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Transplant Recipients and Anal Neoplasia Study: Design, Methods, and Participant Characteristics of a Prevalence Study

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Abstract: Kidney recipients have anal cancer rates 3 times higher than the general population in Australia and New Zealand. High-risk human papillomavirus (HPV) genotypes are implicated in the majority of anal cancers. Establishing the epidemiology of anal HPV infection and precursors of anal cancer in transplant recipient populations is 1 consideration in any potential screening program. The Transplant and Anal Neoplasia Study is a cross-sectional study of the prevalence of anal cytological abnormalities and HPV deoxyribonucleic acid in kidney transplant recipients, as well as evaluating the acceptability of an anal cancer screening intervention. The study aims to recruit 100 kidney transplant recipients, older than 18 years, in Australia. Transplant recipients attending for a protocol biopsy at 3 and 12 months and annually posttransplant are approached to participate. Participants undergo an anal swab, which is then analyzed using liquid-based cytological examination and tested for the detection of 37 anogenital HPV deoxyribonucleic acid genotypes. Participants also complete a demographic and behavioral questionnaire that covers sexual behavior, history of anal symptoms, and possible anal cancer risk factors. Associations will be tested using multiple regression analysis. Recruitment for the study began in 2015 and is ongoing. To date, 96 (77%) of 125 kidney transplant recipients approached have consented to the study. The mean age is 48 (median, 47 y; range, 20–76 y), 59% are male, and Northwest European (58%) represented the largest ethnic group. No participants self-identified as Aboriginal or Torres Strait Islander. High consent rates and positive qualitative results suggest that a larger screening program may be well received by kidney transplant recipients, with increased resources and some modification to the timing of approach. Further results of the study will inform the possible implementation of a larger screening trial for prevention of anal cancers in kidney and other solid organ transplant recipients.

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Anal squamous cell cancer (ASCC), as with cervical squamous cell cancer, is caused by persistent infection with high-risk human papillomavirus (HPV). Both cancers have a precursor stage, high-grade squamous intraepithelial lesion (HSIL). Dramatic reductions in cervical cancer incidence have been observed since screening programs have been implemented to detect and ablate cervical HSIL. There are however notable differences between the etiology of anal and cervical cancer, and there is currently insufficient evidence to fully support a similar screening and treatment program to prevent anal cancer.

In 1968, the World Health Organization commissioned a report,[1] which described selection criteria to guide decisions around whether or not to screen for a particular disease. One of the guidelines states the need to identify a target patient population for screening. There are a number of clearly identified subpopulations with elevated anal cancer incidence rates. Along with gay and bisexual men, people living with human immunodeficiency virus (HIV), and women with previous HPV-related genital disease,[2,3] solid organ transplant recipients are one such subpopulation. In Australia and New Zealand, kidney transplant recipients are estimated to have almost 3 times the risk for anal cancer compared to the general population (standardized incidence ratio, 2.76; 95% confidence interval, 1.51–4.64).[4]

Anal squamous cell cancer is uncommon in the general population, with an estimated global age-adjusted annual incidence rate of up to 1.3 cases per 100000 in men, and 1.7 cases per 100000 in women.[5] However, incidence rates are rising in most high-income countries, including Australia.[5] In Australia, the rate of ASCC in the general population increased from 0.65 to 1.00 per 100000 between 1982 and 2005.[6] Risk of ASCC increases exponentially with age, in both men and women, and women have a higher incidence rate overall.[6] Anal cancer often presents late in the disease process and prognosis is poorer with increasing tumor size. Retrospective analysis of people with anal cancer has shown 61% present with stage 1 or 2 cancer and 35% have lymph node involvement at diagnosis.[7] There is potential for early intervention. Early detection of invasive anal carcinoma is associated with increased recurrence-free survival. Patients with locally advanced tumors had significantly increased risk of cancer-specific death (hazard ratio, 2.55; 95% confidence interval, 1.49–4.38).[8] Recognized risk factors are older age, cigarette smoking, infection with HIV, immune deficiency, receptive anal intercourse, higher lifetime number of sexual partners, a history of other anal sexually transmitted infections, and corticosteroid use for over 6 months.[3,9]

