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Hillary Hunt Date

The Association Between Papanicolaou Test Results and Immunohistochemistry Test Utilization in the Diagnosis of Cervical Precancers

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The Association Between Papanicolaou Test Results and Immunohistochemistry Test Utilization in the Diagnosis of Cervical Precancers

By

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Bachelor of Arts

Seattle University

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Abstract

The Association Between Papanicolaou Test Results and Immunohistochemistry Test Utilization in the Diagnosis of Cervical Precancers

By Hillary Hunt

Background: In the United States, cervical cancer screening recommendations today consist of cytology-based screening every three years in women ages 21-65 or may be every five years in women 30 and older with high-risk HPV testing alone or in combination with cytology. Women with abnormal cytology are triaged to further testing to determine management of potentially precancerous cervical lesions. Immunohistochemistry (IHC) is becoming increasingly common in diagnosing precancerous cervical lesions. The results of IHC testing influence the clinical management of lesions, but there has been little research into its practical implementation, particularly clinical or demographic factors associated with its usage.
Methods: Cross-sectional data from the Human Papillomavirus (HPV) Vaccine Impact Monitoring Project (HPV-IMPACT) was used to evaluate the association between Papanicolaou (Pap) testing results and the usage of IHC testing among women ages 18-39 in five Emerging Infections Program (EIP) sites in the United States diagnosed with cervical precancers. Descriptive statistics were generated, and a logistic regression analysis was used to estimate the association between Pap results and IHC testing, adjusting for presence of high-risk HPV, site, and final diagnosis.
Results: A total of 4,675 cases of CIN2+ were reported to HPV-IMPACT during 2015-2017, among which, approximately 29% had IHC testing (n=1,343). Compared to cases with Pap results of atypical squamous cells of unknown significance (ASCUS), cases with a normal Pap result had higher odds of IHC testing (OR 1.42, 95% CI 1.07, 1.88, p=0.01) and cases with atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesions (ASC-H)/high-grade squamous intraepithelial lesions (HSIL) had lower odds of IHC testing (OR 0.54, 95% CI 0.44, 0.65, p-value <0.01). Cases with a Pap result of low-grade squamous intraepithelial lesions (LSIL) or atypical glandular cells of undetermined significance (AGUS)/adenocarcinoma in situ (AIS) were less likely to have IHC testing compared to ASCUS, but these results were not significant.
Discussion: This study supports the possible association between cytology-based cervical cancer screening results and the use of IHC testing as it is used to diagnose the grade of precancerous cervical lesions among women ages 18-39 years participating in HPV-IMPACT.

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Background/Literature Review

Human Papillomavirus

Human papillomavirus (HPV) infection is the most common sexually transmitted infection in the United States (1). These infections are highly prevalent in young women under 25 years old (2). HPV infections are largely asymptomatic, but if the infection is not cleared by the host and is persistent, the infection can lead to several health consequences (3). Consequences include several types of cancers, genital warts, or recurrent respiratory papillomatosis (RRP) (4-6) . HPV-associated cancers include oropharyngeal or anogenital cancers, with about 91% of cervical cancers worldwide attributable to HPV infection (7). There are over 200 HPV types. Not all types are oncogenic and worldwide, about 70% of cervical cancers are attributable to just two high-risk HPV types, 16 and 18, worldwide (2, 8, 9). Annually, approximately 5% of all cancers are HPV-associated worldwide, with about 80% of these occurring in developing countries (2).

Cervical cancer screening guidelines

Precancerous lesions are asymptomatic, making screening the only method for their detection. Both cervical cancer incidence and mortality rates have decreased over the second half of the 20th century, largely due to the introduction and common practice of the Papanicolaou (Pap) test, an exfoliative cytology-based screening test, and subsequent effective treatment of the precancerous lesions identified through screening (10). Clinical HPV testing, which can detect the presence of high-risk HPV through various technical approaches, has also come into use in cervical cancer screening (11). HPV testing is considered to be more sensitive than cytology-based screening alone, but it is not

recommended for use in all women based on age (12). Although most women become infected with HPV by their mid-20's, cervical cancer requires persistent HPV infection, so the time between initial HPV infection and the progression to cervical cancer is most commonly a decades-long process, resulting in a median age for cervical cancer diagnosis of 49 years in the United States (13). HPV is a necessary causal factor in developing cervical cancer, which makes HPV testing useful for cervical precancer and cancer screening. (2, 14, 15). There is support for the use of HPV testing alone as more effective compared to cytological screening [18], yet other studies emphasize the possibility for overtreatment in using HPV testing alone, especially in young women (16).

Cervical cancer screening guidelines in the United States are set by several organizations, which concur that the recommended frequency of screening for precancerous lesions and HPV co-testing depends on age (10, 17-19). The American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP) produced their own recommendations, which are concordant with USPSTF (10). The United States Preventive Services Task Force (USPSTF) recommends Pap testing in women ages 21-29 years every three years (20). For women ages 30-65, women may receive Pap testing alone every three years, HPV testing alone every five years, or both Pap and HPV testing (co-testing) every five years (20). All organizations agree that HPV testing is not an appropriate screening test for women under 30 years. The high prevalence of HPV infection would prompt an increase in the number of colposcopies, with a relatively small reduction in cancer risk (10). Since the recommendations by ACS, ASCCP, and ASCP

were introduced, more studies have evaluated the effectiveness of HPV testing, supporting its use either in combination with cytology-based screening (Pap testing) or alone (17).

