrosis centrally (short thin arrow). Small enhancing urinary bladder lesion again demonstrated (block arrow)

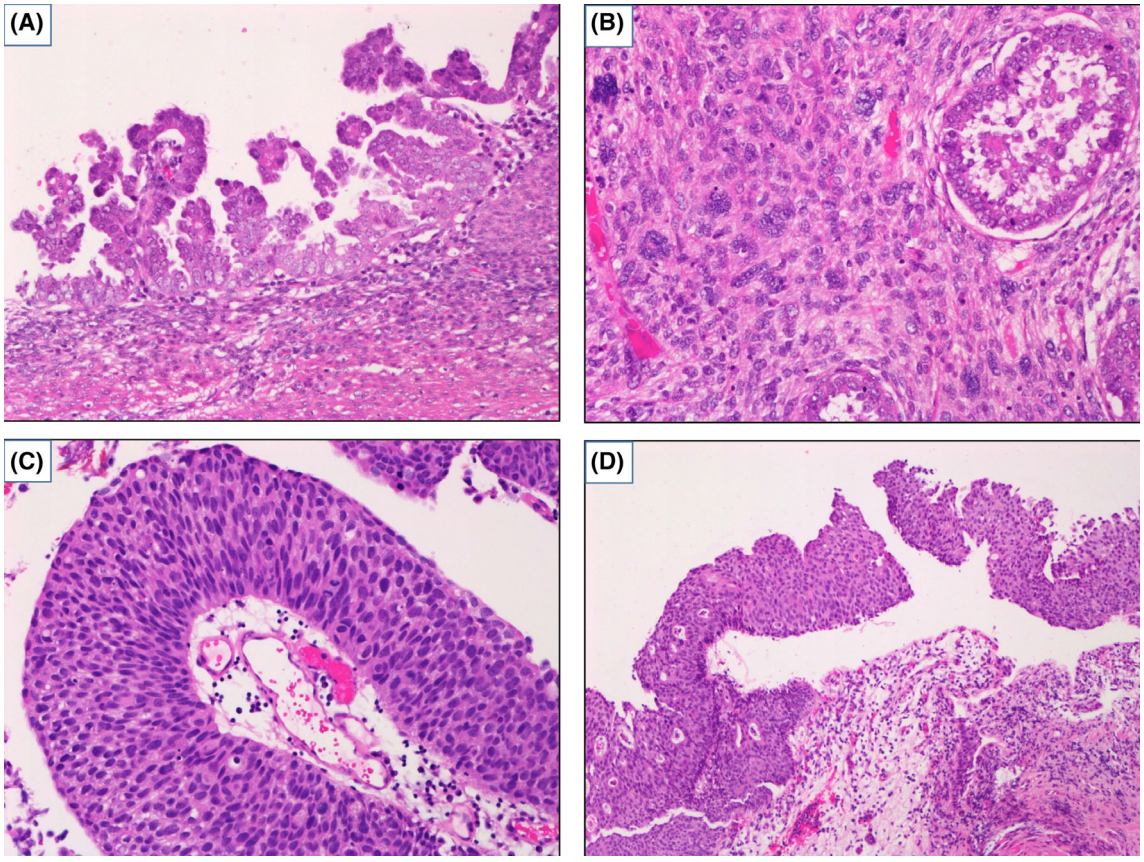

FIGURE 5 Microphotographs of uterine and bladder pathology. Haematoxylin & eosin-stained sections. Uterine carcinosarcoma shows (A) high grade adenocarcinoma ×200 and (B) sarcomatous elements ×200; (C) Bladder biopsy shows high-grade urothelial carcinoma ×200 and (D) carcinoma-in-situ ×100

and enlarged, angular, hyperchromatic nuclei containing coarse chromatin pattern and irregular nuclear outlines that were interpreted as being high-grade urothelial carcinoma cells (Figures 2A and 3A). The second population comprised 3-dimensional cell clusters with enlarged, eccentric nuclei containing coarse granular chromatin pattern and prominent nucleoli, which were interpreted as adenocarcinoma cells with high-grade cytologic atypia, most likely of endometrial origin (Figures 2A and 3A). The urine sample contained more high-grade

urothelial carcinoma cells and fewer adenocarcinoma clusters, whilst the reverse was true for the vaginal sample.

Agar cell blocks were prepared for each sample by concentrating residual material and embedding pellets into 3% molten agar with subsequent preparation of haematoxylin and eosin (H&E) sections (Figures 2B and 3B). A panel of immunocytochemistry (IHC) (Table 1) was performed on the Ventana BenchMark ULTRA (Roche Diagnostics) according to the manufacturer's instructions. The single malignant cells interpreted as high-grade urothelial carcinoma stained positively for GATA3 but negatively for PAX8 indicating urothelial origin (Figures 2C and 3C), whilst the adenocarcinoma clusters stained positively for PAX8 but negatively for GATA3 indicating female genital tract origin (Figures 2D and 3D). A cytological research diagnosis was made of synchronous malignancies of the urothelial and female genital tracts, most likely of endometrial origin, within 48 h of the patient's admission to hospital.