Our understanding of the natural history of ASCC is incomplete. Current models are based on data from gay and bisexual men, with and without HIV infection, and on analogy with cervical cancer in women. Central to ASCC pathogenesis is persistent infection with high-risk HPV types, most commonly HPV 16 and HPV 18,[10] causing cellular changes in anal epithelium and the development of precancerous lesions, termed high-risk squamous intraepithelial lesions (HSIL).[11] However, the rate of progression and clearance of HSIL and low-grade squamous intraepithelial lesions (LSIL) is not well understood, even in high-risk populations. It is estimated that 1 in 600 HSIL becomes malignant.[12] These estimates are based on cross-sectional studies in HIV-positive gay and bisexual men. Longitudinal studies investigating HPV-associated anal lesions and cancers are currently underway.[13]

There are few data on the prevalence and natural history of HPV-associated anal cancers in transplant recipients. A UK study of the prevalence of HPV infection in the anal canal of kidney transplant recipients found a strong correlation between precancerous lesions and HPV infection, with HSIL or LSIL found in 5.8% of recipients, and anal HPV infection in 21.3% of transplant recipients.[14] Earlier studies using biopsy tissue found that anal HSIL, LSIL, and cancer were significantly more prevalent in kidney transplant recipients (20.3%) compared with age-matched controls (0.7%).[15] All patients with anal HSIL, LSIL, or cancer had co-existing HPV infection, and of the 133 recipients, 47% had HPV 16 deoxyribonucleic acid (DNA) detected in their biopsy. Interestingly, HPV infection alone was higher in the control group (12.4% controls vs 3.8% recipients), which could indicate an increased rate of progression in transplant recipients.[15]

Human papillomavirus vaccines are safe and may benefit immunocompromised groups; however, studies of vaccination in solid organ transplant recipients have demonstrated rapid declines in antibody titer 1 year after vaccination. Reduced immunogenicity was associated with vaccination early posttransplant, and higher levels of immunosuppressive drug treatment.[16] The International Papillomavirus Society recommends immunocompromised individuals are ideally vaccinated prior to the development of immunocompromised status, or while immunocompromised in a 3-dose regimen.[17] Until the clinical implication of lower antibody titers in immunocompromised individuals is known, screening remains a critical component of anal cancer prevention.

There are currently no national guidelines for anal cancer screening in the general population or transplant recipients. In Australia, cervical screening programs have successfully reduced cervical cancer incidence and mortality.[18] The current Australian cervical cancer screening program has moved from cytological screening to a more risk-based approach using molecular testing, with liquid based cytology analysis performed only if HPV DNA is detected.[19] One or both of these tests could potentially provide a similar benefit in the prevention of anal cancers in the transplant population, depending on the performance of these tests and the efficacy of anal HSIL treatment. Individuals who test positive for high-risk HPV infection or cytological HSIL could then be offered high-resolution anoscopy to determine the presence or absence of HPV-related lesions by guided biopsy. Individuals with biopsy-confirmed HSIL could be offered closer monitoring, and possible early intervention, to prevent the development of invasive ASCC.

There are currently no evidence-based consensus guidelines on the treatment of anal HSIL. Widely used ablative treatments using laser, cryotherapy, infrared coagulation and cautery, and targeted excision, as well as newly developed strategies such as topical application of various agents, photodynamic therapy, and vaccination have been associated with considerable recurrences.[20] Randomized studies are ongoing to determine whether topical or ablative treatment of anal HSIL is of value in preventing anal cancer in increased risk populations, compared with active monitoring.[21]

Kidney transplant recipients are closely monitored in routine outpatient appointments to assess their progress and the condition of their transplant, providing ample opportunity to

test a potential screening intervention. Few studies, however, have reported the use of anal swabs to assess anal HPV infection and HSIL in high risk groups,[13,14] and the acceptability of anal swab testing in the transplant population is unknown.

