ABSTRACT

Title of Dissertation:	APPLIED EPIDEMIOLOGICAL STUDIES OF HIGH RISK HUMAN PAPILLOMAVIRUS INFECTIONS AND RISK OF CERVICAL PRECANCER
	Maria Teresa Demarco, Doctor of Philosophy in Epidemiology, 2017
Dissertation directed by:	Professor Olivia Carter-Pokras, Department of Epidemiology and Biostatistics

Persistent infection with human papillomavirus (HPV) is a leading cause of cancer among women worldwide. Health care providers face a growing number of competing cervical cancer screening approaches and tests. HPV testing is very sensitive but a secondary test is needed to identify infections with sufficient risk of cervical precancer/cancer.

This dissertation aims to address three questions in the management of HPV infections: (1) to compare the first HPV screening test seeking FDA approval that identifies many individual HPV types (BD Onclarity™) to two FDA approved assays (Roche cobas™ and Qiagen HC2™); (2) to clarify how HPV type influences cumulative risk of clearance, progression or persistence of HPV infections; and (3) to assess whether established etiologic co-factors for cervical precancer, given HPV infection, represent

clinically useful, actionable factors that clinicians “need to know” when considering how to manage the HPV infected woman.

All manuscripts in this dissertation used data from the HPV Persistence and Progression Cohort, conducted by Kaiser Permanente Northern California and the National Cancer Institute. The study population is a group of 33,295 women, ages 30 or older, who are HPV positive at baseline and have results for cytology. Contingency table methods, Kappa statistics and McNemar’s test were used to assess agreement between HPV DNA tests (manuscript 1). Competing risk proportional hazards models were used to estimate eight-year cumulative risks of HPV clearance, progression to precancer, or persistence (manuscript 2). Absolute risks from Logistic-Cox models were used to study whether co-factors acted as clinically relevant risk stratifiers (manuscript 3).

Results from this dissertation suggest that: (1) Onclarity agreement was good to excellent compared with cobas and HC2, and clinical accuracy was high for detection of precancer; (2) cumulative risk of clearance varied little by HPV type, cumulative risk of progression was substantially higher for HPV16, and long-term persistence was uncommon; and (3) the most important predictors of progression from HPV infection to precancer were HPV type and cytologic result.

By clarifying these aspects of methods, and management of HPV-positive women, it is hoped that this dissertation will contribute to the improvement of cervical cancer screening incorporating HPV testing.

APPLIED EPIDEMIOLOGICAL STUDIES OF HIGH RISK HUMAN PAPILLOMAVIRUS INFECTIONS AND RISK OF CERVICAL PRE-CANCER

by

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Dedication


O R D N A O T N A N A I R D N A Y D N A
O C R A M E D S O L R A C N A U J T T L
J I T N A M A T T E T A D L I H N J B P
S O T A B O T R N Y J R I L G Q T L W V
U M S J T W C J G O L L X U Q Y Y M R D
S W B E N S B R S N L T S M D K J M L Y
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T O J B V B R Z N N Y J N D O L B J B M

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List of Abbreviations

ACS - American Cancer Society
ASCCP - American Society for Colposcopy and Cervical Pathology
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL
ASC-US - Atypical squamous cells of undetermined significance
BD - Becton Dickinson
BMI – Body mass index
CIN - Cervical intraepithelial neoplasia
DMPA - Depot medroxyprogesterone acetate
DNA - Deoxyribonucleic acid
FDA - Food and Drug Administration
HC2 - Hybrid Capture 2
HIV - Human immunodeficiency virus
HPV - Human papillomavirus
HR – High-risk
HSIL - High-grade squamous intraepithelial lesion
IARC – International Agency for Research on Cancer
ICO – Institut català d'oncologia
IRB - Institutional Review Board
KPNC - Kaiser Permanente North California
LA –Linear array
LSISL - Low-grade squamous intraepithelial lesion
MRS - Mean risk stratification
NCI - U.S. National Cancer Institute
NH - Non-Hispanic
OCP – Oral contraceptive pills
PaP Cohort - HPV Persistence and Progression Cohort
RNA - Ribonucleic acid
SES - Socioeconomic status
UMD - University of Maryland
USPTF - U.S. Preventive Services Task Force

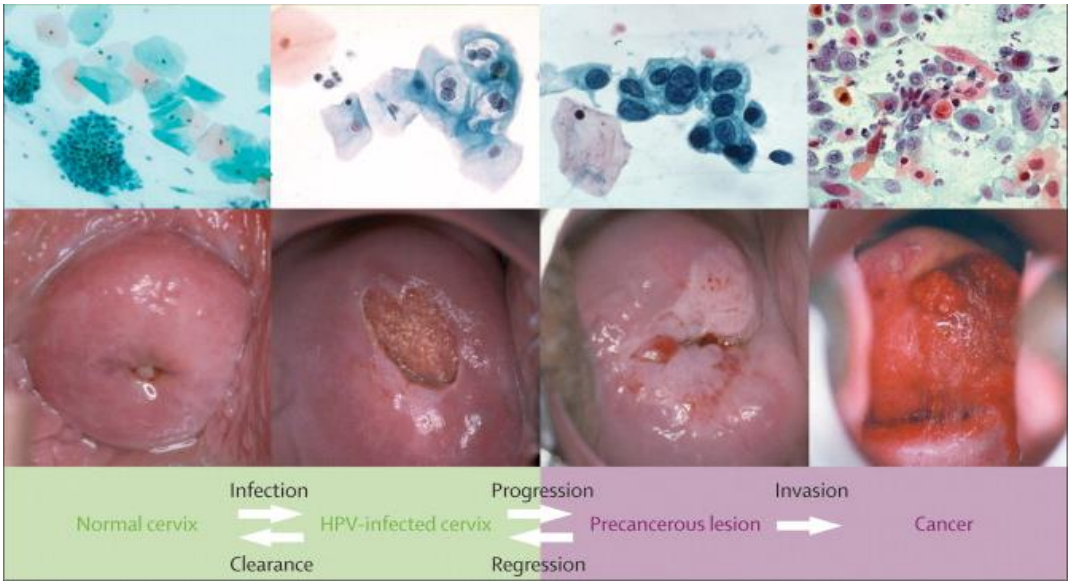
Chapter 1: Introduction

Background

Infection with human papillomavirus (HPV) is a leading cause of cancer among women worldwide with approximately 500,000 new cervical cancer cases and 250,000 deaths each year [1]. HPV is the most common sexually transmitted infection in the United States [2], affecting 6.2 million persons 15 to 44 years of age annually [1]. HPV types 6 and 11 account for 90% of genital warts, and types 16 and 18 are responsible for 70% of cervical cancer in women [2]. HPV is associated with cervical cancer, oral cancer, and less common anogenital cancers such as cancer of the vulva, vagina, penis, and anus [3]. In addition to causing cancer, HPV infections can cause genital warts, common warts, plantar warts, and flat warts [4]. The importance of cervical cancer is accentuated by the relatively young average age at incidence and death.

Cervical cancer is caused by persistent infection with a group of carcinogenic genotypes of HPV (HPV16, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and probably HPV68). This cancer arises via a series of four necessary natural history steps that can be reproducibly distinguished (**Figure 1**) and which provide a rational starting point to discuss optimal cervical cancer prevention efforts [5]. These include HPV infection, viral persistence, progression to precancer, and invasion. “Backward” steps can also occur, such as clearance of HPV infection (e.g., immune control), and regression of an apparent precancer to normalcy.

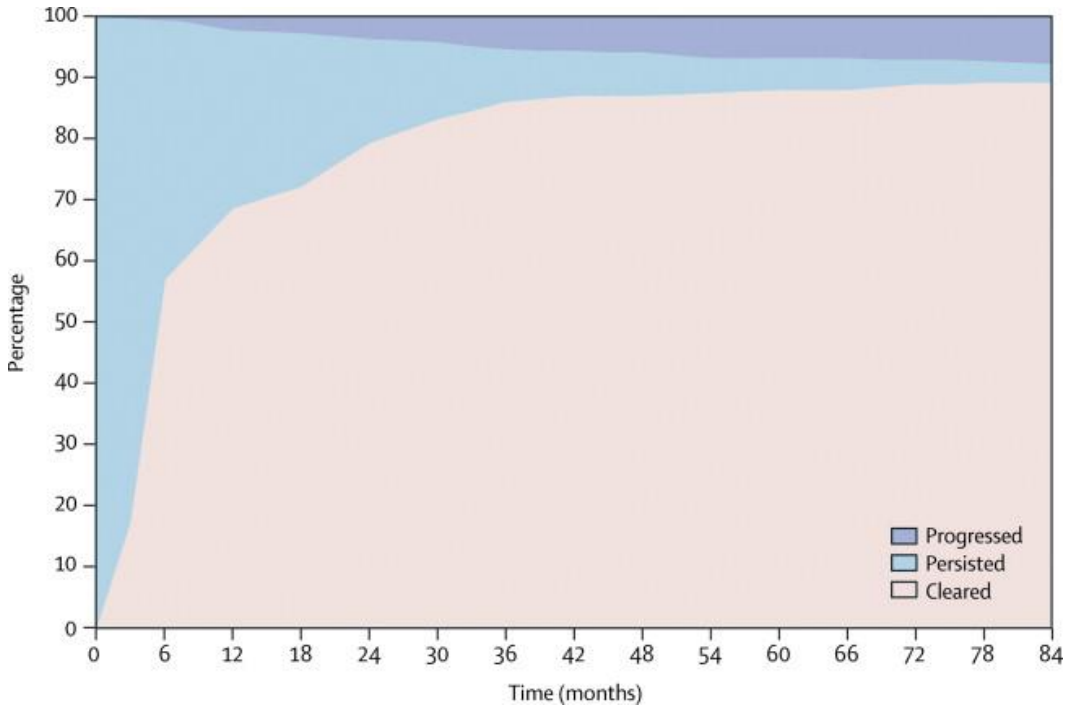
Figure 1. Major steps in the development of cervical cancer



Source: Schiffman, M., et al., Human papillomavirus and cervical cancer. Lancet, 2007. 370(9590): p. 890-907.

The natural history of cervical cancer starts with a sexually transmitted infection with a carcinogenic or high risk (HR) HPV genotype (**Figure 2**) [6, 7]. It is estimated that more than 50% of HR HPV infections "clear" instead within a year, and 90% of infections clear within 2 years [7]. Clearance generally means lifelong immunologic control rather than total elimination of infection.

Figure 2. Average clearance, persistence, and progression (to CIN2+) of carcinogenic HPV infections



Source: Schiffman, M., et al., Human papillomavirus and cervical cancer. Lancet, 2007. 370(9590): p. 890-907.

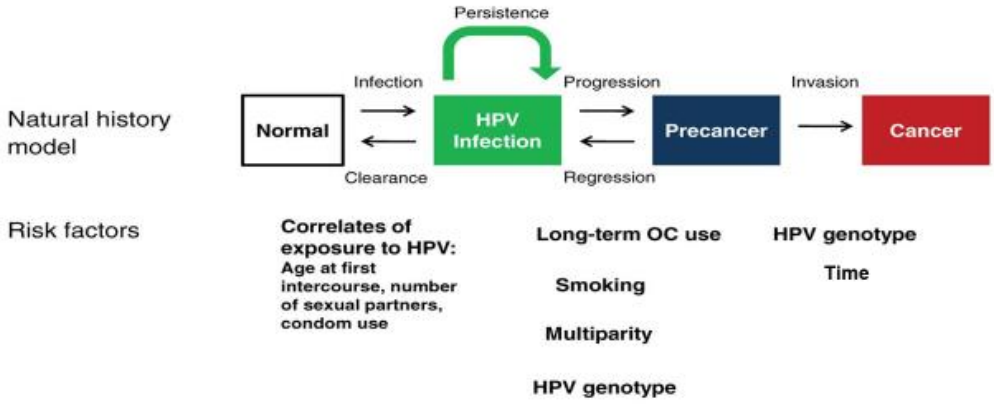
The longer that an HR HPV infection persists, the lower the probability of subsequent clearance and the higher risk of precancer diagnosis [8-12]. Prevalent infections detected in cross-sectional screening persist longer in older women than in younger women, probably because they are more likely to represent older infections [13]. The median time to clearance of HPV infections detected in screening studies is 6–18 months [8, 10-14]. There is no known definition of clinically important persistence, but follow-up strategies targeting abnormalities lasting more than 1 year (and especially 2 years) seem to distinguish infections and associated lesions with greater risk of progression [12, 15]. A small proportion (<10%) of carcinogenic infections persists for more than a few years and this small subset is strongly linked to a high subsequent absolute risk of precancer [12].

There are biomarkers that reflect or predict each stage in the natural history of cervical cancer. For example, biomarkers for HPV infection include direct detection of HPV by viral nucleic acids but also characteristic cytopathology (called LSIL).

HPV type is an example of a biomarker that predicts risk, specifically for progression to precancer [16]. Of the HR types, the most important is HPV16, responsible for only 20% of infections but causing 50% of cervical cancer. HPV18 is the second most important, and is underrepresented in precancers given its importance in cervical cancer [17]. Viral genomic variation is important for etiology and even subtle variations within viral types influence risk of progression and invasion, with relative risks stronger than for behavioral and genetic factors [18].

Behavioral factors that approximately double the risk of progression to precancer among HPV-infected women include long-term hormonal contraceptive use and smoking [19, 20] (**Figure 3**). Some studies detected an increased risk of HPV progression to cervical precancer among users of hormonal contraceptives for more than 5 or 10 years [20-22]. More information is needed about the newer hormonal contraceptive agents with different hormonal formulations. Previous studies of tobacco smoking found a 2-fold increase in risk of cervical cancer or precancer, some with a dose response for the number of cigarettes smoked and duration of smoking [19, 23-25]. It is still unclear if the effect of tobacco is mediated by genotoxicity or immunomodulation [19, 23-25].

Figure 3. Natural history and co-factors for cervical cancer.



Source: Schiffman M, Wentzensen N. Human papillomavirus (HPV) infection and the multi-stage carcinogenesis of cervical cancer. *Cancer epidemiology, biomarkers & prevention.* 2013;22(4):553-560. doi: 10.1158/1055-9965.EPI-12-1406.

Most studies on parity show increases in risk associated with increasing number of pregnancies. The possible role of a hormonal, nutritional or immune status change during pregnancy or potential cervical trauma during delivery as a cofactor of HPV remains to be determined. While nutrients can act as preventive agents in some epithelial cancers, the role of nutrients as cofactors of HPV in cervical cancer has scattered support, with some studies showing protective effects and others no association.

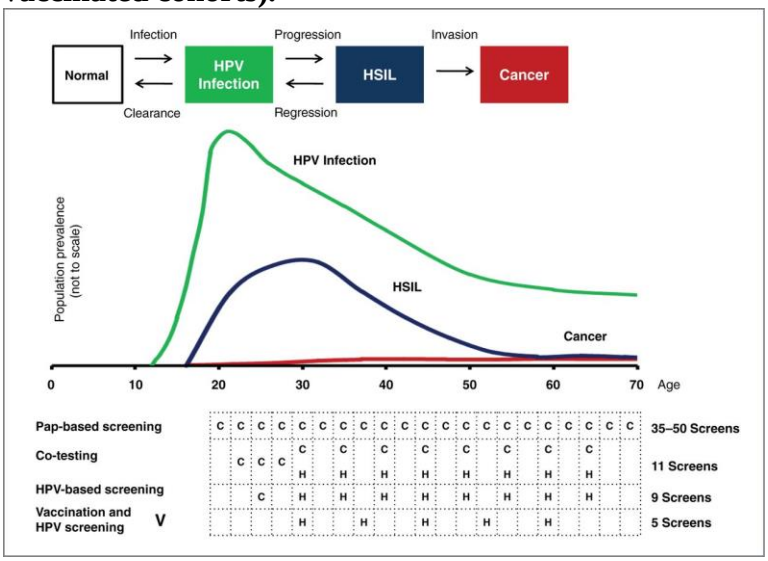
Host genetic factors influencing control of infection are poorly understood. There is some evidence of familial aggregation in cervical cancer, suggesting genetics as a cofactor of HPV, but the association is not confirmed, mechanisms have not been established, and we need to rule our confounding by shared environmental exposures.

Less well-established co-factors for progression to precancer given HPV infection include chronic cervical inflammation and immunosuppression (e.g., HIV and chlamydia). The only factor consistently associated with control of HPV infection is human leukocyte antigen,

supporting the importance of T-cell responses in control of HPV infections and cervical precancers [26].

