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Anal cancer screening in women with a history of human papillomavirus-related lower genital tract cancers: a pilot study

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ABSTRACT

Objectives Women diagnosed with a history of lower genital tract cancer (LGTC) and precancer are at increased risk of anal cancer. Screening for anal cancer in a manner analogous to cervical cancer may detect precursor anal high-grade squamous intraepithelial lesions (HSILs) and prevent progression to cancer.

Methods In a pilot study of anal cancer screening, women with previous LGTC and aged ≥18 years in Sydney, Australia underwent a digital anorectal examination, anal swab for human papillomavirus (HPV) and p16/Ki67 testing and completed a questionnaire. Participants with positive HPV and/or p16/Ki67 results were referred for a high-resolution anoscopy (HRA) and evaluation of their HSILs.

Results Of 52 participants, 46 agreed to screening and 6 provided demographic information only. Median age was 46.5 years (IQR: 36.0–59.0). Anal high-risk HPV (HRHPV) was detected in only seven (15.2%) participants (three HPV16). Eight (17.4%) had positive p16/Ki67 dual staining, with invalid results for 25 (54.4%). Of 10 women referred for HRA, 9 attended and 3 had HSILs, representing 6.5% of the screened population. Questionnaires were completed by 41 participants (89.1%). The majority reported that being screened was reassuring (97.5%) and was positive for their health (95.1%).

Conclusion This pilot study demonstrated a lower-than-expected prevalence of anal HRHPV. Screening with HRHPV and p16/Ki67 staining identified anal HSILs in 6.5% of screened women. Despite some discomfort, screening was viewed as beneficial by almost all participants. The utility of p16/Ki67 dual staining was low, suggesting it may not be a suitable anal cancer screening methodology.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Anal cancer risk is higher among women with a history of lower genital tract cancer. Anal cancer screening uptake, acceptability and rates of positivity need to be determined in this population in Australia.

WHAT THIS STUDY ADDS

⇒ Women were willing to be screened and found the process highly acceptable. A significant minority of screened women had anal high-grade squamous intraepithelial lesions.The utility of p16/Ki67 dual staining may not be a suitable anal cancer screening methodology.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Further research into biomarkers such as DNA methylation, to improve the anal cancer screening algorithm, is warranted.

INTRODUCTION

Anal cancer most commonly occurs in the sixth or seventh decade of life, and its incidence increases with age.[1] An increase in incidence has been recorded in the past few decades.[2] In most geographical locations, anal squamous cell cancer (ASCC) accounts for 70% or more of cases,[3] and its incidence is 1.5-fold to 2-fold higher in women than in men.[4 5] Survival is highly stage dependent, and cure is likely if the cancer is diagnosed early.[4]

Nearly 90% of anal cancers are caused by persistent infection with oncogenic high-risk human papillomavirus (HRHPV).[6] One type of HRHPV, HPV16, causes 90% of anal cancers attributable to HPV.[7 8] It seems likely that in women, HPV is predominantly transmitted from the cervix to the anus, given the anatomical proximity, and the fact that the majority of women with diagnosed anal HPV or anal cancer report never having had anal intercourse.[9] A few specific population groups are at markedly increased risk of ASCC, largely because of either higher exposure to HPV infection in the anal canal (gay and bisexual men, women with a history of precancerous lesions or cancer in the lower genital tract (LGTC)), and/or impaired immune function (people living with HIV (PLHIV), solid organ transplant recipients and those with autoimmune conditions).[10]

Annually, about 18 000 women are diagnosed with anal cancer worldwide. Women with prior LGTC have between 10-fold and 50-fold higher risk compared with women in the general population.[10 11]

Anal cancer screening programmes targeted towards higher-risk groups have been advocated, aiming to reduce cancer rates as has occurred with cervical cancer screening.[12 13] The recently published ANal Cancer/HSIL Outcomes Research (ANCHOR) Study demonstrated a 57% reduction in anal cancer risk by treatment of precursor anal high-grade squamous intraepithelial lesions (HSILs) in PLHIV.[14] Despite this effective treatment for anal HSIL, there is no consensus on anal HSIL screening strategies. HPV testing has been examined as a potential tool to screen for anal HSIL. A single anal HPV test is a sensitive tool for identifying HSIL (88–100%), but specificity is low (22–41%).[15] The specificity of HPV testing can be improved by using type-specific genotyping and testing on two different occasions to confirm persistent infection. Another potential screening tool is p16/Ki67 dual staining, which has been used as a triage test for cervical colposcopy in women with detectable cervical HPV[16 17] and demonstrated better long-term risk stratification than cervical cytology over 5 years of follow-up.[18] It is imperative that high-resolution anoscopy (HRA) infrastructure is established prior to assessment of anal cancer screening strategies.[19] In Australia, HRA services are limited to clinics in Sydney (New South Wales), Perth (Western Australia) and Hobart (Tasmania).