We have identified 2 areas of research requiring further investigation in transplant recipients to inform the development of a screening program for ASCC prevention in this population: natural history of the disease and acceptability of screening procedures. In a manner similar to the Study of Prevention of Anal Cancer (SPANC) study, which was undertaken in gay and bisexual men,[13] the Transplant and Anal Neoplasia (TAN) study aims to investigate the possible roles of anal HPV and cytology tests, and the acceptability of anal screening in the kidney transplant population. The results of this study will inform the possibility of a larger longitudinal study in the transplant population. This article describes the design and methods of the TAN study and includes the cohort characteristics of kidney transplant recipients recruited between 2015 and 2018. A brief thematic analysis of qualitative data, where recipients provided reasons for participation or nonconsent is also included.

PROTOCOL DESIGN AND METHODS

The TAN Study is a single-center, cross-sectional study, based at a large transplant center at Westmead Hospital, in New South Wales, Australia. Specifically, the study aims are to (1) assess the acceptability of anal swab testing, (2) determine the distribution of anal cytological abnormalities, (3) determine the prevalence of high- and low-risk anal HPV infection, and (4) investigate any correlation between HPV infection, abnormal cytology, and sociodemographic and lifestyle characteristics, in kidney transplant recipients.

The TAN study is approved by the Western Sydney Local Health District Ethics Office ((4136) AU RED HREC/14/WMEAD/421). Humans involved in this study were treated in a manner in accordance with the Declaration of Helsinki and the Declaration of Istanbul. Written informed consent is obtained from all individuals before any study specific procedures are performed. The trial is registered with the Australian New Zealand Clinical Trials Registry as of 1 November 2016 (Web address: http://www.ANZCTR.org.au/ACTRN12616001507471.aspx).

Study Population and Recruitment

Participant recruitment began in 2015 and will conclude when 100 kidney transplant recipients have completed study procedures. Kidney transplant recipients, including recipients of kidney and pancreas, aged over 18 years, attending routine posttransplant renal biopsy appointments at the renal medicine and outpatient clinic, Westmead Hospital are prospectively recruited. Prospective participants are identified in the clinic waiting room, or in the renal ward, and approached by study clinicians where they are asked to confirm their eligibility. Those participants unable to provide informed consent, with a history of previous anal cancer, or any other anal pathology associated with significant anal bleeding, are excluded from the study.

Study Procedures

Figure 1 describes the flow of study procedures. Following informed consent, participants are asked to complete a sociodemographic and lifestyle questionnaire and undertake an anal swab. Swabs are taken by a trained investigator, using a moistened Dacron swab which is inserted (3–5 cm) into the anal canal and slowly withdrawn in a spiral fashion over approximately 20 seconds. Material collected on the swab is then immediately suspended in a ThinPrep (Hologic) vial containing 20 mL of PreservCyt (Hologic, Inc., Marlborough, MA) fixative medium. Anal samples in PreservCyt are sent to Douglas Hanly Moir Pathology (DHM, Sydney, Australia) for processing, where an aliquot is obtained under aseptic conditions (to avoid cross-contamination) is for HPV genotyping.[22]

Cytopathology

Samples are processed by ThinPrep 5000 and slides produced then stained with ThinPrep proprietary stain (Hologic Inc.) before being manually assessed by study cytologists. DHM offers specialist anogenital cytology and histopathology, operating within a large private general pathology laboratory in Sydney, Australia. Training of cytology study staff and criteria for assessment have been described previously in the SPANC study.[13] Briefly, all study staff are trained on a set of teaching slides drawn from previous smaller studies of anal canal lesions. To enhance reproducibility of reporting, only 3 anatomical pathologists, with over 15 years' experience in anogenital pathology, are involved in the study. Stained study slides are manually screened by a study cytologist on a standard light microscope. The resulting preliminary report and slide are sent to 1 of 3 study pathologists for final assessment and reporting. Slides must have at least 2000 nucleated squamous cells to be satisfactory for assessment, unless abnormal cells are present, in which case the abnormality is reported. Cytology results are classified using the Australian Modified Bethesda System as negative, possible LSIL, LSIL, possible HSIL, and HSIL. Where possible, participants whose anal swab samples are reported as unsatisfactory are contacted for a repeat swab.