Primary prevention of cervical cancer can be achieved by prophylactic vaccination (**Figure 4**), eliminating the infections that lead to precancer and then to cancer. However, vaccine programs will take many decades to succeed in reduction of morbidity and mortality from invasive cancer; since many countries have not yet initiated a formal vaccination effort and the typical strategy is to vaccinate young adolescent girls (and sometimes boys) [27]. In the meantime, secondary prevention using screening will continue to be the mainstay of cervical cancer prevention.

Figure 4. Prevalence of HPV infections, precancer, and cancer by age. Cervical cancer prevention strategies based on cytology, co-testing, and HPV testing (for unvaccinated and vaccinated cohorts).



Source: Schiffman M, Wentzensen N. Human papillomavirus (HPV) infection and the multi-stage carcinogenesis of cervical cancer. *Cancer epidemiology, biomarkers & prevention*. 2013;22(4):553-560. doi: 10.1158/1055-9965.EPI-12-1406.

Figure 4 (bottom table) shows the evolution of screening strategies from cytology-based screening to co-testing, HPV-based screening without vaccination, and HPV-based screening

with vaccination [7]. In the late 20th century in the US, women were instructed to participate in annual cytology screening (C). Despite the low sensitivity of cytology, repetition (sometimes through 35-50 lifetime screens [28]) produced effective secondary prevention of cervical cancer over time [7]. More focused screening is now possible, given the identification of HPV as the cause of cervical cancer, permitting the extension of screening intervals and reducing the number of lifetime screens [7].

It takes many years for an HPV infection to cause cervical cancer [5]. The typical time course of the natural history produces characteristic age-specific curves for each stage (**Figure 4**, middle graph) with the peak of HPV acquisition in adolescence and early adulthood, the peak of precancer around 25–30 years of age, and the peak of cancer from 45–60 years of age [29]. Thus, it is not optimal to screen adolescent women, when HPV infection is extremely common but the risk of cervical cancer is very low. Accordingly, the recommended age of initiation of cytologic screening has been raised to 21. Beginning at age 30, past the peak of acute HPV infection, co-testing with HPV assays (H in **Figure 4**) and cytology (C in **Figure 4**) is generally preferred in the United States (US) over cytology alone [30]. The recommended interval between screens has been increased from yearly (cytology based screening) to every 5 years (co-testing), although there is resistance to interval extension of this length. Cervical cancer screening (either through cytology or co-testing) currently stops at age 65 for women with normal screening histories [30]. Outside of the US, primary HPV testing is more popular than cotesting, although concern over rare missed cancers is motivating consideration of cotesting in some European countries.

A cervical screening program stratifies risk of cancer (using the surrogate endpoint of precancer) [31] to identify and define women needing treatment. Screening of the general population and triage of women testing positive are tightly linked, and are the focus of this

dissertation. The third part of a screening program is treatment; treatment options will be described to demonstrate understanding, but were not researched for this dissertation.

The risks of cervical precancer determined during screening and triage determine three broad and risk-related management actions. First, if elevated risk is predicted by screening and triage tests, this mandates referral for colposcopic biopsy. Second, moderate risk dictates return for retesting within a short timeframe (~1 year) to rule out progression of HPV infection to precancer. Third, low risk permits return at the next routine screening (~3 or more years).

Analyzing the initial screening step a bit more closely, cervical cancer screening (testing the general population to define the minority at elevated risk) can be done in three ways according to current US guidelines: HPV testing as primary screening; Papanicolaou test or "Pap"-based cytology as primary screening with HPV testing as triage for abnormal cytology; or simultaneous co-testing of all screened women for HPV and cytological abnormalities [7]. For many decades, the main screening method was based on primary cytology screening. Given the deficiencies of cytology (low sensitivity and subjective nature of test due to inter-observer variability), HPV testing was developed, initially as an adjunct to traditional cytology-based screening, to clarify equivocal results. Thus, in 1999, HPV testing was approved to triage patients with atypical squamous cells of undetermined significance (ASC-US, meaning equivocal or borderline) cytology. HPV and cytology cotesting was recommended for general screening a few years later, in 2002. In 2004, the National Institute of Health National Cancer Institute, the American Society for Colposcopy and Cervical Pathology (ASCCP), the American Cancer Society (ACS), and other groups, cooperatively developed interim guidelines to expand the use of HPV to co-test women age ≥30 years [32-34]. In subsequent rounds of guidelines, there has been a growing consensus that cytology will be supplanted as the primary screening

test because of the suboptimal sensitivity for subsequent precancer of a single cytology screen, which necessitates frequent repetition. HPV testing likely will be the primary screening test, and cytology in some form will continue in the medium term to be used, to triage HPV-positive women [35].

In the context of the changing guidelines from cytology to HPV-based cervical cancer screening, this dissertation addresses three current questions in screening and triage:

(1) How do the three most prominent HPV DNA screening assays compare? The analysis covers the DNA tests most likely to compete in the US market;

(2) What is the cumulative risk of clearance vs progression vs persistence of HPV infections for the different genotypes of HPV? This analysis will guide follow-up of infected patients, if HPV genotyping is used instead of just a pooled probe yielding yes/no results for the group of high-risk types; and

(3) Are established etiologic co-factors for cervical precancer clinically useful, actionable factors that clinicians "need to know" when considering how to manage the HPV-infected woman.

Chapter 2: Methods

Data sources

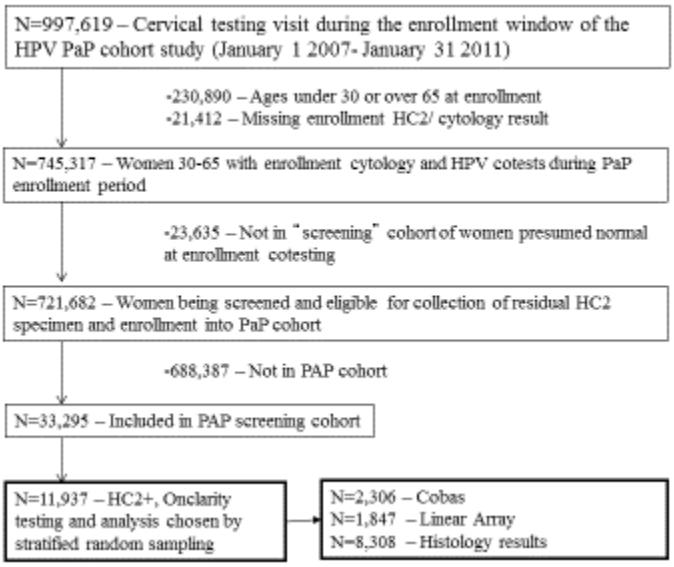
All manuscripts in this dissertation used data from the HPV Persistence and Progression Cohort conducted by Kaiser Permanente Northern California (KPNC) and the National Cancer Institute. At KPNC, women are tested by Hybrid Capture 2 (HC2) for the group of carcinogenic genotypes of HPV (as a pool without genotyping) to triage the equivocal cytologic result of atypical squamous cells of undetermined significance (ASC-US) (since 2001) and as a co-test with cytology in women ages 30 and older (since 2003) [35, 36]. The HPV Persistence and Progression Cohort (The PaP Cohort) was created by banking residual, waste cervical specimens, collected into specimen transport medium (STM; Qiagen), from women tested by HC2 and cytology.

KPNC membership is demographically similar to the US Census–enumerated population in the Bay Area Metropolitan Statistical Area, with racial/ethnic minority groups being well represented. Of the 48.8% of women who self-reported their race/ethnicity in KPNC, 62.1% were Caucasian, 12.4% were Asian/Pacific Islander, 12.2% were Hispanic, and 8.4% were African-American. In the San Francisco/ Bay area, the ethnic makeup of the population is: 48% White, 33% Asian, 6% African American, 15% Hispanics, and 0.5% Native American [33]. The main differences between the KNPC study population and the general population are: 1) lack of representation of extremes in income (particularly lower income groups), 2) since all people have health insurance coverage it is considered a well-screened population where the risk of cervical cancer has historically been lower than the national average. Previous studies that compared the KNPC population with other screening data point out that while the baseline screening results are different, the patterns of progression given HPV and cytology status do not differ [34].

Study population

The study population is drawn from a group of 997,619 women who were routinely co-tested with HC2 and cytology at KPNC during the initial collection period of PaP study. After cervical specimens were tested for HC2, the residual cervical specimens were neutralized and frozen [35, 36]. Figure 2 outlines details regarding the population in the KPNC's PaP study. Baseline data were collected between Jan 1, 2007 and Jan 1, 2011, and follow-up continued until December 31, 2015. While in the general population, the general cervical cancer screening rate is 80%, all women in the study population had at least two visits, varying in frequency based on screening recommendations for their age and health status, following internal Kaiser guidelines concordant with national standards at the time. The 688,387 women "not in PaP cohort" were HPV negative women that, based on the purpose of the study to follow-up persistent and progressing HPV infections, were not of interest to follow-up [33].

Figure 2. Consort diagram of KPNC's PaP cohort population



Participants and selection criteria

Women were excluded if they were under 30, over 65, were missing HC2 or cytology results at baseline, or were not part of the screening population. Given the extremely high agreement between validated tests on HPV negative women, and the fact that cancer or severe precancer are very rarely diagnosed in HPV negative subjects, we excluded participants with negative HPV test results from most of this study. Exclusion of HC2 negative women is based on the difficulties reweighting the very few HC2 negative participants leading to anomalies in the weighted results. The study population for this dissertation are the 11,937 women with positive screening HC2 results who were chosen by stratified random sampling (with strata retrospectively based on precancer outcome) for masked HPV typing (to distinguish at least partly the individual carcinogenic HPV type(s) present) at BD Diagnostics (Sparks MD) by the Onclarity assay or at Roche Molecular Systems (Pleasanton CA) by cobas/Linear Array. Within our study population, 2,322 had additional results for all three important US HPV DNA tests (HC2, cobas (Roche), and Onclarity (BD)). Women with additional results for these three tests had all their samples throughout follow-up tested with these three assays.

HPV infection related variables

In manuscript 1, grouped results were hierarchical, based on type channel-associated risk of CIN3+, as follows: positive for HPV 16, else positive for HPV 18, else positive for other high risk HPV types, else negative. In manuscript 2, the main exposure was non-hierarchical infection with independent HPV types (HPV16, HPV18, HPV45, HPV31, HPV51, HPV52, based on Roche's Linear Array, Onclarity, or cobas). Cobas channels tried to separate particularly risky types such as HPV16 and HPV18. Onclarity channels further separated HPV

types based on biological similarities (sometimes unrelated to risk of progression). A given woman could contribute more than one infection. No attempt was made to adjust for theoretically possible auto-correlation, because infections with multiple HPV types are established to act independently of each other, with minimal interaction at the viral-viral level regarding persistence/clearance/progression [7]. Infection with a specific HPV genotype was defined hierarchically (16, else 18/45, else 31/33/52/58, else 35/39/51/56/59/66/68w) based on previous established HPV risk groups.

Analysis of potential confounders and effect modifiers

For manuscript 3, selection of potential co-factors (age, smoking status, hormonal contraceptive use, Body mass index (BMI), socio-economic status (SES), and race/ethnicity) was based on previous literature [16, 17, 21, 22]. Assessment of whether co-factors act as confounding factors in this dataset was based on comparison of unadjusted and adjusted risk estimates. Potential confounders that produced a change in the estimate greater than 10% were considered possibly important confounding factors in this study and were included in the final multivariable model. Potential effect modifiers were assessed through interaction terms and, if significant, stratification [22]. Co-factors were dichotomized (table below) for inclusion in the final, multivariable model. Original KPNC variables are detailed in Appendix 1. All p-values are considered significant at ≤ 0.05 and 95% confidence intervals are reported when appropriate. For this manuscript, KPNC variables were redefined as follows:

Variables	Categories
Progression of HPV infection to precancer	1: histology results of CIN3+ at follow-up 0: histology results of CIN1 or less at follow-up
HPV genotype	16, else 18, else individual type/channel for all other high risk types
Co-factors	Smoking status (2: current, 1: former, 0: never) Hormonal contraceptive use (1: ever, 0: never) BMI (1: obese class III (BMI>40), 0: not obese class III (BMI<40)) SES (1: below federal poverty level, 0: at or above federal poverty level) Race/ethnicity (1: AA or 0: all other races)
Age	30-44, 45-60

Statistical analyses

Logistic-Cox models were used to estimate the hazard ratios of cumulative risk for HPV infection clearance and progression. Logistic-Cox are semi-parametric prevalence-incidence models that estimate cumulative risk while including covariate effects. Semi-parametric models have both parametric (use parameters to specify an assumed distribution) and non-parametric (no assumptions) components [22]. Logistic-Cox modeling was developed to use electronic health record data for screen-detected disease. Logistic-Cox models address three limitations of standard methods for survival data analysis, such as Kaplan-Meier, by: 1) separating risk of prevalent disease at baseline, 2) accounting for diagnosed and undiagnosed prevalent disease, and 3) estimating absolute risk form two-phase stratified samples nested within a cohort [23].

Human subjects/ ethical considerations

We obtained prior University of Maryland Institutional Review Board (IRB) approval for secondary data analysis. The analyses were based on existing data at KPNC/NCI, which were obtained in a systematic manner. This dissertation is designed to develop or contribute to generalizable knowledge. The study does not involve additional intervention or interaction with human subjects, or access to identifiable private information. In fact, the data used do not contain identifiable private information.

Chapter 3: Manuscript 1 – Comparison of an HPV DNA cervical screening test providing substantial HPV typing to two established assays

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Keywords: HPV DNA tests, screening, Onclarity, HC2, Cobas, Linear Array

Abstract

Given the ongoing shift from cytology to HPV-based cervical cancer screening, it is important to compare the ability of major HPV assays to detect high risk HPV to find precancers. This analysis compared Onclarity™ (Becton Dickinson), seeking Food and Drug Administration (FDA) approval, to two approved tests, Hybrid Capture 2, HC2™ (Qiagen) and cobas™ (Roche). We also compared type-specific results from the research assay Linear Array™ with Onclarity.

We tested cervical samples by Onclarity using a stratified random sample (n=10,090) of discarded clinical specimens from women tested by HC2 in routine screening at Kaiser Permanente Northern California (KPNC), as part of the NCI-KPNC Progression and Persistence study. HPV results were linked to clinical data from electronic health records. A subset of specimens was also tested by cobas and LA (n= 1,965).

We compared: 1) HPV positivity of Onclarity and HC2; 2) HPV type-group agreement between Onclarity and cobas (comparing HPV16, HPV18, and 12 other high-risk types); 3) HPV typing agreement between the nine Onclarity typing channels and Linear Array typing; and 4) clinical accuracy of Onclarity compared to histology.

Onclarity and HC2 showed good agreement on HPV positivity. Onclarity and cobas had excellent agreement on partial typing of HPV infections (kappa: 0.81). Identification of HPV genotypes by Onclarity channels showed strong agreement with LA type-specific results (kappas ranging from 0.80 for HPV39/68/35 to 0.97 for HPV16). Onclarity showed high sensitivity for CIN2+ and CIN3+. We concluded that Onclarity yields typing results and clinical performance similar to already-approved HPV DNA tests.

Introduction

Cytology (Pap testing) has been the mainstay of cervical cancer screening but, increasingly, testing for high-risk HPV types has become a major part of screening programs [7]. A HPV test is more sensitive than cytology for detecting prevalent cervical precancer/cancer, with a decided advantage in predicting subsequent risk over the following decade [37-42]. The two kinds of screening tests can be used simultaneously for maximum sensitivity, but a growing body of evidence indicates that adding cervical cytology to HPV testing offers only a very small benefit compared with HPV testing alone [43].

Given their increasingly prominent role, it is important to know how the major HPV tests compare in performance. Four HPV DNA detection methods (HC2™, cobas™, Cervista™ HPV16/18, Cervista™ HPV HR) and one HPV RNA assay (Aptima™) are already approved by the FDA. A fifth HPV DNA assay, BD Onclarity™, is likely to enter the US market soon. [44]. While all of the approved tests detect the infection with any of the twelve HPV types judged to have proven carcinogenic potential [45], some also provide partial genotyping for the most important carcinogenic types (HPV16 and HPV18), possibly allowing finer stratification of the risk of cervical precancer/cancer [42, 46].

If partial typing is judged important, it is predicted that three major HPV DNA tests (HC2™, cobas™, and Onclarity™) will be used in the US. This paper will focus on these 3 and will not assess Cervista™ (Hologic™) and Aptima™. The Hybrid Capture 2 (HC2™) HPV DNA test (HC2™, Qiagen, Germantown, MD) was the first FDA-approved test and is still commonly used [47-50] to detect the high-risk HPV types, but only as a pool with no partial typing. It tends to exhibit some cross-reactivity and detects genetically related HPV types as well [51].