This pilot study aims to explore anal cancer screening methodologies (anal HRHPV with genotyping, p16/Ki67 positivity) and the uptake of an anal cancer screening programme in women with prior LGTC in Sydney, Australia.

METHODS

The Women and Anal Dysplasia Assessment (WANDA) Study was a pilot study conducted at two clinical sites: Chris O'Brien Lifehouse and Prince of Wales Private Hospital in Sydney, New South Wales, Australia. The study recruited women with a history of cervical, vaginal or HPV-related vulva cancer, aged 18 years or greater, and who were currently undergoing post-treatment follow-up with their treating clinician. Women with HPV-independent vulvar cancers were excluded, as were women with a history of anal cancer, prior HRA, prior or current involvement in the St Vincent's Dysplasia and Anal Cancer Service and prior or current participation in HPV-related anal lesion research.

At the baseline visit, study clinicians provided potentially eligible participants with standardised anal cancer education that described the risk of anal cancer in higher-risk groups, anal cancer natural history and the screening procedures. Women were given a choice to participate in either full anal cancer screening, collection of only demographic data or not to participate. Written informed consent was obtained and reasons for not participating in anal cancer screening were recorded (more than one reason could be reported). The demographic data are listed in table 1.

Participants underwent a digital anorectal examination (DARE). Those with lesions suspicious for anal cancer were referred for further evaluation and ceased their study participation. An anal swab was collected for HPV and p16/Ki67, and a lower vaginal/vulvar swab and cervical/upper vaginal swab were collected for HPV testing.

The swab was rinsed into a vial of PreservCyt fluid (Hologic Corp, Marlborough, Massachusetts, USA), from which a ThinPrep (Hologic Corp, Marlborough, Massachusetts, USA) slide was produced. Instead of the standard practice of staining for cytology, two immunostains (p16 and Ki67) were applied, using the CINtec PLUS kit (Roche MTM Laboratories, Tucson, Arizona, USA), according to the manufacturer's instructions. The slides were initially screened by a senior cytologist and then examined by a cytopathologist, both trained in interpretation of such dual-stained slides. An assessment was made whether the slide cellularity was satisfactory (defined as at least 2000 nucleated squamous cells) and whether any squamous cells were dual-stain positive. This is defined as the coexistence of red nuclear staining (Ki67 proliferative marker positive) and brown cytoplasmic staining (p16, surrogate marker for active HRHPV positive) (figure 1). Dual positivity only occurs in cells transformed by HRHPV. The reporting categories were: positive (one or more positive cells), negative (no positive cells) or unsatisfactory (no positive cells but fewer than 2000 nucleated squamous cells present). Within the positive category, the number of positive cells was recorded.

Within the week following the initial screening tests, participants were sent a link via email to invite them to complete an electronic questionnaire on pain, bleeding and acceptability of the screening tests.

HPV testing was performed using the Roche COBAS 4800. If positive for 'other HRHPV', specific type identification was then determined by EUROArray (HPV18, 26, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59, 66, 68, 73 and 82).

Participants with positive HRHPV results and/or p16/Ki67 dual staining were referred to St Vincent's Hospital, Sydney, for HRA. Women referred for HRA had anal cytology performed and biopsy of any suspicious lesions.

Women with previous LGTC were not involved in the design of the research question or the outcome measures. This is a pilot study and a larger study based on these results will include members of the affected community on protocol steering committees and in dissemination of study findings. In accordance with the journal's guidelines, we will provide our data for independent analysis by a selected team by the editorial team for the purposes of additional data analysis or for the reproducibility of this study in other centres if such is requested.