HPV Genotyping

An aliquot of 1 mL of anal PreservCyt specimens is extracted using the automated MagNA Pure 96 isolation and purification system (Roche Molecular Systems, Alameda, CA) and tested using the Roche Linear Array (LA) HPV genotyping test (Roche Molecular Systems) in a process described previously.[13,23] The LA HPV genotyping test involves polymerase chain reaction (PCR) amplification of target DNA, followed by hybridization with a reverse line blot system for the simultaneous detection of HPV genotypes 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39 (HPV 82v subtype), and CP6108 (HPV 89) as well as amplification of the human beta-globin gene as an internal control. Linear array HPV genotyping has been validated against dot blot methods with good agreement for HPV positivity (kappa = 0.78, $P < 0.001$), and higher sensitivity in low-level samples.[24] Samples that produce a negative internal control are retested with half the amount of sample DNA in order to reduce PCR inhibition sometimes observed with anal canal swab samples. Samples that test negative for both internal control and HPV DNA are considered unassessable for HPV and excluded from analysis. Due to possible cross-reactivity on LA of the HPV 52 probe with types 33, 35, and 58 amplicons, samples positive for 1 or more of

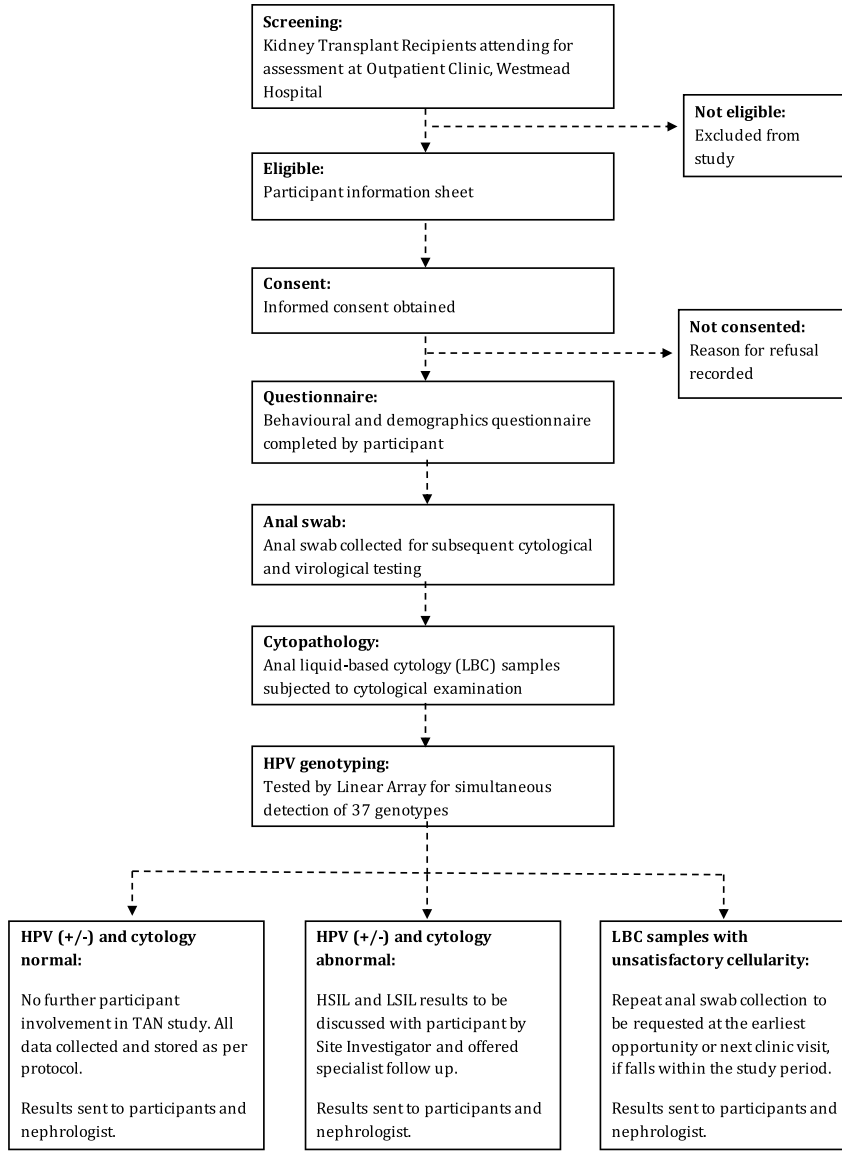

FIGURE 1. Flow of participants and study procedures. Participants with positive cytology were referred to a specialist for follow-up regardless of HPV status. Participants with unsatisfactory cytology were asked to return for a repeat swab collection. HPV, human papillomavirus; HSIL, high-risk squamous intraepithelial lesions; LSIL, low-grade squamous intraepithelial lesions; TAN, Transplant and Anal Neoplasia.

HPV 33, 35, and 58 probes are further tested on a confirmatory HPV 52-specific quantitative real-time PCR.[25]

Study cytology and HPV test results are provided to participants and their Nephrologists and General Practitioners. Outcomes are described in Figure 1. Participants with high grade cytological abnormalities (HSIL and possible HSIL) with or without concurrent HPV DNA detected are referred for specialist follow up at Dysplasia and Anal Cancer Services at St Vincent's Hospital. Participants with low-grade abnormalities (LSIL and possible LSIL), irrespective of HPV status, are invited to return for screening in a year's time. As detection of HPV DNA is not an indication of risk of anal cancer, we did not recommend any follow-up for patients with normal cytology and HPV DNA detected in their sample. However, concerned participants were encouraged to refer to their general practitioners with any concerns.

Questionnaire