The Roche cobas 4800 HPV test (Roche Molecular Systems, Pleasanton CA) [52, 53] provides HPV16 and HPV18 genotyping. It combines 12 other high risk HPVs (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66[1], and 68) as a pooled result [54].

BD is seeking FDA approval for the Onclarity™ HPV assay. Onclarity™ individually identifies six genotypes (16, 18, 31, 45, 51, and 52) and the remaining eight HPV genotypes are reported in three channels (33/58, 56/59/66, and 35/39/68) grouped based on biological similarities of the HPV types. The recently-completed enrollment phase of the BD Onclarity™ FDA registration trial, and published performance data from research studies, suggest that Onclarity™ could become a useful HPV test and expand its Market from Europe to the US in the years ahead [42]. To our knowledge, there are no other HPV tests under consideration by the FDA at this time.

The aim of this analysis is to compare these three HPV DNA screening assays (HC2, cobas™, and Onclarity™), focusing on the newcomer (Onclarity™) with the least published support. Specific analyses address: 1) High risk HPV detection for Onclarity™ versus HC2, 2) HPV type-group agreement between Onclarity™ and cobas (how the assays compare to each other in classification of HPV16, else HPV18, else 12 other high-risk types); 3) Onclarity™ typing accuracy (how Onclarity™ compares to Linear Array, which is a commonly used research HPV genotyping assay); and 4) the clinical accuracy of Onclarity™ compared with histopathologic reference standards of CIN2 and CIN3+).

[1] The inclusion of HPV66 in cervical screening tests is based on a now-corrected IARC evaluation. But HPV66 was incorporated into HPV testing and, given the difficulty in changing test formats, it remains.

Materials and methods

Study design and population

This is a cross-sectional analysis using data from HPV testing of a large convenience subset of specimens from the HPV Persistence and Progression Cohort conducted by Kaiser Permanente Northern California (KPNC) and the National Cancer Institute (Figure 1). At KPNC, women are tested by Hybrid Capture 2 (HC2) for the group of high-risk genotypes of HPV (as a pool without genotyping) to triage the equivocal cytologic result of atypical squamous cells of undetermined significance (ASC-US) (since 2001) and as a cotest with cytology in women ages 30 and older (since 2003) [35, 36]. The HPV Persistence and Progression Cohort (The PaP Cohort) was created by banking residual, discarded cervical specimens, collected into specimen transport medium (STM; Qiagen), from women tested by HC2. The emphasis was on HC2 positive specimens. Women were contacted and 8% opted out of specimen storage and research testing. The study collection from the enrollment phase of the PaP Cohort is a group of 45,000 HPV positive (approximately 80% of HC2 positive specimens from KPNC cotesting during that time) and 10,000 randomly chosen HPV specimens. The core collection used for the present HPV test comparison was drawn from 30,000 specimens from 30-65-year-old women who tested positive by HC2 during routine screening (target population), with a small number of HC2 negative specimens included as well.

Specifically, this analysis uses data from 10,762 women with screening HC2 results who were chosen by stratified random sampling in two previously published investigations for masked retesting at BD by Onclarity. Due to overlap with other HPV testing efforts, 1,965 also have results for cobas (performed at Roche), permitting comparison of three clinical HPV tests.

The LA research typing test was performed with cobas on a common DNA extract, allowing comparisons to specific HPV types.

Variables and statistical analysis

The main analyses compared Onclarity with HC2, cobas, and Linear Array. Grouped results were hierarchical, based on type channel-associated risk of CIN3+, as follows: positive for HPV 16, else positive for HPV 18, else positive for other high risk HPV types, else negative. Worst cervical histopathology subsequent to HPV testing was grouped as cancer, else CIN3/AIS, else CIN2, else ≤CIN1. For evaluation of Onclarity's typing accuracy, we used the typing channels established by the test (16, 18, 31, 45, 52, 51, 33/58, 39/68/35, 59/56/66, negative). The viral load cut points for positive results are those proposed by each manufacturer for the most current version of their tests.

Statistical analysis

We used standard measures of agreement (Kappa statistics and asymmetry chi-square tests) and measures of test accuracy (sensitivity, specificity) to examine: 1) HPV positivity for Onclarity versus HC2; 2) HPV type-channel results for Onclarity and cobas, using the more grouped cobas channels; 3) Onclarity results compared with individual types according to LA; and 4) accuracy of Onclarity for detection of precancer defined as either CIN2+ or CIN3+.

Given the lack of a gold standard among HPV tests, we assessed agreement between HPV tests through kappa statistics (unweighted and weighted based on sampling weights) and tests of symmetry. When comparing results of Onclarity and/or cobas with histology results (used as a

gold standard for disease in this case), we calculated specificity and sensitivity using the binary cut points previously described for each variable.

Results

Most women were between 30 and 45 years old, in the high-income category, never smokers, never users of hormonal contraceptive, had BMI of 25 or greater, had normal cytology, and lower high risk HPV infections (Table 1). Ethnically, less than half of the analytic sample was non-Hispanic White.

We compared Onclarity and cobas following categories defined by cobas channels and assigning infections hierarchically. Agreement between both tests was excellent, with a weighted kappa of 0.81 (Table 2). The disagreements by channel show that cobas is more sensitive detecting HPV 16 (75 extra positive specimens compared to 6 extra positives detected by Onclarity) and HPV 18 infections (29 extra positives for cobas and 5 cases for Onclarity). Onclarity was more sensitive than cobas for the category "other high risk HPV types", excluding HPV 16 and HPV 18, detecting 117 extra positives as opposed to the 40 detected by cobas.

Nonhierarchical analyses (Table 3) show similar patterns for all HPV type channels but with fewer extra cases detected by Onclarity in the "other high risk HPV types" category. We confirmed that the hierarchical analysis was reducing the number of HR12 positives more for cobas than for Onclarity, due to greater sensitivity for HPV16 and HPV18 when multiple concurrent infections were present, as they frequently are. This accentuated the assay differences for HR12 types (data not shown). Agreement was similar across different cytology strata (data not shown).

Table 4 assesses Onclarity's typing accuracy identifying infections in one of its 9 channels and comparing these results with non-hierarchical type specific data from Linear Array. Onclarity had good sensitivity and specificity for all 9 channels. Sensitivity ranged from 80.2% for HPV channel 45 to 92.4% for HPV channel 16. Specificity ranged from 95.1% for channel 39/68/35 to 99.7% for channel 45.

We used histology results as the reference standard of target disease to assess the clinical importance of agreement between Onclarity and cobas (Table 5). For all HPV type channels, agreement between cobas and Onclarity was better for infections with more severe histology results. For the 209 CIN3 cases with HPV16 infections, 89.5% tested positive with both tests, cobas picked up 19 additional infections, and Onclarity 3. Most (82.5%) of the 40 CIN3 cases with HPV18 infections were identified by both tests, cobas picked up 5 additional cases and Onclarity 2. For the 199 CIN3 cases with other high risk HPV infections, 91.5% were positive for both tests, cobas picked 3 extra cases and Onclarity 14.

Discussion

We assessed how Onclarity compares to FDA approved HPV DNA tests and a commonly used research typing assay in a large convenience sample of women from the NCI/KPNC HPV Persistence and Progression study. Our data suggest that Onclarity is comparable to FDA approved cobas in its identification of HPV types 16, 18, and other high risk types. However, cobas was slightly more sensitive and less specific than Onclarity for HPV types 16 and 18. When the disagreements between tests were stratified by histology results, most disagreements occurred in women with <CIN2, but a few cases of CIN3 or cancer are potentially missed by one test or the other. Onclarity's typing was accurate when compared to Linear Array.

Methodological limitations include the lack of a gold standard to compare HPV DNA assays. We compared Onclarity to previously FDA approved HPV DNA assays but cannot assume superiority of either test. Clinically, this is a challenge because we do not know if the infections missed by one test are real or false positives by the other test. We then used histology to assess whether the discrepancies between assays corresponded to women with precancer. Histology is widely used to define precancer but it has limitations: diagnosis is subjective, not all precancers progress to cancers, and there is disagreement on the severity of histology that defines precancer (CIN2 or CIN3) [55, 56].

Study strengths include the large sample size of HPV-infected women with HPV DNA test and cytology results in the KPNC's Pap cohort study, followed longitudinally for up to 10 years. Samples from KPNC were tested using multiple HPV DNA tests available for both clinical management (cobas, HC2) and research purposes (Onclarity, linear array). These two factors provided us with unprecedented statistical power to compare different HPV tests with each other and against enough cases of precancer and cancer [35].

Viral load of HPV infection was not studied in our analysis. Viral load for HPV infection, particularly for HPV 16, is positively associated with risk of progression to precancer [57-60]. Given naturally continuous viral loads, HPV tests use a specific cutpoint to categorize test results into positive or negative. In the current study, we did not assess whether the disagreements between tests belonged to infections with lower viral load.

Given the good agreement in detection between tests, other factors will become more meaningful in the choice of using one test or the other. A key determinant might be the need for typing information. Cobas provides specific information for HPV16, HPV18, and groups all other results in a pooled channel. Onclarity further stratifies the other high-risk HPV types into 9

channels. The main advantage of this additional information is the possibility to break down the high risk other HPV group and keep the 7 HPV types with the lowest risk of progression separate from other types with higher risk. Typing information could then be used as triage for HPV positive women, reducing the cost of traditional colposcopy based triage. Other factors that need to be considered are the cost, availability, and ease of use of the two tests.

Onclarity could provide additional typing information without compromising the levels of detection achieved by cobas. Future studies should evaluate the impact of extended typing information in cervical cancer risk stratification. Based on our findings, cobas and Onclarity give comparable grouped results and both could be used in clinical settings without compromising the quality of detection of HPV infections.

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The field effort was a collaboration of the NCI and KPNC and was supported in part by the intramural program of the NCI. The NCI has received HPV and cytology test results at no cost from Roche Molecular Systems and BD Diagnostics for independent evaluations of these technologies. The statistical analysis was supported by NCI and led independently by the first author and the NCI.

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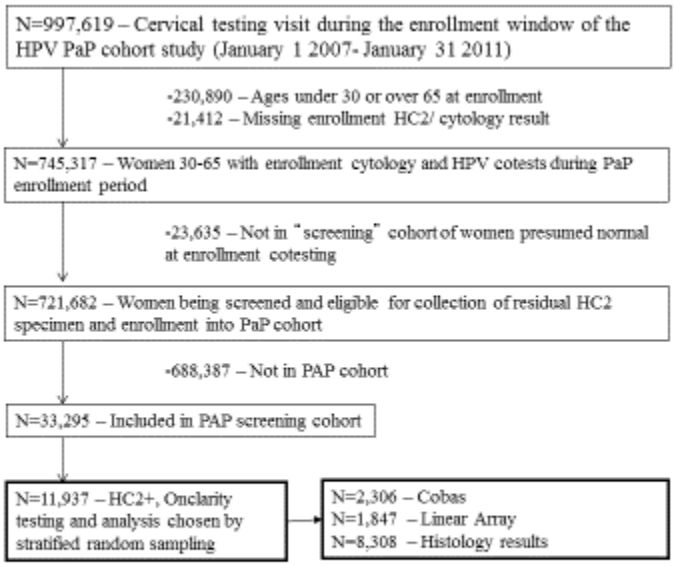
Figure 1: Study population for comparison of Onclarity to cobas and HC2 using NCI- KPNC's Persistence and Progression (PaP) cohort



PaP: Persistence and Progression cohort
HC2: Hybrid Capture 2
KPNC: Kaiser Permanente North California

Table 1: Sociodemographic and clinical characteristics for women in KPNC's PaP cohort (n=11,937) *

		Frequency	Percent
Age in years	30 to 44	18563	60.9
	45 to 54	6993	22.9
	≥55	4929	16.2
BMI (kg/m^2)	Underweight (<18.5)	320	1.1
	Normal (18.5-24)	10993	37.9
	Overweight (25-29)	8850	30.5
	Obese (30-39)	7102	24.5
	Obese class III (≥40)	1722	5.9
Income	Low (≥20% households below poverty level)	3352	10.1
	Middle	10449	31.4
	High (≥80% households >200% poverty level)	18966	57.0
Race/ethnicity	NH White	15574	46.8
	Hispanic	6812	20.5
	NH African American	3083	9.3
	NH Asian/Pacific islander	5892	17.7
	Multiracial	369	1.1
Smoking status	Never	23869	71.7
	Former	4459	13.4
	Current	3448	10.4
Oral contraceptive use (lifetime number of prescriptions)	0	19254	57.9
	1 to 6	3779	11.4
	7 to 21	3338	10.0
	22 to 51	3403	10.2
	≥51	3508	10.5
DMPA use (lifetime number of injections)	0	30898	92.8
	1	673	2.0
	2 to 4	709	2.1
	5 to 9	455	1.4
	≥10	547	1.6
Cytology	WNL	21486	66.0
	ASCUS	6633	20.4
	LSIL	3573	11.0
	ASC-H+	863	2.7

HPV type channel	HPV 16	2044	8.2
	Higher high risk HPV (18, 45, 31/33/52/58)	4420	17.7
	Lower high risk HPV (35/39/51/56/59/66/68w)	18460	74.1

*Acronyms:
BMI: body mass index
DMPA: depot medroxyprogesterone acetate
HPV: human papillomavirus
NH: Non-Hispanic
WNL: within normal limits
ASCUS: Atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesion
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Table 2: Hierarchical HPV type channel agreement between Onclarity and cobas, among HC2 positive women (n=1,965)

Cobas		**Onclarity**			
		HPV 16 (n=403)	**HPV 18** (n=137)	**Other HR HPV** (n=1211)	**Negative** (n=214)
HPV 16 (n=472)	**n**	397	13	38	24
	Row%	84.1	2.8	8.1	5.1
	Col%	98.5	9.5	3.1	11.2
HPV 18 (n=148)	**n**	0	119	21	8
	Row%	0	80.4	14.2	5.4
	Col%	0	86.9	1.7	3.7
Other HR HPV (n=1084)	**n**	6	3	1035	40
	Row%	0.6	0.3	95.5	3.7
	Col%	1.5	2.2	85.5	18.7
Negative (n=261)	**n**	0	2	117	142
	Row%	0	0.8	44.8	54.4
	Col%	0	1.5	9.7	66.4

Kappa Statistics					
Statistic	**Value**	**95% Confidence Limits**		**DF**	**Prob**
Simple Kappa	0.77	0.74	0.79		
Weighted Kappa	0.81	0.79	0.83		
Test of symmetry	115.14			6	<.0001

Table 3: Non-hierarchical HPV type channel agreement between Onclarity and cobas, among HC2 positive women

HPV 16				
Cobas		Onclarity		
		Positive	Negative	Total
Positive	Freq	397	61	458
	Row%	86.7	13.3	
	Col%	98.5	4.0	
Negative	Freq	6	1455	1461
	Row%	0.4	99.6	
	Col%	1.5	96.0	
Total		403	1516	1919

Statistic	Value	95% Confidence Limits		DF	Prob
Simple Kappa	0.90	0.88	0.92		
Test of symmetry	45.15			1	<.0001

HPV 18				
Cobas		Onclarity		
		Positive	Negative	Total
Positive	Freq	140	36	176
	Row%	79.6	20.5	
	Col%	95.2	2.0	
Negative	Freq	7	1736	1743
	Row%	0.4	99.6	
	Col%	4.8	98.0	
Total		147	1772	1919

Statistic	Value	95% Confidence Limits		DF	Prob
Simple Kappa	0.85	0.81	0.90		
Test of symmetry	19.56			1	<.0001

Other HR HPV				
Cobas		Onclarity		
		Positive	Negative	Total
Positive	Freq	1222	101	1323
	Row%	92.37	7.63	
	Col%	90.32	16.5	
Negative	Freq	131	511	642
	Row%	20.4	79.6	
	Col%	9.68	83.5	
Total		1353	612	1965

Statistic	Value	95% Confidence Limits		DF	Prob
Simple Kappa	0.73	0.70	0.76		
Test of symmetry	3.88			1	0.05

Table 4: HPV type/channel percent agreement between Onclarity and non-hierarchical type-specific Linear Array assay, among HC2 positive women*

HPV channel		Virologic standard								
		16	**18**	**31**	**45**	**52**	**51**	**33/58**	**39/68/35**	**59/56/66**
		% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Onclarity	**+**	92.4 (450)	88.2 (149)	92.2 (237)	80.2 (97)	87.5 (259)	85.8 (157)	89.1 (172)	85.1 (309)	86.0 (325)
	-	98.8 (1698)	99.3 (2022)	98.6 (1920)	99.7 (2077)	98.1 (1873)	99.2 (2006)	99.3 (1997)	95.1 (1752)	97.4 (1780)

*This table shows the percent of samples that tested positive (first row) or negative (second row) with both Onclarity and the linear array virologic standard for each individual HPV type.