Table 1 Demographic and laboratory results for 46 participants

	n (%)
Median age	46.5 (IQR 36.0–59.0)
Country of birth	
Australia	27 (58.7)
Other	19 (41.3)
Language spoken at home	
English	39 (84.8)
Other than English	7 (15.2)
Smoking status	
Non-smoker	20 (45.5)
Current smoker	6 (13.6)
Past smoker	18 (40.9)
History of cervical cancer	
No	8 (17.4)
Yes	38 (82.6)
History of vaginal cancer	
No	43 (93.5)
Yes	3 (6.5)
History of vulval cancer	
No	41 (89.1)
Yes	5 (10.9)
History of HPV vaccination	
No	36 (78.3)
Yes	10 (21.7)
Type of HPV vaccine	
9 valent	2 (20.0)
4 valent	8 (80.0)
Age at first vaginal sex	18.0 (16.0–19.0)
Number of lifetime male sexual partners	6.0 (3.5–11.5)
History of anal sex	
No	26 (56.5)
Yes	20 (43.5)
Anal high-risk HPV	
Negative	39 (84.8)
Non-high-risk HPV16	4 (8.7)
HPV16	3 (6.5)
p16/Ki67 dual staining	
Negative	13 (28.3)
Positive	8 (17.4)
Unsatisfactory	25 (54.4)
HRA referral	
No	36 (78.3)
Yes	10 (21.7)
Reason for HRA referral	
High-risk HPV positive	4 (40.0)
p16/Ki67 positive	3 (30.0)
High-risk HPV and p16/Ki67 positive	3 (30.0)
Anal cytological results	
Negative	1 (11.1)
ASCUS	2 (22.2)
LSIL	2 (22.2)
ASC-H	1 (11.1)
HSIL-AIN2	2 (22.2)
Unsatisfactory	1 (11.1)
Histological results	
Negative	5 (55.6)
Flat LSIL	1 (11.1)
HSIL-AIN2	2 (22.2)
HSIL-AIN3	1 (11.1)

AIN, anal intraepithelial neoplasia grade; ASC-H, atypical squamous cells, cannot exclude HSIL; ASCUS, atypical squamous cells of undetermined significance; HPV, human papillomavirus; HRA, high-resolution anoscopy; HSIL, high-grade squamous intraepithelial lesion; IQR, interquartile range; LSIL, low-grade squamous intraepithelial lesion.

RESULTS

Participants were recruited between February 2021 and February 2023. A total of 52 participants were enrolled, with 6 (11.5%) declining anal cancer screening and providing demographic information only. The reasons given for not undergoing the screening tests were concerns about pain (three women), about the results (four women) and unproven benefit (one woman). More than one reason could be provided.

Of the remaining 46 women, all were HIV negative, median age was 46.5 years (IQR: 36.0–59.0), 58.8% were Australian born and just above 20% had received HPV vaccination (10, 21.7%). Most women had prior cervical cancer (38, 82.6%), 5 (10.9%) had vulvar and 3 (6.5%)

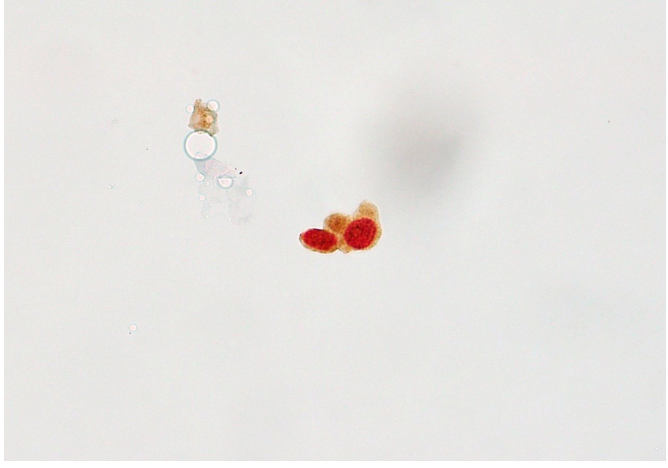

Figure 1 Two dual-stain-positive squamous metaplastic cells. The nuclei stain red with Ki67 and the cytoplasm stains brown with p16.

Table 2 Anal HRHPV and p16/Ki67 results in 46 participants

p16/Ki67 dual staining	HRHPV			Total
	Negative (%)	Non-HRHPV16 (%)	HPV16 (%)	
Negative	12 (92.3)	1 (7.7)	0 (0)	13
Positive	3 (37.5)	3 (37.5)	2 (25.0)	8
Unsatisfactory	24 (96.0)	0 (0)	1 (4.0)	25

HRHPV, high-risk human papillomavirus.

had vaginal cancer. One woman was referred after an abnormal DARE, which was later diagnosed as internal haemorrhoids. Technically valid HPV results were available for all 46 women, and 7 (15.2%) had anal HRHPV detected (3 HPV16). Eight (17.4%) had positive p16/Ki67 staining. The number of participants with invalid results for p16/Ki67 staining was high (25, 54.4%). Of those with valid dual staining results, eight (38.1%) had positive dual staining results. Numbers were too small for statistical analyses but of the three participants with HPV16, dual stain was positive in two and unsatisfactory in one (table 2). Five (12.2%) of 41 women who were tested for cervical HPV had cervical HRHPV detected (all non-HPV16). Six (14.3%) of 42 women who were tested for vulval HPV had vulval HRHPV detected (all non-HPV16).