HPV vaccination

In 2006, the quadrivalent-HPV vaccine was introduced and recommended for adolescent girls aged 11-12 but was made available for those between ages 9-26 (21). Today, a nine-valent vaccine is recommended for both girls and boys at age 11-12 years, requiring only two doses of vaccine if the first dose is received prior to turning 15 years old (22). Those who did not receive the vaccine as adolescents are still recommended to receive the vaccine until age 26 for women and age 21 for men, or until age 26 for men who have sex with men, transgender people, and those with immunocompromising conditions, such as HIV (23). While the introduction of this vaccine is still relatively recent and its full effect on preventing cervical cancer cannot be fully examined based on the extended period of time between HPV infection and cervical cancer development, the incidence of cervical precancers can be used to evaluate vaccine impact (24-27). Significant declines in incidence of cervical intraepithelial neoplasia (CIN) grades 2 or 3 and adenocarcinoma in situ (AIS) (collectively, CIN2+), lesions have been observed and described in young women These declines are attributable to vaccine impact (27).

Traditional Diagnostic Practice

If considered necessary based on initial screening results, a colposcopy is performed to visualize the cervix under magnification. During a colposcopy, an acetic acid or Monsel's solution is applied to the cervix to differentiate areas of abnormal cells. The provider examines the cervix using a colposcope, allowing for a magnified view of the

cervix. Based on the appearance of the abnormal cells, the provider may remove one or multiple samples through punch biopsy. Endocervical sampling with a brush or curette may also be performed. This method is used to increase the probability of the sample taken containing the tissue that best characterizes the true pathology of the cervix, however, the biopsy or excision is only a sample and there is a possibility that an area of dysplasia will not be sampled (28). A pathologist examines tissue samples under microscope, using hematoxylin and eosin (H&E) staining to distinguish areas of cell abnormalities as well as the degree to which the cells are abnormal, and the type and grade of abnormality: cervical intraepithelial neoplasia grade 1, 2, or 3 (CIN1, CIN2, or CIN3), adenocarcinoma *in situ* (AIS), or invasive carcinoma. These classifications are important as they determine the subsequent clinical management of the cervical lesion. Based on the result of the biopsy, women may require further excision or other treatment. Histological examination is the standard diagnostic tool, with a reported sensitivity of approximately 68.9% and a specificity of approximately 97.2% [29]. Specificity is a priority in order to avoid overtreating cervical lesions that would be likely to be transient and clear on their own with time (2, 15). Women diagnosed with CIN1 will likely not receive further treatment at the time and may be triaged to have a repeat Pap smear after one year, as these lesions are likely to regress without intervention. CIN2 or CIN3 may be excised (10, 18, 19). CIN2 lesions also regress often, but the diagnosis triggers treatment, leaving potential for overtreatment. Furthermore, CIN2 is poorly reproducible when evaluating inter-observer agreement on diagnosis. This ambiguity calls into question its clinical significance as a trigger for treatment (29).

Immunohistochemistry in diagnosis of cervical precancers

Immunohistochemistry (IHC) staining has become a helpful tool in diagnosing HPV-associated precancerous lesions relatively recently. IHC utilizes antibodies linked to an enzyme or fluorescent dye that will bind to a certain antigen in the tissue sample and will become visible under a microscope (30). The utility of several biomarkers has been assessed to determine which is the most reliable and accurate for diagnosing precancerous cervical lesions. The most promising of these biomarkers include p16, Ki-67, ProEx C, or L1 (31). Today, p16 is the antigen most commonly tested for based on recommendations from the Lower Anogenital Squamous Terminology Standardization Project for HPV-Associated Lesions (LAST), a set of recommendations created in partnership between the College of American Pathologists (CAP) and the American Society for Colposcopy and Cervical Pathology (ASCCP) (28). A surrogate marker of the presence of high-risk HPV, p16 is a tumor suppressor protein and is overexpressed in precancerous CIN2, CIN3, AIS, and invasive cancer (30-33). The sensitivity, specificity, and predictive value of IHC staining, and p16, in particular, has been the subject of various studies. For CIN2+ lesions, the reported sensitivity of p16 is 86.7% and specificity is 82.7% (34). Ki-67, another biomarker sometimes used instead of or in conjunction with p16 has a reported sensitivity of 41.7% and specificity of 98.2% for CIN2+ lesions (34) While the sensitivity and specificity for Ki-67 are not considerably different from those values for histologic diagnosis, it may still be a useful tool in situations where there is disagreement among pathologists or the histological analysis is indeterminate between low and high-grade lesions (31, 33). Similar to the changing usage

of HPV typing in screening, p16 and Ki-67 are also being evaluated for their possible utility in screening rather than diagnosis [36, 37]. Research into this area suggests that using these biomarkers prior to biopsy may provide an objective measure in determining risk for the presence or development of high-risk lesions, avoiding unnecessary biopsies or excisions [36].

Terminology

The terminology for cervical precancers has changed over time. A common system of terminology is the grade of cervical intraepithelial neoplasia (CIN), which can be divided into CIN1, CIN2, or CIN3 as well as AIS (28). CIN can be suspected from cytologic testing but is confirmed by histological examination. This is the terminology used by HPV-IMPACT. Prior terminology has been based on the level of dysplasia (mild, moderate, or severe) or grade of squamous intraepithelial lesion (low-grade or high-grade SIL) (35, 36). LAST uses histology-based SIL terminology.