The study questionnaire is adapted from the SPANC study baseline questionnaire to suit the transplant population.[13] A copy of the questionnaire is included as supplementary material (Figure S1, http://links.lww.com/TXD/A187). The questionnaire includes sociodemographic questions, residential postcode, age, gender, ethnicity, highest level of education, and current employment status, as well as health and lifestyle questions related to risk factors for developing HPV-associated ASCC. These questions include cigarette smoking, sexual behaviors, history of anogenital warts, and previous abnormal cervical cytology. The questionnaire also includes questions concerning anal health, and concurrent but unrelated anal conditions, such as hemorrhoids and fissures, as well as sexual practices, such as questions about sexual identity, number of lifetime partners, and experience of receptive anal intercourse. Clinically relevant data including date of transplantation, time on the waiting list, and treatment history list are obtained from patients' clinical records.

Analysis

Prevalence of abnormal anal cytological changes is estimated in all participants, and also separate analysis of

TABLE 1.

Participant sociodemographic and lifestyle characteristics

Participant characteristics	n (%)
Participants	96 (100)
Completed questionnaire	95 (99)
Demographics	
Sex	
Female	39 (41)
Male	57 (59)
Age group, y	
18-24	4 (4)
25-34	9 (9)
35-44	29 (30)
45-54	32 (33)
55+	22 (23)
Ethnic and cultural background[a]	
Oceanian	6 (6)
Northwest European	56 (58)
Southern and Eastern European	8 (8)
North African and Middle Eastern	5 (5)
Southeast Asian	11 (11)
Northeast Asian	5 (5)
Southern and Central Asian	3 (3)
Peoples of the Americas	2 (2)
Sub-Saharan African	1 (1)
Aboriginal or Torres Strait Islander	
Yes	0 (0)
No	95 (100)
Socioeconomic characteristics	
Current employment	
Unemployed	41 (43)
Employed	49 (51)
Declined or incomplete	6 (6)
Household income per fortnight	
<AUD 999	36 (38)
>AUD 1000	37 (39)
Declined or incomplete	23 (24)
Highest education received	
Before or at Year 10 of study (16 y)	26 (27)
Year 12 of study (18 y)	21 (22)
Diploma/trade certificate	20 (21)
Undergraduate degree	15 (16)
Postgraduate degree	8 (8)
Declined or incomplete	6 (6)
Lifestyle	
History of smoking	
Never smoked	50 (52)
Current or ex-smoker	42 (44)
Declined or incomplete	4 (4)
Sexual identity	
Heterosexual	84 (88)
Homosexual	1 (1)
Declined or incomplete	11 (11)
Engaged in receptive anal intercourse (ever)	
No	84 (88)
Yes	12 (13)
Clinical	
History of anogenital warts	
No	94 (98)
Yes	2 (2)
HPV vaccinated	
No	94 (98)
Yes, more than 1 y ago	2 (2)
History of sexually transmitted infection	
No	89 (93)
Yes	7 (7)
History of abnormal Pap smear[b]	
No	32 (82)
Yes	7 (18)
Time since transplant	
<3 mo	46 (47)
3 mo to 1 y	11 (11)
1-2 y	17 (18)
2-5 y	7 (7)
Over 5 y	16 (17)