Table 5: HPV test agreement between Onclarity and cobas, by histology, among HC2 positive women

	Agreement on HPV 16					Agreement on HPV 18					Agreement on Other HR HPV				
	Histology					Histology					Histology				
	≤CIN1	CIN2	CIN3	Cancer	Total	≤CIN1	CIN2	CIN3	Cancer	Total	≤CIN1	CIN2	CIN3	Cancer	Total
	% (n)	% (n)	% (n)	% (n)		% (n)	% (n)	% (n)	% (n)		% (n)	% (n)	% (n)	% (n)	
C+O+	74.8 (89)	79.2 (95)	89.5 (187)	87.5 (21)	392	78.5 (51)	72.5 (29)	82.5 (33)	100 (5)	118	84.5 (554)	89.3 (275)	91.5 (182)	80 (4)	1015
C+O-	25.2 (30)	19.2 (23)	9.1 (19)	8.3 (2)	74	16.9 (11)	27.5 (11)	12.5 (5)	0 (0)	27	4 (26)	3.6 (11)	1.5 (3)	0 (0)	40
C-O+	0 (0)	1.7 (2)	1.4 (3)	4.2 (1)	6	4.6 (3)	0 (0)	5 (2)	0 (0)	5	11.6 (76)	7.1 (22)	7 (14)	20 (1)	113
Total	119	120	209	24	472	65	40	40	5	150	656	308	199	5	1168

Chapter 4: Manuscript 2 – Prospective study of the outcome of type specific HPV infection

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Keywords: HPV outcome, clearance, progression, persistence

Abstract

Background: Human papillomaviruses (HPV) are the most commonly diagnosed sexually transmitted agents. Although 70–90% of infections found on screening clear within 12–24 months, persistent infection with high-risk (HR) HPV types can cause cervical precancer and cancer. This paper identifies type-specific patterns in time to clearance, progression, or persistence of six common HR HPV infections.

Methods: We typed residual test specimens from 33,295 HPV-infected women ages 30-64 years in the NCI-Kaiser Permanente Northern California study. The mutually exclusive outcomes studied in this analysis were cumulative risk of: (1) clearance of HPV infection, (2) progression of HPV infection to precancer, and (3) persistence of HPV infection without clearance or progression. Logistic-Cox models were used to estimate cumulative risk.

Results: Most (65-79%) HPV infections cleared within 2 years. Cumulative risk of progression varied by type, with HPV16 substantially more likely to progress than the other types (e.g., 2-year cumulative risk of progression of 1% for HPV51 compared to 4% for HPV16). Most infections cleared or progressed by the end of follow-up; long-term persistence was rare (1-6%).

Discussion: The fate of most HPV infections is determined within two years of detection. HPV genotyping proved useful to stratify prospective risk of precancer among HPV-positive women.

Abbreviations used

HPV - human papillomavirus

HR – High-risk

WNL - within normal limits

ASC-US - Atypical squamous cells of undetermined significance

LSIL - low-grade squamous intraepithelial lesion

ASC-H+ - Atypical squamous cells of undetermined significance - cannot exclude HSIL

NCI/KPNC PaP - National Cancer Institute/Kaiser Permanente Northern California Persistence and Progression

CIN - Cervical intraepithelial neoplasia

AIS - Adenocarcinoma-in-situ

Introduction

Cervical cancer screening is shifting from cytology to HPV testing starting at age 25-30, past the peak of acquisition of HPV infections [61]. HPV testing identifies the group of a dozen high risk (HR) HPV types judged capable of causing cancer, with variation in the specific HPV types detected by the different tests [42]. HR HPV testing has very high sensitivity but most cervical HPV infections are cleared, i.e., suppressed by cell-mediated immunity within 1–2 years of exposure [62, 63].

Compared to newly detected HPV infections, persistent infections are substantially more likely to progress to precancer (histopathologic precursor to invasive cancer) [8-12]. There is no known definition of clinically important persistence, but follow-up strategies targeting abnormalities lasting more than 1 year (and especially 2 years) seem to distinguish infections and associated lesions with greater risk of progression [12, 15].

Among the different HR HPV types, there is no consensus on whether the risk of persistence might differ by type, although some research suggest that some types take longer to clear [64]. However, once an infection persists, HPV progression to precancer is known to differ by type [65]. For current cervical cancer screening and triage efforts that will soon lead the U.S. market, it is important to understand the natural history of individual HPV genotypes.

Identifying type-specific patterns in cumulative risk for clearance, progression to precancer and persistence of HPV infection would help guide clinical decisions related to time to re-test, treat, and stop testing, given a known HPV infection. The National Cancer Institute/Kaiser Permanente Northern California Persistence and Progression (NCI/KPNC PaP) study was created to help inform the use of HPV testing for primary cervical cancer screening. Our study aims to identify type-specific patterns in clearance, development of a precancer (progression), or persistence in a large group of HR HPV-infected women.

Methods

Study design and population

This is a longitudinal analysis using data from HPV testing and follow-up from Kaiser Permanente Northern California (KPNC) in collaboration with the National Cancer Institute (NCI) (Figure 1). At KPNC, women are tested by Hybrid Capture 2 (HC2) for HR HPV (as a pool without genotyping) to triage the equivocal cytologic result of atypical squamous cells of undetermined significance (ASC-US) (since 2001) and as a co-test with cytology in women ages 30 and older (since 2003) [35, 36]. The HPV Persistence and Progression Cohort (The PaP Cohort) was created by banking residual discarded cervical specimens collected into specimen transport medium (STM; Qiagen) from women tested by HC2. The emphasis was on HC2 positive specimens. Women were contacted and

8% opted out of specimen storage and research testing. The core collection used for the PaP cohort was drawn from 33,295 specimens from 30-65-year old women who tested positive by HC2 during routine screening, with a small number of HC2 negative specimens included as well.

Given the interest in evaluating outcomes of type-specific infection, we restricted the analytic sample to 10,762 women with a positive HPV test result by the HC2 test at baseline and information on HPV typing. Masked HPV typing (to distinguish at least partly the individual carcinogenic HPV type(s) present) was done at BD Diagnostics (Sparks MD) by the Onclarity assay or at Roche Molecular Systems (Pleasanton CA) by cobas/Linear Array (a research-use-only typing test) [42, 66]. A small group of samples were HPV-typed using Linear Array or another PCR-based method at one of two academic laboratories. For the current analysis, we further restricted the study sample to the 7,522 infections with one of the following genotypes: HPV16, HPV18, HPV45, HPV31, HPV51, HPV52.

Variables and statistical analysis

This analysis studied outcomes of HPV infection after the initial screening visit. The main exposure was non-hierarchical infection with independent HPV types (HPV16, HPV18, HPV45, HPV31, HPV51, HPV52, based on Roche's Linear Array, Onclarity, or cobas). A given woman could contribute more than

one infection. No attempt was made to adjust for theoretically possible auto-correlation, because infections with multiple HPV types are established to act independently of each other, with minimal interaction at the viral-viral level regarding persistence/clearance/progression [10].

Mutually exclusive outcomes for this analysis were: (1) cumulative risk for clearance of HPV infection, (2) cumulative risk for progression of HPV infection to precancer, and (3) cumulative risk for persistence of HPV infection without clearance or progression. Consistent with previous literature, clearance was defined as HPV positive at baseline and HPV negative at follow-up [67-70]. A single negative test was deemed sufficient to define clearance. Progression was defined with a histopathologic diagnosis of CIN2/CIN3/AIS at follow-up as an imperfect surrogate endpoint for cancer. The uncommon diagnoses of cancer were excluded. Persistence was assigned when no clearance or progression were identified by the end of follow-up.

Statistical analysis

We present descriptive statistics for the study sample by outcome of HPV infection. Logistic-Cox models were used to estimate the hazard ratios of cumulative risk for HPV infection clearance and progression (given non-clearance). Separate analyses were carried out for cumulative risk for clearance and progression of type-specific HPV infections.

To calculate cumulative risks for different events we first determined the timepoints of interest. Based on previous literature, time to clearance was defined using the likelihood of the event falling within the interval defined between the last HPV positive measurement and the first HPV negative measurement for each type-specific HPV infection [10]. Time to progression was defined using the time interval between the last HPV positive measurement and the first CIN2/CIN3/AIS measurement for each type-specific HPV infection. Women who completed the study without clearing their infection, progressing to precancer, or were lost to follow-up were censored as of their last available visit.

Results

Most HPV infections cleared within 2 years (Figure 2). The absolute risk of clearance at year 2 ranged from 63% (HPV31) to 79% (HPV51) across types. After approximately year 2, clearance continued but at a slower rate. Different HPV types followed similar patterns of clearance with a similar inflection time in the curve but slightly different absolute risks. The less carcinogenic types showed slightly increased risk of clearance whereas HPV 16 differentiated itself from other types with the lowest risk of clearance manifested in the later years of follow-up. By the end of follow up (8.5 years), risk of clearance ranged from 86% for HPV16 to 98% for HPV51. The lower clearance for HPV16 was linked to increased risk of progression (see next paragraph).

Cumulative risk of progression exhibited different patterns for different HPV types. For most types studied, risk of progression increased rapidly in the first two years, and stabilized after that (Figure 3). By year 2, absolute cumulative risk of progression ranged from 1% for HPV51 to 4% for HPV16. HPV16 had the highest risk of progression and, unlike most other types, it was linked to continually increase risk throughout follow-up, reaching a cumulative risk of 8% by year 8.5. All other HPV types had much smaller cumulative 8-year risk: 1% for HPV51 to 4% for HPV31.

Persistence (i.e., neither progression nor clearance) as a proportion of initial infections declined steeply in the first two years, slowed in the subsequent two years, and fell under 10% for all types after 5 years (Figure 4). The patterns of persistence did not vary by type and the magnitude of the risk for different types were similar on an absolute scale throughout follow-up (except for HPV16). At year 2, persistence ranged from 20% (HPV51) to 35% (HPV31). By the end of follow-up, when most infections had either cleared or progressed, persistence was rare for any HPV type ranging between 1% for HPV51 to 6% for HPV16.

Discussion

To our knowledge, this is the largest reported study of time to event by HPV type, with a final sample of 2,294 HPV16 infections; and 814 HPV18, 606 HPV45, 1,267 HPV31, 1,050 HPV51, and 1,491 HPV52 infections. Our sample was about 10 times the size of the next largest study on time to outcome of HPV infection, allowing us to conduct statistically precise type-specific analyses for multiple HPV genotypes using very high quality typing data with over 8 years of follow-up.

Our results confirm that the fate of most HPV infections found at cervical cancer screening is evident within 2 years, when most infections have cleared [10]. The cumulative risk for clearance of HPV infection was similar across types other than HPV16, which tends to clear less often than other HR HPV because of substantially greater competing risk of progression. Persistence, with neither clearance nor progression, is ultimately uncommon. HPV16 is most likely to persist without progression but, given the insensitivity of colposcopy, it is difficult to rule out occult progression in the form of a small precancer missed on visual examination.

This study has several limitations. First, the exact risk estimates might be somewhat biased toward progression given that we oversampled for precancer outcomes when choosing which specimens to type. Second, we chose CIN2/CIN3/AIS as the disease endpoint but most cases of CIN2 or even

CIN3/AIS would not progress to cancer if left untreated and the true cancer risk posed by various HR HPV types is misspecified by the prospective risk of CIN2/CIN3/AIS. Third, we cannot estimate absolute time to clearance because we do not have sufficiently dense timing of visits, and are likely to miss the true time of transition between the last positive and first negative results. Fourth, we define clearance as a single negative test for a specific HPV type which would be inaccurate in the case of immunologic control of a persistent infection or a false negative test (i.e.: diagnostic accuracy of cervical smears). Fifth, variables related to past medical history and coinfections were not part of this analysis.

An earlier generation of clinicians was told that, if a CIN1 lesion was observed, roughly one-third would progress, one third would persist and one third would clear [12, 69, 70]. However, CIN1 is a poorly reproducible sign of HPV infection [41, 71, 72]. Our typing based on HPV molecular assays permits us to observe more accurately that 90% of HPV infections clear, 5% progress, and <5% persist over approximately eight years of follow-up. HPV16 uniquely tends to lead to higher cumulative risk of progression and lower cumulative risk of clearance. Given the large numbers of infections followed in the PaP cohort, and agreement with other prior work, these patterns seem solid and believable.

The PaP study was designed to study determinants of HPV persistence and progression to precancer. Precancer is a theoretical perfect precursor and surrogate endpoint for risk of cancer. Our choice of CIN2/CIN3/AIS as the

disease endpoint was pragmatic, given that when found such lesions are usually treated. Although CIN2/CIN3/AIS are imperfect surrogate endpoints for cancer, their use permits ethical prospective study in that observation lasting purposely until cancer develops would not be acceptable.

We know from the direct typing of cancers from around the world as performed by IARC and ICO that HPV18 and HPV45 are the second and third most important types, respectively, when cancer is the outcome [1, 10]. HPV18 and HPV45 require integration into the host cellular genome to pose a risk for cancer [66, 73, 74]. The eight years of follow-up in our study do not permit observation of the cancer risk visible only in the longest cohorts spanning 15 or more years [35, 65, 75].

High-risk HPV types are heterogeneous. The IARC grouping of established carcinogenic HPV types was never meant to assess carcinogenic potential or need for inclusion in diagnostic assays [3, 76]. The inflection point for the highest risk HR HPV types, especially HPV16, is 2-3 years of type-specific persistence. After that length of persistence, the rate of clearance is slow while rates of progression remain elevated. It might be worth considering in ASCCP cervical cancer screening guidelines that treatment is indicated when persistence of this sub-group of infections is prolonged past this point. On the other hand, for the lowest risk HR types, the cumulative risk of progression is low even after eight years of follow-up, supporting caution before excisional

treatment. Future research should assess the role of age and cytology results as potential effect modifiers in cumulative risk of progression. In summary, this study identified both overall similarity but some type-specific differences in the patterns of progression and clearance of HPV infections, suggesting that availability of HPV typing details could be useful for cervical cancer screening/management programs.

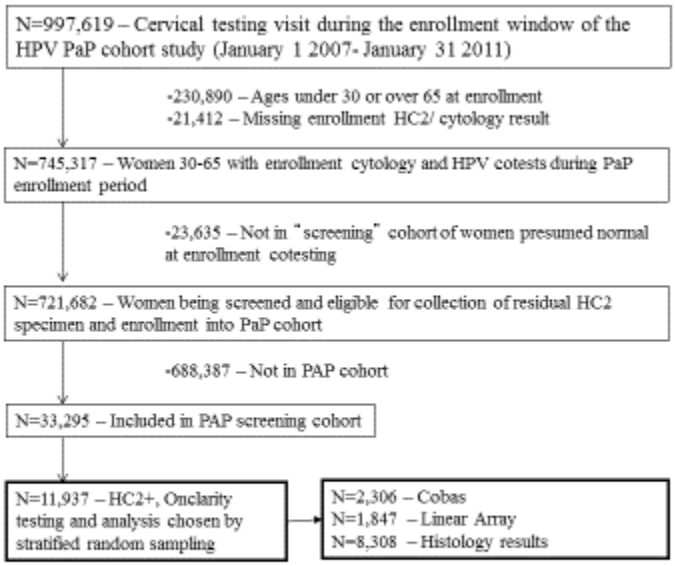
Acknowledgements

The field effort was a collaboration of the NCI and KPNC and was supported in part by the intramural program of the NCI. The NCI has received HPV and cytology test results at no cost from Roche Molecular Systems and BD Diagnostics for independent evaluations of these technologies. The statistical analysis was supported by NCI and led independently by the first author and the NCI.

Philip E. Castle has received commercial HPV tests for research at reduced or no cost from Roche, Qiagen, Norchip, and MTM. He is a paid consultant for BD, GE Healthcare, Roche, Gen-Probe/Hologic, and Cepheid and is compensated as a member of a Merck Data and Safety Monitoring Board for HPV vaccines.