LAST Guidelines

IHC staining can be a useful diagnostic tool in some cases, but it is not always necessary to use in addition to histological examination. LAST is a proposed change in terminology to create a uniform system as well as guidelines to implement the responsible usage of IHC staining for the sake of enhancing reproducibility and consistency (28). HSIL are the focus of potential intervention, as the goal of screening and treatment procedures is to identify precancerous lesions that may become invasive cancer in the future and not all precancerous lesions will develop into cancer (2, 3, 11, 37).

LAST guidelines emphasize enhancing diagnostic reproducibility through usage of biomarkers, especially p16, in certain situations. LAST outlines three situations in which p16 staining should be used for diagnosing cervical lesions: (1) H&E staining is inadequate for distinguishing between HSIL and conditions that may mimic precancer such as inflammatory lesions or atrophy; (2) The pathologist considers the tissue sample to represent CIN2 under the old terminology; (3) The pathologist is unable to distinguish the tissue sample as LSIL or HSIL, or if there is professional disagreement across these categories; or (4) The patient has a previously diagnosed as histologically HSIL as these women are at higher risk for high-grade disease (38).

Regarding the first recommendation, using IHC in situations where morphological examination is inadequate, it is necessary to have a tool to rule out conditions that may mimic the appearance of cervical precancers but hold no risk for developing into cancer. The next recommendation, using IHC for cases considered to be CIN2, is also important to clinical management of cervical lesions. A diagnosis of CIN2 is between low-grade lesions that are likely to regress without intervention, CIN1, and lesions that have a higher probability of progressing into cancer in the future, CIN3. By using IHC staining on samples designated as CIN2 based on morphology alone, the pathologist can better distinguish the sample as LSIL or HSIL, both determining the need for and influencing the type of intervention (34, 39). The third recommendation, use of IHC when a pathologist is uncertain or if there is disagreement between pathologists regarding the lesion being classified as either LSIL or HSIL, is similar to the second as IHC staining provides an objective measure when the tissue does not clearly represent LSIL or HSIL. Finally, the fourth recommendation, to use IHC when an individual has had a past HSIL

diagnosis, reinforces the suggestion that IHC staining is unnecessary in most cases where the diagnosis is clearly CIN1 or CIN3, except when there is *a priori* knowledge that the patient is at a higher risk, usually based on a prior diagnosis of HSIL or AIS.(38, 40). LAST addresses the importance of caution when there is uncertainty between LSIL and HSIL. As many lesions diagnosed as LSIL will clear on their own, overdiagnosing LSIL as HSIL will likely result in overtreatment (41). Tested tissue samples are considered positive with strong and diffuse block positive p16 staining (28). About half of LSIL samples will result in strong block staining, as they indicate high-risk HPV infection. This high proportion of low-grade lesions that are positive for p16 highlight the importance of careful histological examination to avoid overdiagnosis, as LSIL will likely regress without intervention (32, 34, 42). LAST recommendations emphasize that "each cytologic or histologic sample is only a statistical representation of the patient's true biology" (28, 41), which addresses the potential shortcomings of biopsy in diagnosis because there is a probability that the sample taken does not represent the true biology of the patient.

The guidelines put forth by LAST have support from many pathologists (39, 43-45), but debate is not absent from this discourse (46-48). Those in support cite evidence in favor of diagnostic accuracy and reproducibility (49), supporting the basis that the recommendations are appropriate based on the biology of HPV and clinical management of cervical lesions. Despite being considered an objective method for designating CIN2 lesions as LSIL or HSIL, there is still possibility for misclassification. Recommendation two (2) is the most controversial as studies have shown there is potential for an increased number of false negatives in samples with high-grade morphology consistent with CIN2

along with p16 negativity (47). Studies demonstrating concern over aspects of these recommendations do not altogether discount the importance of their generation and implementation, but rather, provide support for cautious and judicious utilization (47, 48).

Rationale for Research

The LAST guidelines were first published in 2012 and several studies have examined their effect on the subsequent utilization of IHC, particularly p16 staining, in the diagnosis of cervical precancers in clinical studies. There is sparse literature, however, regarding how IHC staining is being used throughout the United States. It is important to understand factors associated with IHC usage in order to evaluate its efficacy and appropriateness. The primary goal of this research is to analyze the clinical and demographic characteristics associated with utilization of IHC staining in diagnosing cervical precancers among women at five Emerging Infections Program (EIP) sites across the United States. This study will focus on the association between the results of Pap testing, the initial screening that prompts a need for further assessment of lesions, and IHC testing in cervical precancers. This aim will assist in addressing the missing area of literature describing when IHC staining is being used to diagnose precancerous cervical lesions. We hypothesize there will be variation in the frequency of IHC utilization by result of Pap testing. Further, we hypothesize there will be greater IHC utilization for low-grade lesions, including ASCUS or LSIL, compared to high-grade lesions, including ASC-H/HSIL or AGUS/AIS, as there is greater ambiguity in final histological diagnosis of these low-grade lesions which may encourage the reviewing pathologist to seek testing beyond histological examination.

Methods

Data

The data for this analysis came from the HPV Vaccine Impact Monitoring Project (HPV-IMPACT), a collaboration among CDC, state health departments, and academic partners participating in the Emerging Infections Program (EIP). HPV-IMPACT is a continuing, population-based surveillance project designed to describe trends in cervical precancers following the implementation of the HPV vaccine, ultimately describing the impact of the HPV vaccine. HPV-IMPACT collects data on cervical precancers, including lesions classified as CIN2, CIN3, AIS, or any combination of these, collectively CIN2+. There is no mandatory reporting of cervical precancers nationally and each site implemented its own reporting system in which local laboratories in each catchment area report to HPV-IMPACT staff for their site [21].