Routine investigations included transvaginal and transabdominal ultrasound scans, which showed an enlarged uterus, thickened endometrium and a large endometrial mass that demonstrated increased vascularity. Endometrial sampling by suction aspiration with a pipelle endometrial sampler was attempted on the gynaecology ward, but the procedure was poorly tolerated and abandoned. A subsequent hysteroscopy under general anaesthetic was suspicious for malignant disease and an endometrial biopsy yielded multiple fragments of p53-abnormal atypical glandular epithelium with crowded, highly complex glands demonstrating a cribriform architecture and marked cytological atypia. The stroma contained highly pleomorphic malignant cells. The overall appearances were consistent with carcinosarcoma of female genital tract origin. A subsequent magnetic resonance imaging (MRI) scan demonstrated a large heterogeneous endometrial mass with prompt arterial enhancement and restricted diffusion, and a small enhancing mucosal lesion within the right lateral wall of the urinary bladder (Figure 4). A staging chest, abdominal and pelvic computerised tomography (CT) scan showed enlarged pelvic and aortocaval lymph nodes and a 2.6 cm lesion within the posterior aspect of the left femur, suspicious for metastatic disease. The patient underwent total abdominal hysterectomy, bilateral salpingo-oophorectomy and bilateral pelvic lymph node sampling. Histology confirmed endometrial carcinosarcoma with involvement of the cervix, parametrium, both fallopian tubes and ovaries, and metastatic spread to 3 of 8 right and 2 of 7 left pelvic lymph nodes (Figure 5A,B). The ultrasound guided biopsy of the femoral lesion obtained necrotic, non-diagnostic tissue. Her case was discussed at the Gynaecology Multidisciplinary Team meeting and a diagnosis of at least FIGO 2009 Stage 3C1 uterine carcinosarcoma was made.

Further investigation of the urinary bladder lesion included attempted outpatient flexible cystoscopy, which was cancelled on first attempt pre-hysterectomy, due to a concurrent urinary tract infection, and on second attempt post-hysterectomy, because it was poorly tolerated. A cystoscopy was subsequently performed under general anaesthetic and found a 1–2 cm carpet-like lesion affecting the right lateral wall and base of the bladder. A directed biopsy obtained fragments of bladder mucosa showing a high-grade urothelial carcinoma (G3pTa) and carcinoma-in-situ (Figure 5C,D). Further active treatment for either cancer was abandoned because the patient developed vaginal and pulmonary recurrence shortly after her hysterectomy. A clinical decision was made in partnership with the patient and her family to pursue best supportive care, and she sadly died 3 months later.

3 | DISCUSSION/CONCLUSION

We have previously shown proof-of-principle that endometrial cancer can be detected in urine and vaginal samples by cytology.[5] Here we show that cytology and adjunctive immunocytochemistry can identify synchronous cancers of different urogenital aetiologies in the same biofluid sample. Whilst synchronous cancers of the urogenital tract are uncommon, they can cause diagnostic confusion, treatment delays and poor outcomes. Here we demonstrate that the distinctive morphology and immunoprofiles of tumours originating from different anatomical sites can be identified from a single biofluid sample by cytology. This can help rationalise subsequent diagnostic pathways, facilitate treatment planning and ensure that healthcare resources are spent appropriately. Rapid cancer diagnosis is an important goal for public sector healthcare providers since it improves efficiency and reduces costs.[6] It is also important for patients because early diagnosis of cancer is associated with less treatment-related morbidity, improved survival outcomes and better long-term quality of life.[7]

Here, urine and vaginal cytology was conducted within the confines of a research study, but their use in routine clinical practice could offer significant advantage. Non-invasive sampling combined with the established discipline of cytopathology provides an opportunity for a simple, community-based triage tool that rapidly distinguishes benign from malignant causes of urogenital bleeding. Women with positive urogenital cytology could be referred urgently for invasive diagnostic work-up, whilst those with negative results could be safely reassured. This would ensure that scarce healthcare resources are directed towards those with cancer and healthy women are spared the burden of tests they ultimately do not need. In an era of personalised medicine, the 'one-size-fits-all' approach to diagnosis is outdated and needs urgent reform. Diagnostics for uterine cancer have benefitted little from innovation over the past 50 years, with women being exposed to tests that lack specificity (transvaginal ultrasound scan) or are unacceptably invasive (hysteroscopy, endometrial biopsy).[4] The James Lind Alliance Womb Cancer Priority Setting Partnership recognised this unmet clinical need, finding "which women with abnormal vaginal bleeding should be referred urgently for investigations?" to be the second most important endometrial cancer research priority according to patients, their carers, healthcare professionals and members of the public.[8] The importance of finding new solutions to cancer diagnosis was also highlighted by "what simple, non-invasive, painless, cost-effective, and convenient tests can be used to detect cancer early" being ranked the most important research priority in a similar gap analysis for Detecting Cancer Early.[9]

Three previous case reports have demonstrated the potential for urogenital cytology to inform uterine cancer diagnosis,[10–12] and its use is well established for the diagnosis, surveillance and monitoring of bladder cancer patients.[13,14] The identification of cancer cells in these easily-obtained biofluids emphasises their potential as so-called 'liquid biopsies' and associated proteomic, genomic or metabolomic biomarkers could facilitate high-throughput automated testing for cancer early detection.[15] Future developments, including single cell technology, digital pathology platforms and machine learning algorithms are on the horizon. These digital solutions may be trained to recognise malignant cells by morphology, and when combined with adjunctive immunocytochemistry, may pinpoint site(s) of origin.[16] The utility of urogenital cytology as a screening tool for asymptomatic patients is untested but could provide the opportunity for home-based self-sampling for high risk patients, for example in Lynch syndrome, who may be at increased risk of both endometrial and urothelial tumours, and further research is needed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Emma J. Crosbie was the principal investigator for the DETECT study and is its guarantor. Helena O'Flynn and Neil A. J. Ryan obtained written, informed consent from the patient, recruited her to the trial and carried out study procedures. Nadira Narine, David Shelton and Durgesh Rana performed cytological analyses. Dina Awad reviewed the MRI and CT scan images. Nadira Narine, Helena O'Flynn and Emma J. Crosbie wrote the manuscript. All authors provided critical comment, edited the manuscript, and approved its final version.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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