We acknowledge support by the University of Maryland School of Public Health (Drs. Carter-Pokras, He, Dallal, Chen) for participation in preparation of this manuscript.

Figure 1. Study population in KPNC's Persistence and Progression (PaP) study



PaP: Persistence and Progression cohort
HC2: Hybrid Capture 2
KPNC: Kaiser Permanente North California

Figure 2. Type-specific cumulative risk for clearance of HPV infection over 8.5 years of follow-up.

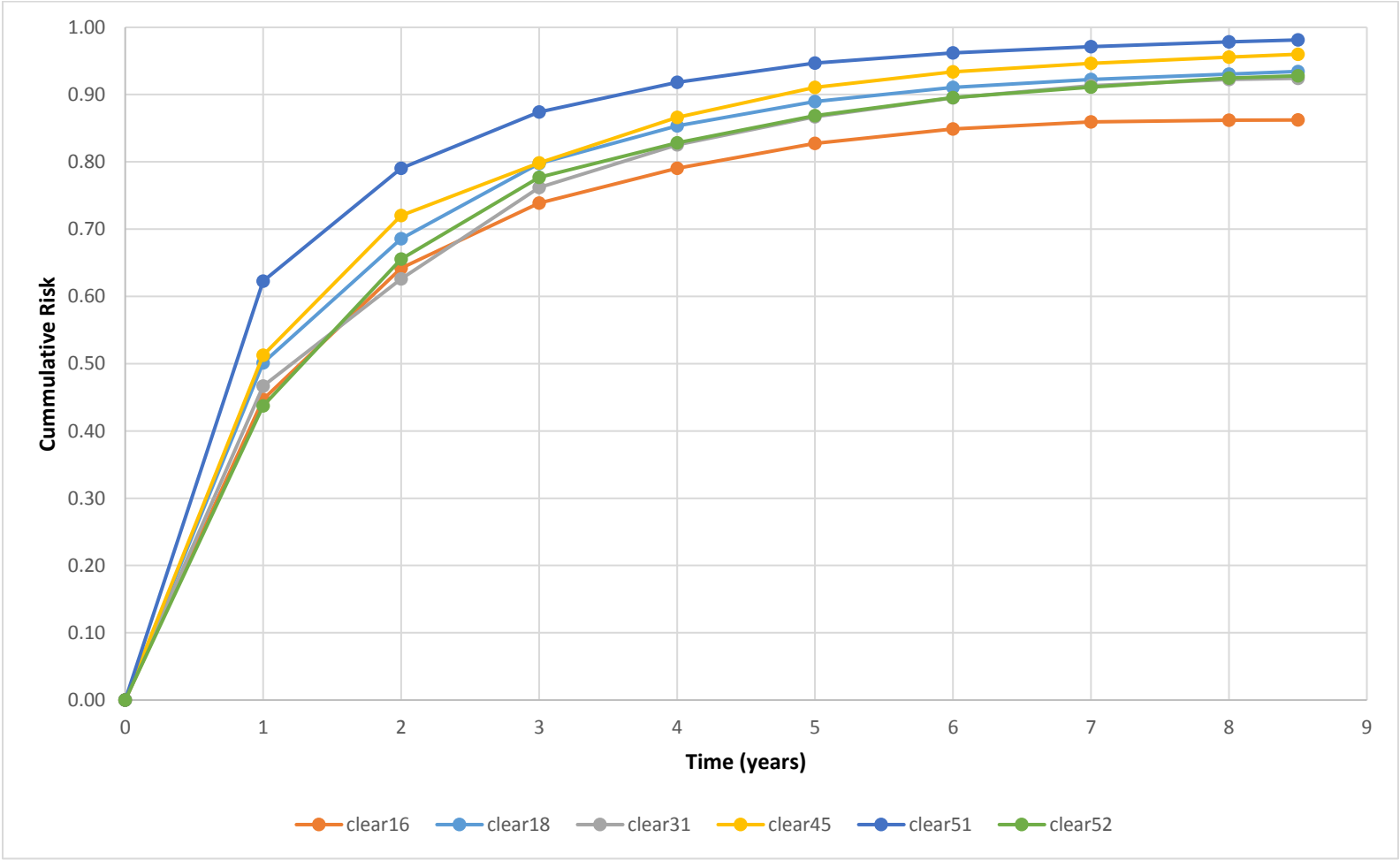


Figure 3. Type-specific cumulative risk for progression (CIN2/CIN3/AIS) of HPV infection to precancer over 8.5 years of follow-up.

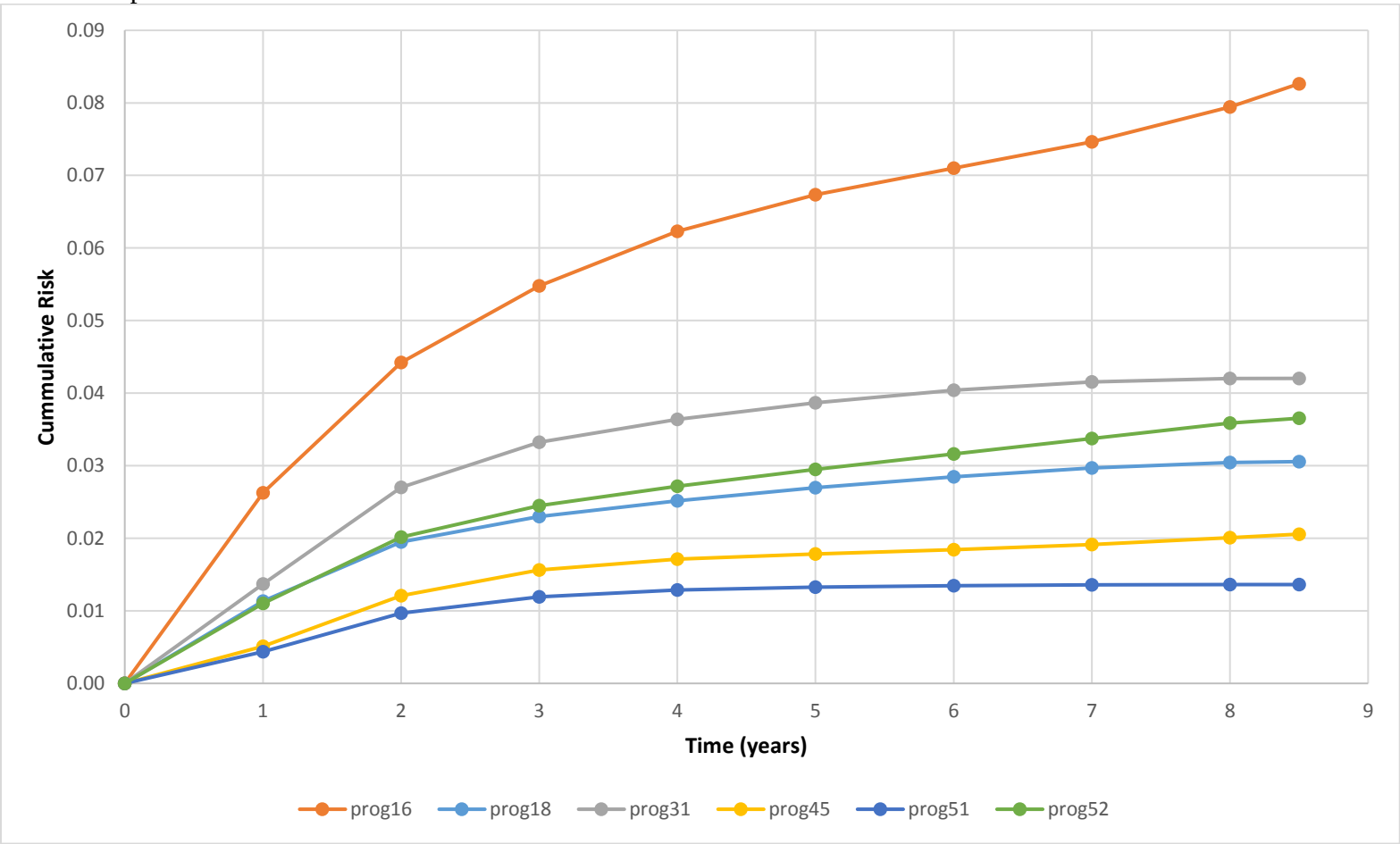
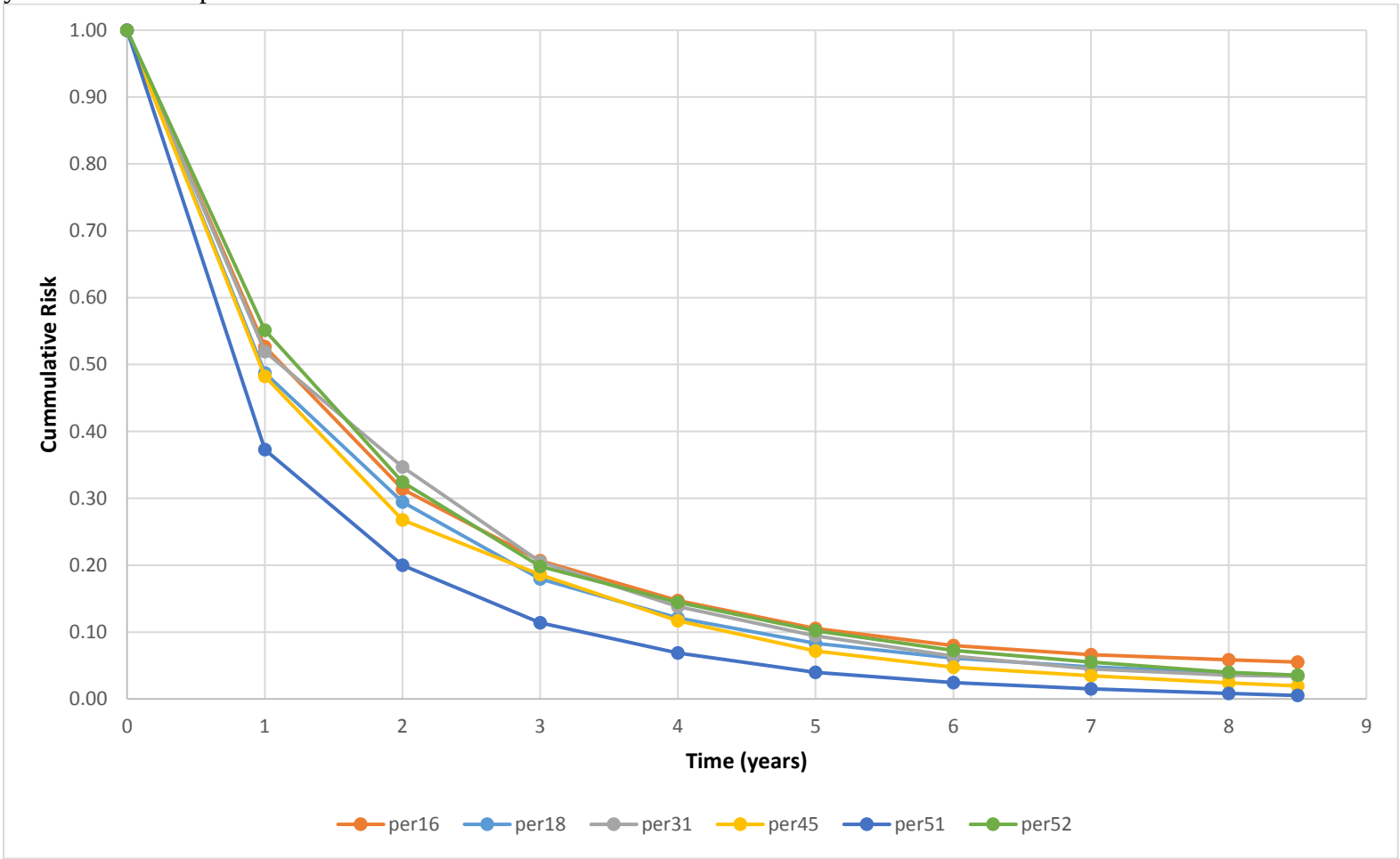


Figure 4. Type-specific cumulative risk for persistence of HPV infection without clearance or progression over 8.5 years of follow-up.



Chapter 5: Manuscript 3 – Clinical utility of considering HPV cofactors from etiologic studies of cervical cancer in cervical screening and management

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Precis

HPV type and severity of cytological change are the main predictors of the risk of progression from HPV infection to CIN3+.

Keywords: screening, progression, cervical cancer, absolute risk, cofactors

Abstract

Persistent infection with HPV is the necessary cause of cervical cancer. Until HPV vaccination controls cancer rates, screening for cervical cancer will remain critical, shifting from cytology to HPV or co-testing. U.S. screening guidelines for cervical cancer are based on absolute risk of treatable precancer estimated from large clinical cohorts and trials. Critical questions include the level of detail necessary for HPV and cytology test results, and whether other cofactors are worth including in risk prediction models for clinical management of HPV-infected women. Our aim was to assess which established co-factors for cervical precancer, given HPV infection and cytology results, and whether they represent clinically useful, actionable risk predictors.

We analyzed data from HPV-infected women in the NCI-Kaiser Permanente Northern California cohort study. We identified the 3-year risk of CIN3+ of 10,450 HPV infections. Variables considered included: HPV type, cervical cytology result, age, race/ethnicity, BMI, smoking, and hormonal contraceptive use. Weighted Logistic Cox models (combining prevalent odds ratios and incident hazard ratios) were used for multivariable absolute risks of CIN3+.

HPV type and severity of cytological change were the main determinants of the risk of progression from common HPV infection to uncommon CIN3+. Other cofactors had minimal (age, race/ethnicity) or no significant independent

(hormonal contraceptives, smoking, BMI) effects on 3-year risk of progression to CIN3+.

The fate of most infections was determined mainly by HPV test and cytology results rather than behavioral cofactors. Future studies should consider other likely predictors: previous medical history, vaccination status, and HIV status.

Abbreviations used

BMI: body mass index

DMPA: depot medroxyprogesterone acetate

HPV: human papillomavirus

NH: Non-Hispanic

NILM: negative for intraepithelial lesion or malignancy

ASC-US: Atypical squamous cells of undetermined significance

LSIL: low-grade squamous intraepithelial lesion

ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Introduction

Cervical cancer screening is shifting from cytology to co-testing with HPV assays and cytology, or even HPV testing alone [61]. Current cervical cancer guidelines mainly use cytology and HPV status (positive/negative) to stratify risk and guide clinical management [30]. Screening guidelines will soon need to address how to incorporate new components of cervical cancer screening such as HPV typing, secondary tests that predict which infected women need treatment, and the impact of vaccinated cohorts entering the screening population [42].

The American Society for Colposcopy and Cervical Pathology (ASCCP) is beginning the process of updating US consensus cervical cancer screening guidelines. The new ASCCP guidelines will be incorporated into an electronic application; patients' information will be entered during the gynecologic exam to guide clinical management [30]. The risk prediction tool will use the information entered to find precise precancer risk estimates and will output the associated, recommended clinical action (normal-interval rescreening, short-term retesting, colposcopy referral, or treatment). Providers will also have the option to see the detailed risk estimates and justification for the decision-making process.

To generate accurate precancer risk estimates for the many possible combinations of test results and cofactors, we need data from large cohorts and trials. It is central to assess the importance of each possible risk predictor to be included in the risk prediction tool. The clinical importance of socio-

demographic and behavioral characteristics previously identified as potential etiologic cofactors for progression from HPV infection to precancer can be estimated in terms of impact on absolute risk of precancer/cancer. The risks that mandate increasingly intensive clinical action must take into account societal risk tolerance and resources [30].

While HPV infection is the necessary cause and predictor of risk of precancer/cancer, established co-factors for HPV persistence and progression to precancer include viral (HPV genotype, viral load), behavioral (smoking status, contraceptive use, HIV co-infection), and genetic host factors. These co-factors have been established by large case-control studies and prospective evidence but their importance in terms of clinical management is still unknown [19, 20, 24, 25, 77-82].

In the context of the development of new ASCCP guidelines, this analysis aimed to assess whether, given HPV infection and cytology results, some of the previously established cofactors for cervical precancer (BMI, smoking, hormonal contraceptive use) represent clinically useful, actionable risk predictors that clinicians need to consider to manage HPV-infected women.

Methods

Study design and population

This was a cohort analysis using data from HPV testing of a large set of residual specimens following HPV testing conducted at Kaiser Permanente Northern California (KPNC) and the National Cancer Institute (Figure ?). At KPNC, women were tested by Hybrid Capture 2 (HC2) for the group of high-risk genotypes of HPV (as a pool without genotyping) to triage the equivocal cytologic result of atypical squamous cells of undetermined significance (ASC-US) (since 2001) and as a cotest with cytology in women ages 30 and older (since 2003) [35, 36].