Five sites from the Emerging Infections Program (EIP) contributed to this data. Sites include eight contiguous cities in Alameda County, California; New Haven County, Connecticut; Monroe County, New York; Davidson County, Tennessee; and a 28-zip code area within Multnomah and Washington Counties, in the Portland-metropolitan area of Oregon. Each catchment area includes about 300,000 women aged 18 and older. Altogether, these sites cover 1.5 million women in the United States [19].

For each case of CIN2+, a case report form was completed by the site containing basic clinical and demographic information. For women ages 18-39 years, an enhanced case report form was completed using laboratory and medical records to gather information related to vaccination and further clinical and demographic data. For these women, a

tissue specimen was also sent from the local lab to CDC. CDC pathologists used the specimen to confirm the diagnosis and perform HPV DNA typing.

The case definition for HPV-IMPACT is a histologically-confirmed diagnosis of CIN2+ in women aged 18 years or older diagnosed on January 1, 2008 or later. As there are may be several procedures that lead to a final diagnosis (i.e. Pap test, colposcopy, biopsy, loop electrosurgical excision procedure, or hysterectomy), a case may have more than one event; however, each case only has one case-defining event which is defined as the event with the earliest, highest-grade diagnosis within a six-month period. The diagnosis date of the earliest CIN2+ event was defined as the incidence date. Another CIN2+ diagnosis more than six months after this event-period would be considered a separate event and would not be included in incidence calculations.

Immunohistochemistry testing

The outcome, IHC usage, was classified dichotomously as either "yes" or "no". Cases with any type of IHC testing were classified as "yes" and included tests for the biomarkers p16, Ki-67, BD Pro Ex C, or any other documented IHC test. IHC testing could be documented as "yes" without listing a specific IHC test. Cases with either documented absence of testing or no documentation of IHC testing were classified as "no".

Results of Papanicolaou (Pap) testing

The exposure variable was based on the result of the case's Pap test. Pap results could be classified as normal; atypical squamous cells of unknown significance (ASCUS/ASC); low-grade squamous intraepithelial lesion (LSIL); atypical squamous cells, cannot exclude HSIL (ASC-H) or high-grade squamous intraepithelial lesion (HSIL); or atypical

glandular cells of unknown significance (AGUS/AGC), or adenocarcinoma in situ (AIS). The classification of ASCUS was used as the referent because it was the lowest grade lesion with sufficient sample size.

Clinical covariates

HPV testing was classified dichotomously as “yes” or “no”. Cases with any type of documented HPV testing as screening were classified as “yes” and types of tests include Cervista, Aptima, HC2, cobas, or any other HPV test. HPV testing could be documented as “yes” without listing a specific type of test. Cases documented as not having testing or those with no documentation of testing where classified as “no”. The results of HPV testing were also collected and were classified as high-risk positive (HPV+), high-risk negative/unknown result, or not tested, using not tested as the referent. A variable for overall screening was created to combine the results of Pap and HPV testing. Cases with high-risk negative/unknown results and those that were not tested were combined into one level (HPV-) for the creation of this variable. This screening variable contained values of normal Pap, HPV+; normal Pap, HPV-; ASCUS/ASC, HPV+; ASCUS/ASC, HPV-; LSIL, HPV+; LSIL, HPV-; ASC-H/HSIL, HPV+; ASC-H/HSIL, HPV-; AGUS/AIS, HPV+; and AGUS/AIS, HPV-. ASCUS/ASC, HPV+ was used as the referent. HPV vaccination was classified as “yes”, “no”, or “unknown” based on documented receipt of the vaccine. A secondary variable for vaccine status based on vaccine eligibility was created to categorize cases who were ineligible for the vaccine based on their age when the vaccine was first introduced compared to those who were eligible. This variable for vaccination status categorized cases as ineligible if they were born before the year 1980 as they would already have been over the age where the

vaccine was recommended, 26 years, when the vaccine was introduced; unvaccinated, which were cases who were age-eligible but not vaccinated; vaccinated; or unknown. Variables relating to vaccination were included in the descriptive analysis but excluded from the model because of a substantial amount of cases with unknown vaccination status (n=2,765). Final diagnosis was categorized based on the grade of cervical intraepithelial neoplasia as CIN2, CIN2/3, CIN3, AIS, AIS + CIN2, AIS + CIN2/3, or AIS + CIN3. All classifications containing AIS were collapsed into a single category of AIS, resulting in final classifications of CIN2, CIN2/3, CIN3, and AIS. The lowest grade, CIN2, was used as the referent.

Demographic covariates

A continuous variable for age was calculated for each case by subtracting the date of birth from the date of the first event. The continuous variable for age was then used to create a categorical variable classified as 18-24, 25-29, 30-34, and 35-39 years, using ages 18-24 years as the referent. Race and ethnicity were combined into one categorical variable. If a case was classified as Hispanic, their race/ethnicity was Hispanic. Cases who were not classified as Hispanic were then categorized as non-Hispanic white, non-Hispanic black, non-Hispanic Asian, non-Hispanic American Indian/Alaska Native, or other, hereafter referred to as white, black, and Asian. Cases classified as American Indian/Alaska Native were combined into the category of other due to small numbers. White race was used as the referent. Insurance status was initially categorized as private, Medicaid, Medicare, Indian Health Service, Military or VA, self-pay, other, or none. Medicaid, Medicare, Indian Health Service, and Military or VA were combined to create a classification of public insurance. Self-pay was combined into the classification of

none. Final classifications included private, public, other, or no insurance. Other insurance was included in the descriptive analysis but combined with no insurance for the model and private insurance status was used as the referent. The site from which cases were reported was a categorical variable including California, Connecticut, New York, Oregon, and Tennessee, using Tennessee as the referent as it had the lowest proportion of IHC utilization among its reported cases. Year of diagnosis was included in the descriptive analysis and cases were stratified into years of 2015, 2016, and 2017 based on when CDC collected the first tissue specimen from the reporting site.