Of 10 women referred for HRA (4 HRHPV positive, 3 p16/Ki67 positive and 3 positive for both), all had cervical cancer (mean of 6 years prior). One woman moved interstate and was followed up there. At HRA for the remaining nine, no anal cancer was detected, five had normal histology, one had low-grade squamous intraepithelial lesions and three HSILs (two HSIL-anal intraepithelial neoplasia grade (AIN)2, one HSIL-AIN3). Anal cytology results are listed in table 1. Numbers were small but there was no trend indicating increasing dual staining positivity with higher HSIL risk (data not shown).

41 of the 46 (97.5%) participants completed acceptability questionnaires. More participants found the experience of having an anal swab more unpleasant than the DARE (32.5% vs 22.5%). Most participants reported that being screened was reassuring (95.1%) and was positive for their health (95.1%). Seven participants (17.1%) found the DARE quite/very uncomfortable and three (7.3%) found it quite painful (none reported it was very painful). Six participants (14.6%) found the anal swab quite a lot/very uncomfortable and three (7.3%) found it quite a lot/very painful. Women were asked to describe their experience of being on the study. Universally, their experience was positive, with comments such as 'I wasn't aware that anal cancer existed, and I was very happy to do the screening test.' and 'The tests are nowhere near as uncomfortable, painful, time-consuming, or upsetting as having cancer. If they help to reduce that risk, I would 100% do it again.'

DISCUSSION

Summary of main results

In this pilot study of anal cancer screening among women with previous LGTC, almost 90% of women offered screening accepted it, and all anal swabs were technically satisfactory. Anal HRHPV detection (15.2%) and p16/Ki67 positivity (17.4%) were relatively uncommon. About one in five (21.7%) were referred for HRA, and one-third of those who had HRA had anal HSIL, representing 6.5% of the screened population. Despite procedure-associated pain and discomfort being reported by several participants, anal cancer screening was viewed as a beneficial process by almost all participants.

Results in the context of published literature

A meta-analysis published in 2015 of the prevalence of anal HPV and related disease in women found a prevalence of anal HRHPV infection of 23–86% in HIV-negative women with HPV-related lower genital tract pathology compared with 5–22% in women with no known HPV-related pathology. Histological anal HSIL was 0–9% among women with lower genital tract pathology, a range which included the histological HSIL prevalence in our study.[11] A recently published study of 324 women with low genital tract high-grade dysplasia/cancer, with the majority diagnosed with cervical dysplasia (229, 70.1%) or cancer (21, 6.5%), detected a higher prevalence of anal HRHPV (92, 28%) than our study. This may reflect the large number of participants with cervical HSIL and smaller proportion with cancer, who may be younger/more sexually active than our cohort. Anal cytology was performed and was abnormal in 70 participants (23%). Of the 55 (79%) who underwent HRA, 2 had HSILs, representing 0.62% of the screened population, 10-fold lower than was found in our study.[20]

Strengths and weaknesses

The strength of this study was the recruitment of women with different cancer diagnoses, age range and countries of origin, which makes the results generalisable to women with previous LGTC in Australia. Limitations include the small sample size. Despite study clinicians receiving intensive training in anal swab collection, the fact that over half of the p16/Ki67 tests were unsatisfactory may compromise dual staining use in screening in this population.

Implications for practice and future research

The utility of dual staining with p16/Ki67 was low, suggesting it may not be a suitable screening methodology unless specimen adequacy can be improved. The cause of the high rate of invalid results was unsatisfactory cellularity (as defined above) in many samples. Anal swab samples are acknowledged to be of generally lower cellularity than cervical samples and feedback to clinicians is often needed to improve their ability to obtain a cellular sample.

Other HPV-related biomarkers such as DNA methylation have shown promising results in anal cancer risk

stratification and may be more suitable triage tests.[21 22] Anal cancer screening guidelines will soon be available,[19] including recommendations for women with previous LGTC. The high level of acceptability of screening among women with LGTC found in our study is encouraging. The recent ANCHOR Study results of efficacy of treatment of HSIL in PLHIV[14] add to the impetus for provision of effective anal cancer screening in other high-risk populations, including women with previous LGTC.

Conclusions

This pilot study demonstrated a lower-than-expected prevalence of anal HRHPV. Screening with HRHPV and p16/Ki67 staining identified anal HSIL in 6.5% of screened women, a rate of anal HSIL comparable with that found in other studies of women with LGTC.[11] Despite some discomfort, participants in general felt screening was positive for their health and were motivated to undergo screening. The utility of p16/Ki67 dual staining was low, suggesting it may not be a suitable anal cancer screening methodology.

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Patient consent for publication Not applicable.

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