The HPV Persistence and Progression Cohort (The PaP Cohort) was created by banking residual, discarded cervical specimens, collected into specimen transport medium (STM; Qiagen), from women tested by HC2. The emphasis was on HC2 positive specimens. Women were contacted and 8% opted out of specimen storage and research testing. The study collection from the enrollment phase of the PaP Cohort consisted of a group of 45,000 HPV positive residual specimens (approximately 80% of HC2 positive specimens from KPNC cotesting during that time) and 10,000 randomly chosen HPV specimens. The core collection used for the present HPV test comparison was drawn from nearly 30,000 specimens from 30-65-year-old women who tested positive by HC2

during routine screening, with a small number of HC2 negative specimens included as well.

Given the interest in evaluating progression of type-specific infection to CIN3+, we restricted this analysis to women with a positive HPV test result by HC2 test at baseline and information on HPV typing. Specifically, this analysis used data from 10,762 women with positive screening HC2 results who were chosen by stratified random sampling in two previously published investigations for masked HPV typing (to distinguish at least partly the individual carcinogenic HPV type(s) present) at BD Diagnostics (Sparks MD) by the Onclarity assay or at Roche Molecular Systems (Pleasanton CA) by cobas/Linear Array. A small group were HPV-typed at one of two academic laboratories.

Variables

This analysis studied progression of HPV infection to CIN3+ histopathology within 3 years after the initial screening visit. Infection with a specific HPV genotype was defined hierarchically (16, else 18/45, else 31/33/52/58, else 35/39/51/56/59/66/68w) based on previous established HPV risk groups. Cytology result categories were: NILM (within normal limits), ASC-US (atypical squamous cells of undetermined significance) or LSIL (low-grade squamous intraepithelial lesion), and ASC-H+ (including the higher risk results:

atypical squamous cells of undetermined significance - cannot exclude HSIL, AGC or atypical glandular cells, HSIL, AIS, and cancer).

Potential sociodemographic and behavioral co-factors selected based on previous literature included: age (30-44, 45-54, 55 or older), race/ethnicity (non-Hispanic White, Hispanic, non-Hispanic African American, non-Hispanic Asian/Pacific Islander, multiracial), body mass index in kg/m^2 (underweight, normal, overweight, obese, very obese), smoking status (current, former, never), hormonal contraceptive use (total number of oral contraceptive pill packs or depot medroxyprogesterone acetate injections dispensed in the 10 years prior to and including enrollment date). The relative risk estimates used the original categories and were used to collapse categories with similar risks for subsequent analyses. Co-factors were dichotomized for inclusion in the final, multivariable model.

Statistical analysis

We presented descriptive statistics for the study sample, by outcome of HPV infection (progression to CIN3+, clearance, or persistence). Statistical methods included the calculation of 3-year absolute risk of progression to CIN3+, combining logistic regression in cross-sectional analysis of prevalent CIN3+ cases with Cox models in prospective analysis of CIN3+ incident cases. Thus, logistic-Cox models were used to generate combined 3-year cumulative risk of CIN3+.

We chose 3-years because that is the time it takes for most infections to progress or clear, and CIN3+ because it is a better surrogate endpoint for cancer than the CIN2+ cut point currently used for clinical guidelines. All p-values were considered significant at ≤ 0.05, and 95% confidence intervals were reported when appropriate.

Results

The women studied in this subset of the NCI-KPNC PaP cohort study tended to be middle-aged (median 40 years), in a high-income category, never smokers, never users of oral contraceptive or DMPA, had a BMI of 25 or greater, had currently normal cytology, and were positive for HPV infections of the lower risk carcinogenic types (see Table 1). Ethnically, less than half of the analytic sample was non-Hispanic White. Restricted to cases, i.e., women with a CIN3+ diagnosis in the first 3 years after screening, sociodemographic characteristics followed the same pattern but clinically, most CIN3+ diagnosed women had cytology of ASC-H+ or infection with HPV16.

The estimated univariate relative risk of progression from HPV infection to CIN3+ was higher for younger women and current smokers, lower for non-Hispanic African American, higher for women infected with higher risk HPV types (31/33/52/68, 18/45, or 16), and higher for women with abnormal cytology (see Table 2).

The cumulative risks were derived from prevalent odds ratios and incident hazard ratios. Cross-sectional univariate analyses showed higher odds of CIN3+ histology for women under 45 years old, very obese, current smokers, non-African Americans, with abnormal cytology (ASCUS/LSIL or ASC-H+), and higher risk carcinogenic HPV types (16, 18/45, or 31/33/52/58) (see Table 3). Prospective analyses showed increased hazards ratios for younger women, non-African Americans, women with abnormal cytology (ASCUS/LSIL not statistically significant and ASCH+ statistically significant), and infections with higher risk carcinogenic HPV types (16, 18/45, or 31/33/52/58) (see Table 3).

Multivariable analyses showed that, when adjusting for all other variables, the significant co-factors for progression from HPV infection to CIN3+ were age (decline with increasing age), race/ethnicity (lower risk in African-Americans), cytology (higher risks in ASC-H+), and HPV type (see Table 4).

Given adjustments for age and race/ethnicity, for each of the 12-combined category of cytology and HPV type, cumulative risks did not change clinical action (based on thresholds currently used in cervical cancer screening guidelines) when incorporating previously established behavioral cofactors (BMI, smoking status, OCP or DMPA use) (Table 5 and Appendix III).

Discussion

The outcome of most HPV infections was mainly determined by HPV test and cytology results rather than behavioral cofactors. Given information on HPV type and cytology, after adjusting for age, none of the co-factors in our study changed the estimated risks of CIN3+ enough to alter the clinical actions suggested by the currently recommended risk-action thresholds. Furthermore, in our dataset, cytology results of NILM, ASC-US, or LSIL did not change risk significantly given HPV test results. However, cytology (and other cofactors) would be more important predictors of CIN3+ if HPV status were not known (data not shown).

We chose CIN3+ as the surrogate endpoint for screening. Ongoing debate over the choice of CIN3+ or CIN2+ as the definition of precancer balance the larger number of outcomes for analyses using CIN2+ vs. the more definite, diagnosis of precancer as CIN3+ [83]. Current US consensus guidelines use CIN3+ to guide most clinical actions, and so did we, although sensitivity analyses using CIN2+ yielded equivalent conclusions. Specifically, ancillary analyses of the same methods using CIN2+ suggest that the choice of the surrogate endpoint may change the magnitude of the effects but not directions of associations.

Previous literature identifies behavioral factors that approximately double the risk of progression to precancer among HPV-infected women, including long-term (5 or more years) oral contraceptive use and smoking (some with a dose

response for the number of cigarettes smoked and duration of smoking) [19-25]. Our analysis studied these etiologic cofactors and found them to not be clinically relevant in changing the absolute risk estimates of progression given HPV infection, at least in the low-risk, well-screened KPNC population.

Discrepancies with previous studies could be explained by several reasons. First, we are not interested in the statistical significance of cofactors but in whether the difference is large enough to change clinical action. Since there are only 3 possible clinical actions (normal screening, shorter interval re-testing, and referral to colposcopy), factors that may still change the risk of progression may not change the risk enough to cross clinical action thresholds. Second, in addition to HPV positivity as considered in the literature, we studied the effect of cofactors given HPV type and cytology results. Given that cytology changed the risk of progression by up to 27 times and HPV type changed the risk of progression by up to 9 times when compared with normal results, the additional risk stratification of cofactors that change the risk of progression by 50% or less is smaller.

Some previously established etiologic cofactors were not available in our dataset. Most studies on parity show increases in risk associated with increasing number of births and some studies have cited chronic cervical inflammation from chlamydia infection and immunosuppression (e.g., HIV) as risk factors for precancer. We did not have access to these cofactors for this analysis.

Furthermore, we did not have detailed information on some of the variables studied (i.e., smoking status).

This study aimed to identify important variables to consider in the ongoing construction of the risk "matrix" that will underlie the next round of ASCCP-sponsored US consensus guidelines. The ASCCP guidelines and resultant risk tool will minimize the burden of data entry while providing precise risk estimates and associated simple management recommendations. In this search for parsimony, our results suggested that including HPV typing, cytology, and age would minimize the burden on the patients while maximizing the outcomes of the absolute risk based model. Based on our data, some often-asked, additional screening questions would provide limited additional information and could be omitted for the most part.

The first question in the ASCCP application should be whether current HPV status (positive/negative) is available. If unavailable, the precision of the risk estimates is compromised precluding optimal screening guidelines. If available, the next priorities would be availability of HPV typing (second) and cytology (third) for triage.

Absolute risk based prevalence-incidence models are the first step in the decision-making process of whether cofactors are important in the clinical decision making process. Additional statistical methods that take into account risk and disease prevalence would permit a formal statistical test of which factors are

worth keeping. Finally, risk action thresholds will vary by setting based on societal values of safety, efficiency and availability of resources.

Acknowledgements

The field effort was a collaboration of the NCI and KPNC and was supported in part by the intramural program of the NCI. The NCI has received HPV and cytology test results at no cost from Roche Molecular Systems and BD Diagnostics for independent evaluations of these technologies. The statistical analysis was supported by NCI and led independently by the first author and the NCI.

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We acknowledge support by the University of Maryland School of Public Health (Drs. Carter-Pokras, He, Dallal, Chen) for participation in preparation of this manuscript.

Table 1. Sample sociodemographic and clinical characteristics of women in the sample (n=10,450) *

		Total		CIN3+	
		n	%	n	%
Age in years	30 to 44	6669	63.8	507	71.6
	45 to 54	2216	21.2	126	17.8
	55 or more	1565	15.0	75	10.6
Race/ethnicity	NH White	4830	46.2	370	52.3
	Hispanic	2134	20.4	139	19.6
	NH African American	956	9.2	38	5.4
	NH Asian/Pac	1881	18.0	142	20.1
	Multiracial	106	1.0	5	0.7
Income	Low	1076	10.3	57	8.1
	Middle	3249	31.1	213	30.1
	High	5965	57.1	433	61.2
BMI	Underweight (<18.5)	97	1.1	6	1.0
	Normal (18.5-24)	3455	39.1	234	40.2
	Overweight (25-29)	2681	30.3	170	29.2
	Obese (30-39)	2150	24.3	143	24.6
	very obese (≥40)	464	5.2	29	5.0
Smoking	Never	7359	70.4	487	68.8
	Former	1410	13.5	100	14.1
	Current	1110	10.6	82	11.6
OCP	0	6018	57.6	398	56.2
	1 to 6	1164	11.1	70	9.9
	7 to 21	1016	9.7	73	10.3
	22 to 51	1044	10.0	73	10.3
	51 or more	1208	11.6	94	13.3
DMPA	0	9635	92.2	654	92.4
	1	207	2.0	14	2.0
	2 to 4	236	2.3	18	2.5
	5 to 9	143	1.4	9	1.3
	10 or more	229	2.2	13	1.8
Cytology	NILM	5835	55.8	192	27.1
	ASCUS/LSIL	3783	36.2	85	12.0
	ASCH+	627	6.0	414	58.5

HPV type	16	2030	19.4	349	49.3
	18/45	1256	12.0	88	12.4
	31/33/52/58	3140	30.1	189	26.7
	35/39/51/56/59/66/68w	4024	38.5	82	11.6

Abbreviations used:
BMI: body mass index
DMPA: depot medroxyprogesterone acetate
HPV: human papillomavirus
NH: Non-Hispanic
NILM: negative for intraepithelial lesion or malignancy
ASCUS: Atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesion
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Table 2. Univariate relative risk of progression from HPV infection to CIN3+ within 3 years by sociodemographic and clinical characteristics (n= 10,450)

		RR	95% CI	
Age in years	30 to 44	1.00	-	-
	45 to 54	0.77	0.69	0.86
	55 or older	0.66	0.58	0.76
Race/ethnicity	NH White	1.00	-	-
	Hispanic	0.96	0.87	1.07
	NH African American	0.60	0.50	0.72
	NH Asian/Pac	1.00	0.90	1.12
	Multiracial	0.74	0.46	1.17
Income	Low	1.00	0.94	1.05
	Middle	1.00	0.94	1.05
	High	1.00	-	-
BMI	Underweight (<18.5)	0.89	0.56	1.41
	Normal (18.5-24)	1.00	-	-
	Overweight (25-29)	0.94	0.84	1.06
	Obese (30-39)	1.04	0.92	1.17
	very obese (≥40)	0.87	0.69	1.09
Smoking	Never	1.00	-	-
	Former	1.11	0.99	1.25
	Current	1.30	1.15	1.46
OCP	0	1.00	-	-
	1 to 6	0.87	0.75	1.00
	7 to 21	0.99	0.86	1.14
	22 to 51	0.91	0.79	1.06
	51 or more	1.00	0.88	1.14
DMPA	0	1.00	-	-
	1	1.05	0.79	1.38
	2 to 4	0.78	0.56	1.08
	5 to 9	1.08	0.78	1.50
	10 or more	0.87	0.64	1.18
Cytology	NILM	1.00	-	-
	ASC-US/LSIL	1.59	1.43	1.77
	ASC-H+	5.96	5.41	6.58

HPV type	16	6.93	6.00	8.01
	18/45	3.40	2.85	4.06
	31/33/52/58	2.93	2.51	3.42
	35/39/51/56/59/66/68w	1.00	-	-

Table 3. Univariate measures of association between sociodemographic and clinical characteristics and CIN3+ prevalence and incidence.

		Cross-sectional analysis of prevalent CIN3+ cases			Prospective analysis of CIN3+ incident cases		
		OR	95% CI		HR	95% CI	
Age in years	45 or older	0.6	0.55	0.66	0.75	0.59	0.91
	30 to 45	1	-	-	1	-	-
BMI	very obese (≥40)	0.76	0.58	0.94	1.01	0.55	1.48
	all others	1	-	-	1	-	-
Smoking status	Former	0.95	0.83	1.08	1.33	0.97	1.69
	Current	1.38	1.2	1.55	1.22	0.84	1.59
	Never	1	-	-	1	-	-
Race/ethnicity	NH African American	0.63	0.53	0.74	0.48	0.25	0.72
	all other races	1	-	-	1	-	-
OCP	1 or more	1	0.91	1.08	1.04	0.83	1.25
	Never	1	-	-	1	-	-
DMPA	1 or more	1.05	0.89	1.21	0.93	0.57	1.29
	Never	1	-	-	1	-	-
Cytology	ASC/LSIL	5.32	4.6	6.05	1.22	0.96	1.48
	ASCH+	27.55	23.19	31.9	3.29	2.33	4.26
	NILM	1	-	-	1	-	-
HPV type	16	6.11	5.34	6.87	8.83	6	11.65
	18/45	1.97	1.63	2.3	2.83	1.66	4
	31/33/52/58	2.65	2.32	2.98	2.96	1.96	3.95
	35/39/51/56/59/66/68w	1	-	-	1	-	-

Abbreviations used:
BMI: body mass index
DMPA: depot medroxyprogesterone acetate
HPV: human papillomavirus
NH: Non-Hispanic
NILM: negative for intraepithelial lesion or malignancy
ASCUS: Atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesion
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Table 4. Multivariate measures of association between sociodemographic and clinical characteristics and CIN3+ prevalence and incidence.