Statistical analysis

The selected characteristics were summarized in a descriptive analysis. Frequencies and proportions were calculated for all variables. Descriptive statistics were stratified by both the outcome: IHC testing versus no IHC testing; and the exposure: normal, abnormal, LSIL, and HSIL Pap results. Associations were evaluated using chi-square methods.

The aim of the model in this study was to obtain an estimate of the relationship between Pap testing results and the usage of IHC testing, adjusting for clinical or demographic factors that may confound the association. Demographic and clinical covariates were identified for potential inclusion in the model based on previous studies found in a literature review and the construction of directed acyclic graphs (DAGs). Clinical covariates to be considered were HPV testing and results, combined screening results, vaccination status, and final CIN2+ diagnosis. Demographic covariates to be considered for inclusion were age, race/ethnicity, insurance status, and site.

All demographic and clinical variables except vaccination status and year of diagnosis were included in an initial logistic regression model and variables for the final model were selected based on the results of backward selection. If a variable remained significant at alpha = 0.05, it was included in the model.

The model was assessed for multi-collinearity among covariates used a Condition Index cut-point (CI) of 30 and a Variance Decomposition Proportion (VDP) cut-off of 0.5. Discrimination of the model was assessed using receiver operating characteristic (ROC) curves by calculating their area under the curve (AUC). Following the multi-collinearity and confounding assessments, the final model included the covariates for high-risk HPV, final diagnosis, and reporting site. Model fit was assessed using the Hosmer-Lemeshow Goodness-of-fit test. All analyses were completed in SAS, version 9.4 (SAS Institute, Cary, NC).

Results

Sample

A total of 8,647 cases of CIN2+ have been reported to HPV-IMPACT since 2015 (Figure 2). Of these cases, 7,637 were reported during the years 2015-2017. There were 1,807 cases excluded for age greater than 39. There were 682 women with no documented Pap testing and 43 with missing or unknown results. Another 430 cases were excluded for missing data on IHC testing as legacy cases. The final sample size was 4,675 CIN2+ cases.

Descriptive statistics

Clinical and demographic characteristics are described in Tables 1 and 2. Over a quarter of cases (28.7%) had IHC testing. The most common Pap test result was ASC-H/HSIL at 34.7%. Pap results of ASCUS/ASC and LSIL had similar proportions with 28.7% and 28.9%, respectively, 6% had a normal result, and AIS was the least common result at 1.7%. Most cases, 74.7% had HPV testing as a part of screening. Among these cases who were screened using HPV testing, 72.1% were positive for high-risk HPV. Combining the results of Pap and HPV testing, 5.5% of cases had a normal Pap result and were HPV+, less than 1% had a normal Pap and were HPV-. The most common combination was a Pap result of ASCUS/ASC and HPV+ at 26.8% while the same Pap result and HPV- accounted for 1.9% of the sample. By other combined screening results, 17.2% were LSIL, HPV+; 11.7% were LSIL, HPV-; 21.4% were ASC-H/HSIL, HPV+; 13.2% were ASC-H/HSIL, HPV-; 1.2% were AGUS/AIS, HPV+; and less than 1% were AGUS/AIS, HPV-. Data for vaccination was unknown for over half the sample, 58.7%. Among those with known status, 18.6% were vaccinated, 11.5% were unvaccinated, and

11% were age-ineligible for vaccination. About half (50.6%) of cases had a final diagnosis of CIN2, 17.7% were diagnosed with CIN2/3, 29.7% with CIN3, and 2% with AIS.

Table 3 describes the frequency of IHC utilization within clinical covariates. Women with a normal Pap had the highest proportion of IHC utilization, 43.1%. Among women with a Pap result of ASCUS/ASC, 32.4% had IHC testing and a similar proportion (32.8%) of women with an LSIL Pap had IHC testing. IHC was used the least among women with a Pap result of ASC-H/HSIL (19.7%) and was used in 30.9% of women with a Pap result of AGUS/AIS. Among women who had an HPV screening test, 27.3% had IHC testing and 32.9% that did not have an HPV screening test had IHC testing. By high-risk HPV positive status, 27.2% of those who were positive for HPV had IHC testing and 30.3% of those who were not positive for high-risk HPV had IHC testing. Proportions of IHC usage in combined screening results were similar between women who were HPV+ or HPV- with normal Pap results (42.8% and 45.8%) as well as those with ASCUS/ASC (32.1% and 36.4%). Among women with a Pap result of LSIL, 27.3% of women who were HPV+ had IHC testing and 40.8% who were HPV- had IHC testing. In women with a Pap result of ASC-H/HSIL, 17% of women who were HPV+ had IHC testing and 24.2% of women who were HPV- had IHC testing. Among women who had a Pap result of AGUS/AIS, 28.6% who were HPV+ had IHC testing and 36% who were HPV- had IHC testing. By vaccination status, 31.9% of women who were vaccinated, 26.4% of unvaccinated women, 34.1% of vaccine-ineligible, and 27.2% of women with unknown vaccination status had IHC testing. By final diagnosis, 37.2% of women diagnosed with

CIN2, 28.4% of women diagnosed with CIN2/3, 12.9% of women diagnosed with CIN3, and 52.1% of women diagnosed with AIS had IHC testing.