[a]Classifications based on the Australian Standard Classification of Cultural and Ethnic Groups (Australian Bureau of Statistics, 2016).

[b]Women only.

HPV, human papillomavirus.

cytologically predicted LSIL and HSIL. The HPV prevalence will be estimated in the same fashion, for infection of all genotypes in all participants, and for low- and high-risk genotypes. Further subanalysis for transplant status, length of time on immunosuppression, and behavioral factors is also being conducted. Regression analysis is being performed to examine interactions and associations between HPV DNA detection and cytological changes, and with sociodemographic and lifestyle characteristics, and other clinical factors.

Thematic analysis of participants' responses for participation is conducted by 2 study investigators following guidelines by Braun and Clarke.[26] Investigators individually review and code qualitative responses before jointly examining their codes for common themes. These themes will be systematically validated against individual participant responses for particular cases that do not fit into themes, and the group as a whole for particular themes that may incompletely represent responses provided. A preliminary analysis has been performed.

Consent rates, completion of study procedures (anal swab and questionnaire), the proportion of samples with unsatisfactory cytology results, and referral versus attendance rates for high-resolution anoscopy are also reviewed alongside a summary of participant demographics.

Characteristics of the Enrolled Population to Date

Of 125 kidney transplant recipients approached to participate in the study, 96 (77%) consented to study procedures. Reasons for participation or refusal were captured and further discussed below. Of 96 participants consented to date, 95 (99%) completed the study questionnaire. The sociodemographic and lifestyle characteristics of consented kidney transplant recipients are presented in Table 1. The median age of participants at the time of consent was 47 years (mean, 48; range, 20–76 y). Fifty-nine (59%) of participants were

male. The largest ethnic group was Northwest European (58%), followed by Southeast Asian (11%) and Southern and Eastern European (8%). No participants identified as Aboriginal or Torres Strait Islander. The majority of participants (67%) had completed a high school certificate, with 24% having completed a bachelor's degree. Many participants (43%) reported being currently unemployed, with 38% reporting a household income under AUD 1000 per fortnight, consistently under the minimum wage between 2015 and 2017.[27] Forty-two (44%) participants reported being a past cigarette smoker, and no participants were current smokers. Study participants identified as heterosexual (88%) with 12 (13%) participants, all women, reporting having ever engaged in receptive anal intercourse. Of the 39 women, 18% reported a history of abnormal cervical Pap smear. Seven (7%) participants reported a history of sexually transmitted infection, including 2 (2%) with a history of anogenital warts. The average time since transplantation was 1.8 years (SD, 3.17 y) with a range of 0.3 to 17.8 years.

The acceptability of an anal cancer screening test suggested by the high study consent rate (77%) is reflected in participants' reasons for agreeing. Themes related to participation are shown in Table 2, with key examples. Altruism, including for the benefit of other transplant recipients, was reported. Furthermore, the potential to improve health outcomes with the detection and prevention of cancer, and a belief in medical research to achieve this, was a central belief of many participants. Encouragement from family and clinical staff and the simplicity of study procedures were common facilitators of participation. Many participants reported an awareness of their increased risk of cancer as transplant recipients. Some reported participating due to a desire to know more about

TABLE 2.