		Cross-sectional analysis of prevalent CIN3+ cases			Prospective analysis of CIN3+ incident cases		
		OR	95% CI		HR	95% CI	
Age in years	45 or older	0.74	0.66	0.82	0.84	0.66	1.02
	30 to 45	1	-	-	1	-	-
Race/ ethnicity	NH African American	0.73	0.59	0.87	0.47	0.25	0.68
	all other races	1	-	-	1	-	-
Cytology	ASC/LSIL	5.28	4.54	6.03	1.24	0.96	1.51
	ASCH+	25.48	21.29	29.67	2.95	2.06	3.84
	NILM	1	-	-	1	-	-
HPV type	16	5.34	4.58	5.34	8.75	6.01	11.48
	18/45	1.72	1.39	1.72	2.93	1.76	4.11
	31/33/52/58	2.43	2.09	2.43	2.99	2	3.97
	35/39/51/56/59/66/68w	1	-	-	1	-	-

Abbreviations used:
HPV: human papillomavirus
NH: Non-Hispanic
NILM: negative for intraepithelial lesion or malignancy
ASCUS: Atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesion
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Table 5. Unadjusted and adjusted cumulative risk of CIN3+ by HPV type and cytology

HPV type	cytology	Frequency	Distribution (%)	Unadjusted probability	Adjusted probability
16	NILM	1169	9.23	12.07	12.17
	ASCUS/LSIL	1151	9.09	31.17	31.38
	ASCH+	526	4.16	68.42	68.64
	Unknown	51	0.40	16.51	16.89
18/45	NILM	745	5.89	4.09	4.13
	ASCUS/LSIL	479	3.78	11.91	11.86
	ASCH+	210	1.66	37.56	38.02
	Unknown	23	0.18	5.58	5.85
31/33/52/58	NILM	1781	14.07	4.81	4.86
	ASCUS/LSIL	1540	12.17	15.35	15.44
	ASCH+	489	3.86	44.88	45.12
	Unknown	68	0.54	7.34	7.24
35/39/51/56/59/66/68w	NILM	2229	17.61	1.85	1.86
	ASCUS/LSIL	1816	14.35	6.53	6.55
	ASCH+	312	2.46	23.63	23.77
	Unknown	70	0.55	2.84	2.80

Abbreviations used:
HPV: human papillomavirus
NH: Non-Hispanic
NILM: negative for intraepithelial lesion or malignancy
ASCUS: Atypical squamous cells of undetermined significance
LSIL: low-grade squamous intraepithelial lesion
ASC-H+: Atypical squamous cells of undetermined significance - cannot exclude HSIL

Chapter 6: Conclusions & Public Health Significance

Secondary prevention of cervical cancer by screening is a process, not simply an application of a screening test. The screening process consists of the screening itself, triage of the screen-positive woman to avoid excessive colposcopic referrals, colposcopic-guided biopsy, treatment and post-treatment surveillance of women with precancer or cancer, and surveillance of women found at colposcopy not to have treatable precancer [5, 53, 84-89]. The first goal of cervical cancer screening is to stratify risk of cancer among women from the general population, thought *a priori* to be at low risk.

Manuscript 1 shows that the two most recent tests for HPV DNA to seek FDA approval, cobas and Onclarity, are both meaningful tools to detect HPV DNA in cervical cancer screening with pooled type results roughly comparable to HC2. The Onclarity test, currently under consideration at FDA, produces partial typing results that are quite closely associated with those generated by the FDA approved cobas assay (kappa statistic approximately 0.80), suggesting both tests could be used without major changes in outcomes in clinical settings, and that other factors might be more meaningful in the choice of using one test or the other (e.g. need for typing information, availability, cost). When comparing both tests to histology both tests agreed in the detection of most infections linked to histopathologic diagnoses of precancer, with cobas picking up a few additional

HPV 16 infections with CIN3+. Onclarity could provide additional typing information without excessively compromising the levels of detection achieved by cobas.

Given the good agreement in detection between tests, other factors will become more meaningful in the choice of using one test or the other. A key determinant might be the need for typing information. Cobas provides specific information for HPV16, HPV18, and groups all other results in a pooled channel. Onclarity further stratifies the other high-risk HPV types into 9 channels. The main advantage of this additional information is the possibility to break down the high risk other HPV group and keep the 7 HPV types with the lowest risk of progression separate from other types with higher risk. Typing information could then be used as triage for HPV positive women, reducing the cost of traditional colposcopy based triage. Other factors that need to be considered are the cost, availability, and ease of use of the two tests.

The management of HPV screen-positive women is the major unresolved issue in switching to HPV primary screening. Triage of HPV-positive women by cytology is an option but cytology has limitations in the assessment of risk stratification. Among HPV-positive women, cytology is a good risk stratifier when results are severely abnormal (HSIL+) but a much weaker risk stratifier for lesser cytology abnormalities (i.e., LSIL or ASC-US versus WNL). This sizable last group remains a management problem. One large part of the problem is how

long to follow without colposcopic referral those women who remain HPV positive, but do not present with treatable precancer.

To inform the question of how long to follow HPV infections requires knowledge of typical time to clearance, and its determinants. Manuscript 2 shows that patterns of cumulative risk of clearance/ progression/ persistence differ by HPV type. Our results support the conclusion that selected typing information could be an important risk stratifier for clinical guidelines to establish timing between screening intervals and the limit to reasonably safe follow-up of persistently HPV positive women. The data also suggests that there is clinical utility to the typing data provided by the new Onclarity assay and that future assays might be designed to provide partial typing as well.

Manuscript 3 shows that, information on HPV (with partial typing) and cytology results are the most clinically essential risk stratifiers in cervical cancer screening, at least among the factors assessed by this study at KPNC. Etiologic co-factors such as smoking status, hormonal contraceptive use, BMI, SES, and race/ethnicity play a much smaller role in progression from HPV infection to precancer (defined as CIN3+).

Discrepancies with previous studies could be explained by several reasons. First, we are not interested in the statistical significance of cofactors but in whether the difference is large enough to change clinical action. Since there are only 3 possible clinical actions (normal screening, shorter interval re-testing, and

referral to colposcopy), factors that may still change the risk of progression may not change the risk enough to cross clinical action thresholds. Second, in addition to HPV positivity as considered in the literature, we studied the effect of cofactors given HPV type and cytology results. Given that cytology changed the risk of progression by up to 27 times and HPV type changed the risk of progression by up to 9 times when compared with normal results, the additional risk stratification of cofactors that change the risk of progression by 50% or less is smaller. Furthermore, some cofactors in previous studies could be acting as proxies for access to health care services, which is not a concern in this population of well screened women.

The obvious strength of this series of studies is the unique size of the study population of HPV/cytology tested women with excellent follow-up linkages. For example, the analyses in Manuscripts 2 and 3 are based on populations ten times larger than previous similar work. On the other hand, the studies are complicated from the interval censoring and irregular follow-up times typical of real-life clinical practice. This required the use of sophisticated modeling methods achievable fortunately through collaboration with expert statisticians.

The studies have several limitations:

1. There is no gold standard to compare HPV DNA assays. We compared Onclarity to previously FDA approved HPV DNA assays but cannot assume superiority of either test. Clinically, this is a challenge because we do not

know if the infections missed by one test are real or false positives by the other test.

2. Given that HPV samples are collected from cervical smears, problems in the acquisition of samples could lead to diagnostic accuracy. Since the study population for all manuscripts is comprised of HPV positive women, and the same sample is used for all HPV tests, this limitation would not affect the diagnosis at baseline but could impact the determination of clearance or persistence at follow-up visits in manuscript 2.
3. A given woman could contribute more than one infection. The main limitation related to multiple infections is in manuscript 2, where progression could be assigned to multiple infections co-existing in one woman, giving the false impression of progression for an infection that is not causing the precancer.
4. In our study of cofactors, we were restricted to variables available in KPNC's enrollment questionnaire, limiting the availability of variables (leaving out key factors such as HPV vaccination status, past medical history, HIV status, parity) and restricting details for the variables studied.

This work will serve to support the transition to HPV-based cervical screening. Manuscript 3, in particular, is a prelude to a multi-year risk estimation effort that will underlie the next round of US cervical cancer screening and management guidelines [90]. As part of this research agenda, future studies

should explore topics related to: changes in viral load for different HPV tests (manuscript 1), time to outcome of HPV progression for all HPV types and channels available in the market, with statistical methods to compare quantitatively the differences in the competing risks for different HPV types (manuscript 2), effect in risk of progression by additional cofactors not available in KNPC and statistical methods to quantify the clinical risk stratification of cofactors, and statistical methods that take into account absolute risk and disease prevalence (manuscript 3).

Results from this dissertation can inform or complement recommendations pending from the American Cancer Society (ACS), the American Society for Colposcopy and Cervical Pathology (ASCCP), and the U.S. Preventive Services Task Force (USPSTF) for potential changes in cervical cancer screening guidelines. Further dissemination of these findings can be through application of ASCCP guidelines in clinical settings, through clinicians informing patients on the risk factors for cervical cancer progression, and by publication of findings in peer-reviewed journals. In particular, this work adds to the literature on performance of new HPV assays with detailed genotyping information useful for inexpensive triage of HPV infections, intervals for screening and follow-up of HPV-infected patients, and highlights that HPV test and cytology results are the essential data needed to achieve the best possible clinical management of HPV-infected women.

Appendices

Appendix I. Timeline

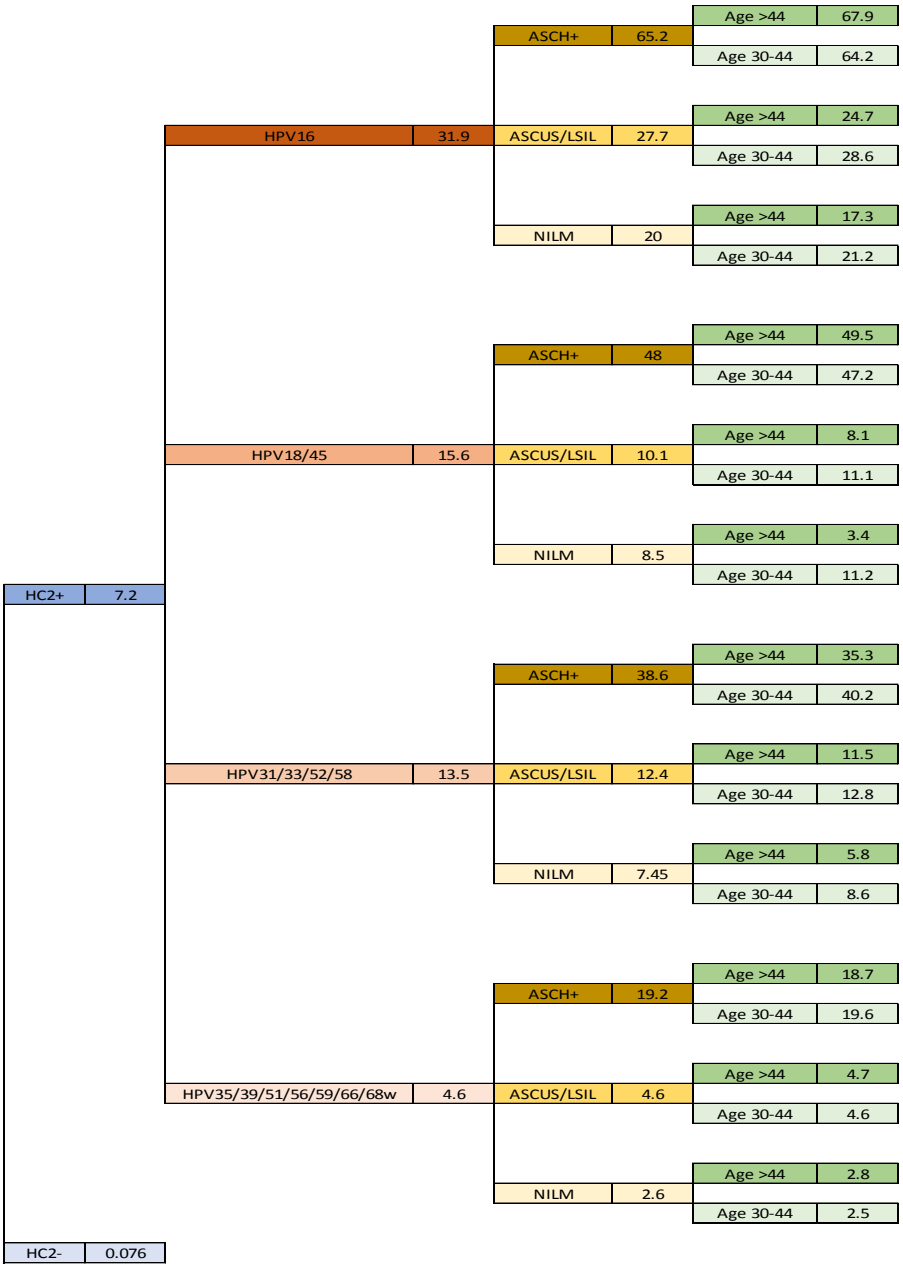
03/12/16	Pre-proposal sent to committee members
03/26/16	Expected feedback from committee members
08/11/16	Proposal sent to committee members
08/31/16	Proposal defense @ 11:00am in 2234CC
09/15/16	UMD IRB submission
10/01/16	Analysis starts
02/17/17	Deadline to submit dissertation committee form
03/16/17	Dissertation sent to committee members
03/30/17	Dissertation defense @ 2:00pm in 2234CC
04/18/17	Deadline to submit dissertation

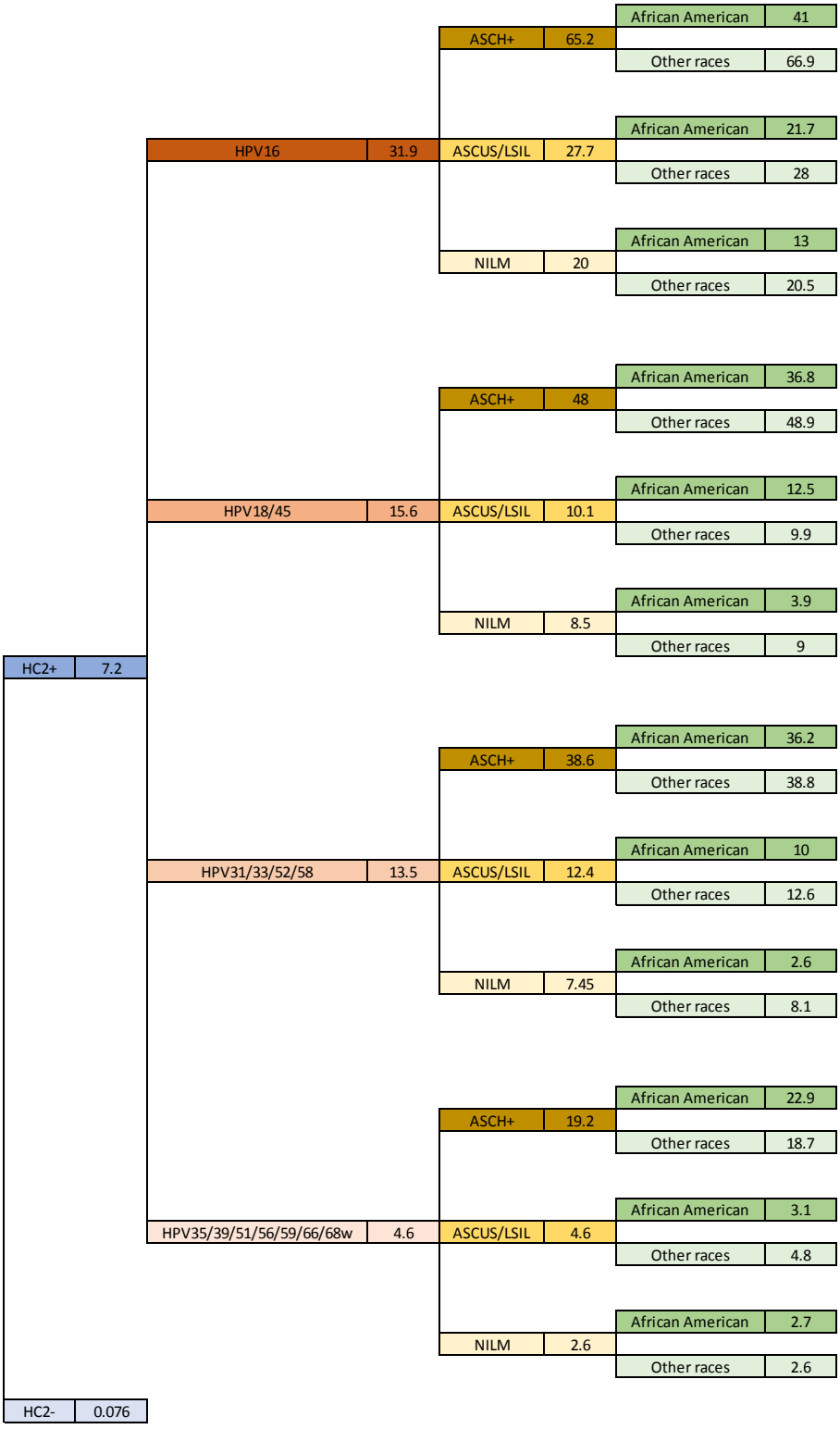
Appendix II. Original list of variables in the KPNC Progression and Persistence cohort

Variable	Description	Original categories
HC2_RES	Hybrid Capture II result	Missing/No test Negative Positive
CYTO_DX	Pap diagnosis	Missing NILM ASC-US/LSIL ASC-H+
RACE_ETH	Kaiser coded Race/ethnicity variable Hispanic else race1-4	White African-American/ Black Latino/ Hispanic Asian (Filipino, Chinese, Southeast Asian, Japanese, Korean, South Asian, Other Asian) Hawaiian/Pacific Islander Nat Amer/ Alaska native Middle Eastern Other
INCOME	>=80% in Census Tract are >200% of Poverty Level >=20% Households in Census Tract are Below Poverty Level	$15,000 or less $15,001 - $25,000 $25,001 - $35,000 $35,001 - $50,000 $50,001 - $65,000 $65,001 - $80,000 $80,001-$100,000 $100,001-$150,000 Over $150,000
SMOKING_STATUS	Closest to Enroll date (10 yrs prior-6 months post)	Never smoked regularly Current smoker Former smoker Unknown/Missing
BMI_CATEGORY	BMI Category closest to Enrollment date	Missing Underweight Normal Overweight Obese Very obese
TOT_ OCP_PACKS	Total #OCP packs dispensed in 10 yrs prior to and including Enrollment date	1-181

WRST_HIST	Worst histology during study period	None WNL atypia glandular atypia CIN1/LSIL CIN2/HSIL CIN3 CIN3/AIS AIS cancer, other cancer, UK hist adeno SCC adeno sq

Appendix III. Stratified absolute risk of CIN3+ by HPV type, cytology, and cofactors.