Table 4 describes the frequency of IHC testing within demographic covariates. Among women ages 18-24 years, 29.5% had IHC testing, while 27.5% of women ages 25-29 years, 27.4% of women ages 30-34 years, and 33.1% of women 35-39 years had IHC testing. By race/ethnicity, 29.3% of white women, 29.1% of black women, 27.3% of Asian women, and 35.4% of women of all other races had IHC testing. Among women with private insurance, 28.8% of women had IHC testing while 26.64% of women with public insurance, 24.5% of women without insurance, and 26.6% of women with any other type of insurance had IHC testing. By site, 27.1% of women from California, 22.7% of women from Connecticut, 48.1% of women from New York, 40.4% of women from Oregon, and 17.9% of women from Tennessee had IHC testing. Over time, 27% of women had IHC testing in 2015, 31.4% in 2016, and 27.6% in 2017.

Table 5 contains clinical characteristics stratified by IHC testing status. Cases with IHC testing were more likely to have a normal (9%), ASCUS/ASC (32.3%), or LSIL (33%) Pap result and less likely to have a Pap result of ASC-H/HSIL (23.8%) compared to those without IHC testing with 4.8% normal, 27.2% ASCUS/ASC, 27.3% LSIL, and 39.1% ASC-H/HSIL (X^2=111.9, p<0.01). A smaller proportion of cases with IHC testing (71%) had HPV testing compared to 76.2% without IHC testing. Cases with IHC testing were less likely to be HPV+ (68.3%) compared to 73.6% without IHC testing (X^2=13.9, p<0.01). Cases with IHC testing had greater proportions of combined screening results of normal Pap, HPV+ (8.2%); ASCUS/ASC, HPV+ (29.9%) or HPV- (2.4%); or LSIL, HPV- (16.7%) and lower proportions of LSIL, HPV+ (16.4%); ASC-H/HSIL, HPV+

(12.7%) or HPV- (11.2%) compared to cases without IHC testing with 4.4% normal, HPV+; 25.5% ASCUS/ASC, HPV+ or 1.7% HPV-; or 9.7% LSIL, HPV- (X^2=152.2, p<0.001). By vaccination status, cases with IHC testing were more likely to be vaccinated (20.7%) and more likely to have documented status (55.7% unknown) compared to cases without IHC testing in which 17.8% were vaccinated and 60% had unknown vaccination status (X^2=16.4, p<0.01). The most common final diagnosis overall, CIN2, was more common among cases with IHC testing (65.5%) as was a diagnosis of AIS (3.7%) compared to cases without IHC testing with 44.5% CIN2. AIS was also more commonly diagnosed among cases with IHC testing, 3.7% compared with those without IHC testing, 1.4%. Additionally, cases with IHC testing were less likely to be diagnosed with CIN3 (13.3%) compared to 36.3% without IHC testing (X^2=278.8, p<0.01).

Table 6 contains demographic covariates stratified by IHC testing status. Cases with IHC testing were less likely to be 25-29 years (36.3%) or 30-34 years (31.2%), but more likely to be in the oldest age group, 35-39 years (21%), compared to 38.6% of cases 25-29 years, 33.3% of cases 30-34 years, or 17.1% of cases 35-39 among cases without IHC testing (X^2=10.6, p=0.01). Greater proportions of cases were reported from New York (21.7%) and Oregon (23.8%) and lower proportions were reported from California (18.7%), Connecticut (21.2%), and Tennessee (15.6%) among cases with IHC testing compared to 9.5% from New York 14.1% from Oregon, 20.3% from California, 29.1% from Connecticut, and 27.1% from California among cases without IHC testing (X^2=250.4, p<0.01). The proportion of cases reported in 2016 (38.2%) was greater among cases with IHC testing compared to those without testing (33.6%), but proportions were lower in 2015 (30.6%) and 2017 (31.2%) among cases with IHC testing compared to

33.4% in 2015 and 33% in 2017 among cases without testing (X^2=9.1, p=0.01). There were no significant differences in distributions of race/ethnicity or insurance status by IHC testing status.

Crude associations

Table 7 presents the unadjusted associations between IHC test utilization and both Pap test results and the covariates that were included in the final model. Compared to ASCUS/ASC Pap testing results, normal Pap results were associated with higher odds of having IHC testing (OR 1.58, 95% CI 1.21, 2.05), and ASC-H/HSIL was associated with lower odds of IHC testing (OR0.51, 95% CI 0.43, 0.61). Women with a Pap result of AGUS/AIS also had lower odds of receiving IHC testing compared to women with an ASCUS/ASC result, but this result was not statistically significant. A Pap result of LSIL was not associated with a significant difference in odds of IHC testing compared to ASCUS/ASC. Women with a positive high-risk HPV test had lower odds of receiving IHC testing compared to cases that were not tested for HPV (OR 0.76, 95% CI 0.66, 0.88), but the association between negativity for high-risk HPV and IHC testing was not statistically significant.

