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ORIGINAL ARTICLE

# Non-16/non-18 high-risk human papillomavirus types predominate in anal cytology categories of negative for intraepithelial lesion and atypical cells of undetermined significance

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**KEYWORDS**
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**Introduction** Screening for anal carcinoma continues to grow despite controversy regarding its efficacy. High-risk human papillomavirus (HR-HPV) has been adopted as a cotest with anal Papanicolaou tests. We sought to identify the prevalence of HR-HPV types in the most common anal cytology specimens: negative for intraepithelial lesion or malignancy (NILM) and atypical squamous cell of undetermined significance (ASC-US).

**Materials and methods** Anal cytology specimens were identified and tested for HR-HPV using Roche cobas 4800 HR-HPV analysis (Roche Molecular Systems, Inc., Indianapolis, Ind) and, if positive, typed further for: HPV-16, -18, and/or non-16/non-18 "other" HR-HPV type.

**Results** There were 642 specimens from 538 patients. The most common interpretation was NILM (48.6%) and ASC-US (25.7%). Of NILM cases, 47% were HR-HPV+ (53% in men, 33% in women, $P = 0.03$, $\chi^2$). In ASC-US cases, 73% were HR-HPV+ (74% in male patients, 70% in female patients). The most common HPV subtype was non-16/non-18 HR-HPV "other" types in 89% of cases. HPV-16 and HPV-18 were positive in 35% and 18% of cases, respectively. In patients with non-16/non-18 HR-HPV+ anal cytology, 16 of 79 had biopsies histologically diagnosed as at least high-grade squamous intraepithelial lesion (HSIL+). However, the relative risk of having HSIL+ was 2.3-times higher for anal cytology positive for HPV-16, -18, with/without coinfection with non-16/non-18 HR-HPV than those positive for non-16/non-18 "other" HR-HPV types alone.

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**Conclusions** Non-16/non-18 "other" HR-HPV types are most prevalent in anal cytology interpretations of NILM and ASC-US. Patients with HR-HPV+ NILM or ASC-US, negative for HPV-16/-18, are at lower relative risk of having subsequent histologic HSIL+, but a percentage of these patients still harbor HSIL+ on biopsy.


## Introduction

Anal carcinoma, specifically squamous cell carcinoma (SqCC) is rare with an incidence of approximately 1.7 per 100,000 in the United States.[1] Although there is some controversy over the efficacy of anal carcinoma screening,[2] anal Papanicolaou (Pap) tests with high-risk human papillomavirus (HR-HPV) testing continue to increase, especially in patients at high risk of developing anal carcinoma.[3] Screening specifically targets identification of precursor lesions, such as anal squamous intraepithelial lesion (SIL) prior to development of invasive disease. Currently, however, there are no national guidelines for screening of anal neoplasia[4]; how, or if, patients are screened is determined regionally.

Risk factors for developing anal SqCC and SIL include history of anogenital HPV infection, receptive anal intercourse, multiple sexual partners, smoking, and chronic inflammatory conditions (anal fissures, fistula, and hemorrhoids) in both men and women.[5-7] Human immunodeficiency virus (HIV)-positive men who have sex with men (MSM) are commonly infected with multiple HPV types, which increases the risk of finding SIL on cytology.[8] Conditions associated with immunosuppression, such as transplantation and HIV-associated adult immunodeficiency syndrome (AIDS), also increase the risk for developing anal carcinoma.[9]

In anal SqCC, HPV-16 is the most common subtype identified, present in 60% of all tumors in male patients and 73.7% of tumors in female patients.[10] Depending on the study, HPV-18[10] is second most common type at 7.8% and 7.3% in male and female patients, respectively; however, more recent data suggest that HPV-33 may be more common.[11] With an increase in anal cytology specimens, there have been a growing number of requests for HR-HPV results. In this study we sought to determine the distribution of HPV subtypes in anal cytology, especially in the most commonly interpretive categories: atypical squamous cell of undetermined significance (ASC-US) and negative for intraepithelial lesion or malignancy (NILM).

## Materials and methods

After receiving institutional review board approval (no. 2013P002696/MGH), the Massachusetts General Hospital pathology laboratory information system was searched for all cases of anal cytology performed from May 1, 2012 through October 31, 2013. All anal cytology specimens were collected and prepared using liquid-based cytology (BD SurePath, Franklin Lakes, NJ). Patient demographics, cytologic interpretation, and, if performed, HR-HPV results were recorded. During the study period, 13 pathologists interpreted anal cytology samples (11 with cytology subspecialty certification and 2 with subspecialty interest in cytology). The Bethesda System for Reporting Cervical Cytology[12] was used for the interpretation category and included the following: negative for intraepithelial lesion or malignancy (NILM), low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), squamous intraepithelial lesion—unqualified (SIL-U), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot rule out HSIL (ASC-H), and unsatisfactory (UNSAT). Rarely, the category of low-grade squamous intraepithelial lesion with rare cells cannot exclude HSIL (LSIL-H) was used outside of the current Bethesda System interpretive categories.

For cases of ASC-US, the HR-HPV result was reflex ordered. For cases of NILM and less commonly other interpretations, HR-HPV was ordered at the request of clinicians. HR-HPV was performed after interpretation and sign-out of the anal cytology. HR-HPV was assessed using Roche cobas 4800 HR-HPV analysis (Roche Molecular Systems, Inc., Indianapolis, Ind), an automated in vitro real-time polymerase chain reaction with nucleic acid hybridization—based test for 14 HR-HPV types and for the human β-globin gene, performed in-house by cytotechnologists. Results were reported as HR-HPV-negative, -indeterminate, or -positive, with positive cases further characterized by HPV subtype: 16, 18, and/or non-16/non-18 "other" HR-HPV type (pooled HR-HPV types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68).

The laboratory information system was then searched for anal biopsies in patients with anal cytology who were biopsied between 90 days (3 months) prior to the anal Pap test to 185 days (6 months) following. All anal biopsies were interpreted by pathologists with subspecialty interest in gastrointestinal pathology; the cytology results were available at the time of reporting. Results were categorized as per the LAST (Lower Anogenital Squamous Terminology) Standardization Project: negative for intraepithelial lesion or malignancy (NILM), low-grade squamous intraepithelial lesion (LSIL, anal intraepithelial lesion [AIN]-1), high-grade squamous intraepithelial lesion (HSIL, AIN-2, AIN-3, squamous cell carcinoma in situ [SqCCIS]), invasive squamous cell carcinoma.[13]

Statistical analysis was performed using Microsoft Office Excel (Microsoft Co, Redmond Wash).