HC2+
7.2
HC2-
0.076
HPV16
31.9
HPV18/45
15.6
HPV31/33/52/58
13.5
HPV35/39/51/56/59/66/68w
4.6
ASCH+
65.2
ASCUS/LSIL
27.7
NILM
20
ASCH+
48
ASCUS/LSIL
10.1
NILM
8.5
ASCH+
38.6
ASCUS/LSIL
12.4
NILM
7.45
ASCH+
19.2
ASCUS/LSIL
4.6
NILM
2.6
African American
41
Other races
66.9
African American
21.7
Other races
28
African American
13
Other races
20.5
African American
36.8
Other races
48.9
African American
12.5
Other races
9.9
African American
3.9
Other races
9
African American
36.2
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38.8
African American
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Other races
12.6
African American
2.6
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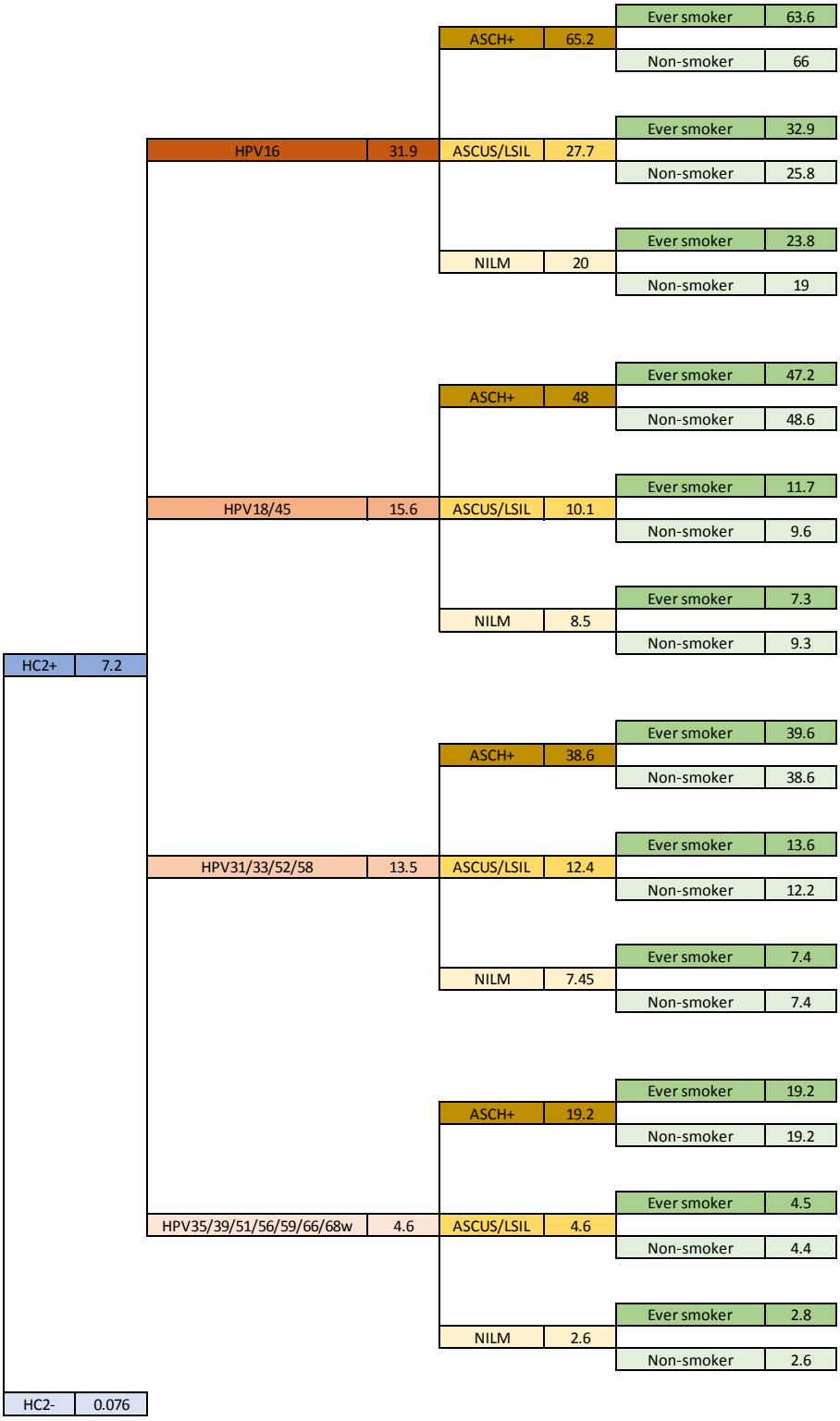
2.7
Other races
2.6


HC2+ 7.2
HC2- 0.076
HPV16 31.9
HPV18/45 15.6
HPV31/33/52/58 13.5
HPV35/39/51/56/59/66/68w 4.6
ASCH+ 65.2
ASCUS/LSIL 27.7
NILM 20
ASCH+ 48
ASCUS/LSIL 10.1
NILM 8.5
ASCH+ 38.6
ASCUS/LSIL 12.4
NILM 7.45
ASCH+ 19.2
ASCUS/LSIL 4.6
NILM 2.6
Ever smoker 63.6
Non-smoker 66
Ever smoker 32.9
Non-smoker 25.8
Ever smoker 23.8
Non-smoker 19
Ever smoker 47.2
Non-smoker 48.6
Ever smoker 11.7
Non-smoker 9.6
Ever smoker 7.3
Non-smoker 9.3
Ever smoker 39.6
Non-smoker 38.6
Ever smoker 13.6
Non-smoker 12.2
Ever smoker 7.4
Non-smoker 7.4
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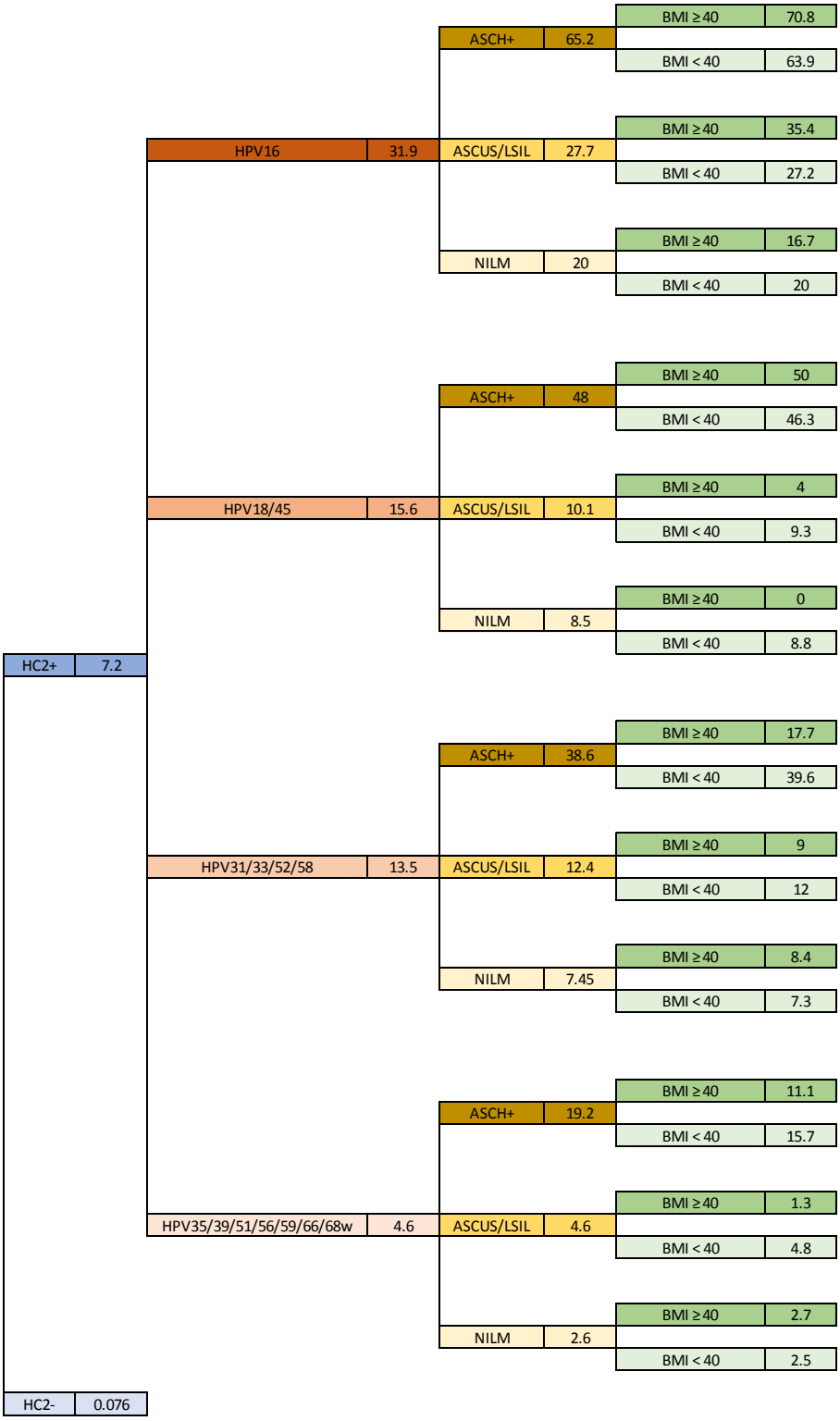
Ever smoker 2.8
Non-smoker 2.6


HC2+ 7.2
HC2- 0.076
HPV16 31.9
HPV18/45 15.6
HPV31/33/52/58 13.5
HPV35/39/51/56/59/66/68w 4.6
ASCH+ 65.2
ASCUS/LSIL 27.7
NILM 20
ASCH+ 48
ASCUS/LSIL 10.1
NILM 8.5
ASCH+ 38.6
ASCUS/LSIL 12.4
NILM 7.45
ASCH+ 19.2
ASCUS/LSIL 4.6
NILM 2.6
BMI ≥ 40 70.8
BMI < 40 63.9
BMI ≥ 40 35.4
BMI < 40 27.2
BMI ≥ 40 16.7
BMI < 40 20
BMI ≥ 40 50
BMI < 40 46.3
BMI ≥ 40 4
BMI < 40 9.3
BMI ≥ 40 0
BMI < 40 8.8
BMI ≥ 40 17.7
BMI < 40 39.6
BMI ≥ 40 9
BMI < 40 12
BMI ≥ 40 8.4
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BMI ≥ 40 11.1
BMI < 40 15.7
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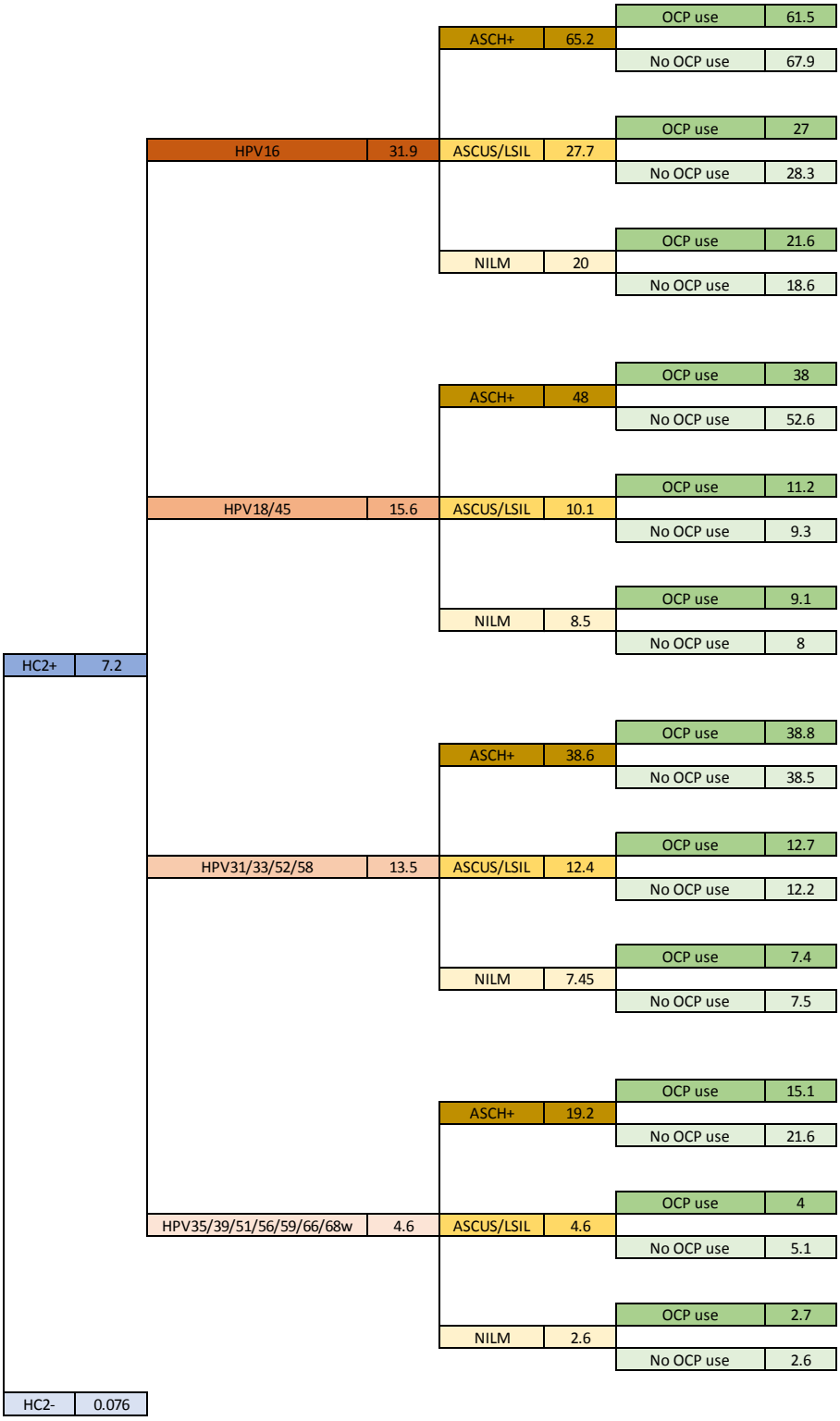
BMI < 40 4.8
BMI ≥ 40 2.7
BMI < 40 2.5


HC2+ 7.2
HC2- 0.076
HPV16 31.9
HPV18/45 15.6
HPV31/33/52/58 13.5
HPV35/39/51/56/59/66/68w 4.6
ASCH+ 65.2
ASCUS/LSIL 27.7
NILM 20
ASCH+ 48
ASCUS/LSIL 10.1
NILM 8.5
ASCH+ 38.6
ASCUS/LSIL 12.4
NILM 7.45
ASCH+ 19.2
ASCUS/LSIL 4.6
NILM 2.6
OCP use 61.5
No OCP use 67.9
OCP use 27
No OCP use 28.3
OCP use 21.6
No OCP use 18.6
OCP use 38
No OCP use 52.6
OCP use 11.2
No OCP use 9.3
OCP use 9.1
No OCP use 8
OCP use 38.8
No OCP use 38.5
OCP use 12.7
No OCP use 12.2
OCP use 7.4
No OCP use 7.5
OCP use 15.1
No OCP use 21.6
OCP use 4
No OCP use 5.1
OCP use 2.7
No OCP use 2.6

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