Examples of participant provided reasons for involvement in the study

Themes	Participant responses
For the broader benefit of others	"To help the future in HPV and cell abnormalities…"
	"The project may do some good for patients in the future…"
	"Anything to help further studies involving [transplant] patients."
	"Because I think it's a project that can help with future studies and can help many people."
	"To help the doctors complete their studies on improvements to transplant patients."
	"Anything to help all future transplant patients."
Belief in/enjoyment of research	"I am happy to participate in studies to benefit the transplant patients."
	"I have participated in many studies in the past as I strongly believe in medical research:)"
	"I like to take part in this type of testing or be part of focus groups."
	"Because without these studies [and] research medicine cannot move forward."
	"I believe studies/testing to improve health and/or treatments is important."
Encouragement from others	"Asked to do so by Doctor to check cancer and to study for cancer prevention"
	"Because my partner recommended I do to have an anal screening and contribute to medical research."
Personal gain	"…it would be in my best interest to get tested for anything I can to be one step ahead of any treatment required to prolong my life and my transplant journey."
	"Because it is very important to know what is going on in your body…"
	"Sounds worthwhile. I would ultimately like to know if I have HPV 'issues', particularly as I am immuno-suppressed."
	"Interested to find out about my long term health"
	"So that I will have peace of mind."
	"I just thought it would be beneficial to know one way or the other if I am at risk or have cancer in that region."
	"Just to rule out anal cancer or the risk of it"
	"I want to know and prevent [any] affect."
No personal cost	"Hey, if it helps understand or prevent cancer. I wasn't doing anything else."
	"Why not it's just a quick test."
	"Would not hurt. Assist in research."
Knowledge of increased cancer risk	"To test myself for future problems due to being on life-long medications."
	"Due to having kidney transplant there is a risk of anal cancer."
	"I am aware that renal transplants can last a long time so reducing the risk of other diseases is important"
	"I understand I am susceptible to many cancers/illnesses as a transplant recipient…"
Personal experience	"My mother died cancer causes 6 years ago. Wife had bowel cancer died 2 years brother has melanoma brain lung liver receiving treatment"
	"Help improve knowledge and treatment of anal cancer in [transplant] patients as have had various cancers previously…"
To improve health care	"Any future procedural changes coming out of study to help patients have better quality of care is a positive."
	"To have better detection of cervical cancer and anal cancer"
	"If it helps in early detection it's got to be a good thing"
	"As a transplant recipient I recognize the significance of this study and research for medical science. I believe it can only benefit the human race and assist in prevention of severe conditions/diseases."
Fear	"Scared of cancer after transplant"

HPV, human papillomavirus.

their personal health, rule out cancer risk, and have peace of mind. One participant reported wanting to do the tests out of "fear of cancer."

Those who declined to enroll in the study reported the invasiveness of study procedures, not feeling at risk of anal cancer, and general lack of interest as reasons for not participating in the study (Table 3). A potentially modifiable barrier to participation is timing. Many gave bad timing as a reason for not participating, with reports of feeling overwhelmed by the number of concurrent tests involved in their care (Table 3). Conversely, good timing or no personal cost was reported by some enrolled recipients as a reason for their participation (Table 2).

DISCUSSION

The TAN study is a single-center, cross-sectional study that aims to establish the prevalence of anal HPV and cytological changes, as well as the acceptability of anal swab testing, in kidney transplant recipients. Recruitment for the study began in 2015 and will conclude once 100 participants have completed the study. As of February 2018, 125 kidney transplant recipients have been approached for participation and 96 (77%) have consented to participate in the study. Consented participants were recruited from a large transplantation unit in Australia and are broadly representative of the Australian kidney transplant population in terms of age and sex.[28]

The prevalence of HPV-associated anal cancers and precursors has not yet been investigated in an Australian transplant population. Based on studies conducted in Denmark, United Kingdom, and Germany, we anticipate 24% to 30% of transplant recipients will have abnormal anal cytological findings and 45% to 50% will test positive for high-risk HPV DNA.[10,15,29,30] A UK study of 108 kidney transplant recipients found that anal precancerous lesions was associated with immunosuppression, and other patient factors including smoking, receptive anal intercourse, and history of genital warts.[14] We hypothesize that our population will similarly have anal cytological changes associated with duration of immunosuppression, history of anogenital warts, and lifestyle characteristics including a history of smoking and history of receptive anal intercourse.