Compared to a final diagnosis of CIN2, a diagnosis of CIN2/3 had lower odds of IHC testing (OR 0.67, 95% CI 0.56, 0.79) as did a diagnosis of CIN3 (OR 0.25, 95% CI 0.21, 0.30). A diagnosis of AIS was associated with higher odds of IHC testing compared to CIN2 (1.84, 95% CI 1.22, 2.78). Utilization of IHC testing varied by site. Compared to cases from Tennessee, where IHC testing was used the least, all cases had higher odds of IHC testing. While odds were lowest in Tennessee, there were increasingly higher odds of IHC testing in Connecticut (OR 1.35, 95% CI 1.11, 1.66), California (OR 1.71, 95%

CI 1.39, 2.12), Oregon (OR2.12, 95% CI 2.53, 3.85), to the highest odds in New York (OR 4.27, 95% 3.42, 5.33).

Adjusted associations

In an adjusted, multivariable logistic regression analysis (Table 7), compared to Pap results of ASCUS/ASC, normal Pap results were associated with higher odds of IHC testing (1.42, 95% CI 1.07, 1.88). Pap results of ASC-H/HSIL were associated with IHC testing compared to ASCUS/ASC (OR 0.54, 95% 0.44, 0.65). Compared to ASCUS/ASC both LSIL and AGUS/AIS were associated with lower odds of IHC testing, but these results were not significant. Compared to cases that did not have HPV testing, being high-risk positive was associated with lower odds of IHC testing (OR 0.75, 95% CI 0.63, 0.88) as was being high-risk negative, but this association was not statistically significant. Compared to CIN2, a final diagnosis of either CIN2/3 or CIN3 was associated with lower odds of IHC testing (OR 0.27, 95% CI 0.23, 0.33) and a diagnosis of AIS was associated with higher odds of IHC testing (OR 2.28, 95% CI 1.45, 3.60). Cases reported from California had higher odds of IHC testing compared to Tennessee (1.60, 95% CI 1.28, 1.99). Compared to Tennessee, higher odds of IHC testing were associated with Oregon (OR 3.07, 95% CI 2.47, 3.84) and New York (OR 3.61, 95% CI 2.85, 4.57). The relation between IHC testing in Connecticut compared to Tennessee were not statistically significant.

Discussion

The results of the current study suggest an association between the results of Pap testing and the use of IHC testing in the diagnosis of cervical precancers among women ages 18-39 in five catchment areas across the United States. A crude model as well as a model adjusted for high-risk HPV positive screening , the catchment area and final diagnosis describe an association in which, compared to a result of ASCUS/ASC, Pap results that are ASC-H/HSIL or AGUS/AIS are less likely to precede the usage of IHC, whereas a normal result is more likely to precede the usage of IHC.

It is unclear why the odds of IHC testing were greatest for women with a normal Pap result. In relation to clinical management, a normal Pap result would not prompt any further diagnostics or treatment that would call for excision. Co-testing, by using both Pap and HPV testing, may help explain these results as the presence of high-risk HPV may contribute to the decision for colposcopy or other procedures. Among the 281 women with a normal Pap result, nearly half (n=121) had IHC testing performed. Most women with a normal Pap result (n=260) had HPV testing, among which 257 were high-risk positive.

A causal association cannot be made in this study, but these associations provide support for future research into how pathologists are implementing the usage of IHC tests in diagnosing cervical precancers. IHC may be useful in some situations but is not always necessary. Among cases with a clear morphologic interpretation of negative for intraepithelial lesion or malignancy (NILM), CIN1, or CIN3, IHC testing is not needed as their diagnosis and subsequent management is distinct and its usage may even result in overtreatment (45, 46).

There was an association between HPV-IMPACT site and IHC usage. Compared to Tennessee, cases from both New York or Oregon were several times more likely to have IHC testing. No causal association can be made from this data, but the differences by site could be attributable to access to resources, such as laboratory equipment, trained staff, or characteristics of a specific provider. Not all providers, including primary care providers or obstetrician/gynecologist physicians, follow cervical cancer screening guidelines and many screen women more or less often than recommended (50-52). Differences may also relate to the priorities of the cervical cancer screening program. Depending on the site, emphasis of a screening program may be on catching and treating any high-grade cervical lesions while others may have concerns about overtreating. IHC testing may be used to support either priority. By using IHC testing, the pathologist may increase their understanding of a woman's risk for a precancerous lesion to progress or regress, contributing to the knowledge needed to decide on the clinical management of the lesion.

Strengths and limitations

A strength of this study is the source from which the data is drawn. HPV-IMPACT is a population-based surveillance system designed to capture cervical precancers. Data on cervical precancers and the events preceding their diagnosis are not routinely collected nationally as cervical precancers are not a mandatory reportable condition, making the data collected through HPV-IMPACT unique and essential in evaluating the incidence of cervical precancers. It is important to have this systematic data collection to establish a baseline rate of cervical precancers as the increasingly widespread uptake of the HPV vaccine will affect future cervical cancer screening guidelines.

A limitation of this study is the amount of missing data. HPV-IMPACT sites abstract information from medical records, which may be missing or incomplete. HPV vaccination affects the proportion of women who will develop persistent high-risk HPV infections, impacting their risk of developing cervical precancers. This study was unable to include HPV vaccination in the regression analysis, however, due to a significant amount of cases with unknown vaccination status. Despite the common use of HPV testing in cervical cancer screening, the variable was not significantly associated with IHC testing. It would be informative to further examine the association between HPV screening test results and IHC testing in the future. Data from this study did allow for the description of associations between the Pap test results, clinical characteristics, and demographic characteristics to IHC testing, however, it is not suitable for establishing a causal relationship between Pap results and IHC testing.