As in the larger Australian kidney transplant population, the majority of participants recruited to date were of Northwest European background; only 3% of participants identified as Pacific Islander and none self-identified as Aboriginal or Torres Strait Islander. This is in contrast to national data, where in 2015, 3.8% of transplanted recipients reported being of Aboriginal or Torres Strait Islander descent.[28] Cervical HPV infection and cancer is also higher in Aboriginal and Torres Strait Islander women compared to the general Australian population.[31]

TABLE 3.

Examples of reasons for not participating in study

Theme	Reason
Issue with timing	"Not today will take questionnaire and information sheet home—next visit?"
	"Too many things and not in the right mood for an extra test—maybe in future"
	"Too many events and procedures"
	"Too many things have happened"
	"Too many things happening, will be happy to consider in the future"
	"Not up to it. Panic attacks coming to hospital"
Too personal	"I do not want anything near my bum, I cannot compromise on that, good luck with your study"
	"Has been poked and prodded too much this week and does not feel like participating"
	"Too personal"
Does not feel at risk	"Does not feel risk of anal cancer applies to him and declined participation"

Strengths and Limitations of the Study

There are currently no widely accepted guidelines for anal cancer screening programs due to an incomplete understanding of the natural history of anal HPV and anal neoplastic lesions, and the unknown effectiveness of treatments. The strength of the TAN study is that it is the first cohort study to investigate HPV genotype and cytology, with sociodemographic and lifestyle characteristics in transplant recipients in Australia. The potential benefit may be identification of individuals who can then be offered enhanced monitoring. Furthermore, by understanding any correlations between HPV and cytological changes, and linking this to sociodemographic and lifestyle characteristics and clinical history, we hope to be able to more closely target screening tests to those most at risk.

The recruitment clinic sees 5 to 10 eligible patients per week. We anticipated that 650 to 1300 eligible patients would be seen over the 2-and-a-half-year period. Slow study recruitment can be attributed to the need to develop clinical expertise in the research team for study procedures. A shortage of clinical staff trained to perform the anal swab and limited number of hours available for recruitment have been identified as hindering recruitment. However, through a mixed methods approach, the TAN Study has been able to identify potentially modifiable reasons for nonparticipation, including an issue with the timing of approach, that could increase participation in future studies.

Timing was identified as a factor in their reasons for agreeing or declining to participate by participating and nonparticipating transplant recipients. Recipients were approached at the time of attending for a protocol posttransplant biopsy, an outpatient procedure after which they are required to lay prone for up to 4 hours. These are scheduled at 3 and 12 months and then annually posttransplant and are reflected in the distribution of time since transplant in recruited participants. However, time since transplant did not seem to affect consent rates, as participants were fairly equally distributed between recent (<3 mo) and distant (>1 y) transplants. It is possible that the timing in relation to their outpatient procedures that was more relevant. Whether recipients were approached before or after biopsy was not recorded in this study and could inform recruitment strategies for future studies. It is important to note that data saturation was not reached in the limited information sought from study participants. Further analysis of data from focus groups and interviews in this population would better describe barriers and facilitators to an anal cancer screening program.

As a cross-sectional study, the TAN study will provide insight into the epidemiology of anal HPV infection and neoplasia in transplant recipients, and the acceptability of a potential anal screening procedure. Longitudinal studies to determine the natural history of anal HPV infection and cancer are currently underway in other immune compromised groups[13]; however, additional follow-up studies should be conducted in kidney transplant recipients to determine if time to cancer is similar. Additionally, determining whether anal screening programs would be beneficial immunocompromised groups will need to await the results of randomized controlled trials currently underway.[21]

The TAN Study brings together a multidisciplinary team that includes epidemiologists, immunologists, vaccinologists, virologists, cytopathologists, and experts in sexual health and nephrology. The study results will contribute to the understanding of anal HPV and HSIL prevalence in kidney transplant recipients and could inform the development of a potential targeted screening program in this population.

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