Future directions

The association between HPV testing and IHC testing may become especially useful in the future as screening recommendations evolve alongside a changing prevalence of HPV types in the United States following widespread uptake of the HPV vaccine. Further research into this area would benefit from a prospective design that includes all women who received a biopsy, regardless of their eventual diagnosis, to facilitate drawing inferences regarding reasons for IHC test utilization. Additionally, future studies should include an analysis that is able to account for vaccination status in regression models. While there is no difference in recommendations for how vaccinated or unvaccinated women with cervical precancers are managed, the proportions of women with high-risk HPV may be differential based on vaccination status. In this study, there was a large

number of cases missing data for vaccination. This may be because there is no registry for HPV vaccination and vaccination records were incomplete for many women in this study.

While the current study examined the use of IHC in the histologic diagnosis of cervical precancers, a further study of interest may be in evaluating the use of IHC testing in the process of screening rather than diagnosis (53-55). Several studies reporting the strength of association between positive p16 or ki-67 staining and the progression of precancerous lesions support the need for more research in this area as it could prevent the need for biopsy or excision if it is used to triage patients to repeat Pap testing or biopsy during the diagnostic process (56).

Public health impact

As IHC testing gains popularity as a diagnostic tool for precancerous cervical lesions, it is necessary to understand the clinical and demographic characteristics associated with its usage. This is especially important as screening practices and guidelines evolve in the HPV vaccine era. Continued surveillance and more research into its practical implementation are needed to ensure its equitable use as well. The results of this study do not show statistically significant differences in IHC usage across racial/ethnic categories or across insurance status, however, it is imperative to ensure disparities do not arise here as they already exist in initial screening practices (57, 58) and this study demonstrates variation in the results of Pap testing across racial/ethnic and insurance status. The development of disparities in IHC usage could lead to differences in treatment as some minorities may be under or over-treated as a result of variation.

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Tables

Table 1. Select Clinical Characteristics of CIN2+ Cases, HPV-IMPACT, 2015-2017

Characteristic	All CIN2+ (n = 4,675) No.	%
IHC testing		
Yes	1,343	28.73
No	3,332	71.27
Pap results		
Normal	281	6.01
ASCUS/ASC	1,340	28.66
LSIL	1,352	28.92
ASC-H, HSIL	1,621	34.67
AGUS/AGC, AIS	81	1.73
HPV testing		
Yes	3,492	74.70
No	1,183	25.30
High-risk HPV type		
Positive	3,370	72.09
Negative	122	2.61
Not tested	1,183	25.30
Combined screening		
Normal, HPV+	257	5.50
Normal, HPV-	24	0.51
ASCUS/ASC, HPV+	1,252	26.78
ASCUS/ASC, HPV-	88	1.88
LSIL, HPV+	803	17.18
LSIL, HPV-	549	11.74
ASC-H/HSIL, HPV+	1,002	21.43
ASC-H/HSIL, HPV-	619	13.24
AGUS/AIS, HPV+	56	1.20
AGUS/AIS, HPV-	25	0.53
Vaccination status		
Vaccinated	871	18.63
Unvaccinated	539	11.53
Ineligible	519	11.10
Unknown	2,746	58.74
Final dx		
CIN2	2,364	50.57
CIN2/3	828	17.71
CIN3	1,389	29.71
AIS	94	2.01

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; No., number; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 2. Select Demographic Characteristics of CIN2+ Cases, HPV-IMPACT, 2015 - 2017		
	All CIN2+ (n = 4,711)	
Characteristic	**No.**	**%**
Age		
18-24	523	11.19
25-29	1773	37.93
30-34	1527	32.66
35-39	852	18.22
Race/ethnicity		
White	2,605	60.09
Hispanic	719	16.59
Black	675	15.57
Asian	271	6.25
Other	65	1.50
Missing	340	
Insurance status		
Private	2,657	65.00
Public	1,126	27.54
No insurance	98	2.40
Other	207	5.06
Missing	587	
Site		
California	926	19.81
Connecticut	1,254	26.82
New York	607	12.98
Oregon	790	16.90
Tennessee	1,098	23.49
Year of diagnosis		
2015	1,524	32.60
2016	1,632	34.91
2017	1,519	32.49

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; No., number; IHC, immunohistochemistry

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 3. Frequency of IHC Testing by Exposure Status and Clinical Characteristics, HPV-IMPACT, 2015-2017

Characteristic	IHC Staining (n=1,412)	Total N (n=4,765)	%
Pap results			
Normal	121	281	43.06
ASCUS/ASC	434	1,340	32.39
LSIL	443	1,352	32.77
ASC-H/HSIL	320	1,621	19.74
AGUS/AIS	25	81	30.86
HPV testing			
Yes	954	3,492	27.32
No	389	1,183	32.88
High-risk HPV type			
Positive	917	3,370	27.21
Negative	37	122	30.33
Not tested	389	1,183	32.88
Combined screening			
Normal, HPV+	110	257	42.80
Normal, HPV-	11	24	45.83
ASCUS/ASC, HPV+	402	1,252	32.11
ASCUS/ASC, HPV-	32	88	36.36
LSIL, HPV+	219	803	27.27
LSIL, HPV-	224	549	40.80
ASC-H/HSIL, HPV+	170	1,002	16.97
ASC-H/HSIL, HPV-	150	619	24.23
AGUS/AIS, HPV+	16	56	28.57
AGUS/AIS, HPV-	9	25	36.00
Vaccination status			
Vaccinated	278	871	31.92
Unvaccinated	142	539	26.35
Ineligible	177	519	34.10
Unknown	746	2,746	27.17
Final dx			
CIN2	880	2,364	37.23
CIN2/3	235	828	28.38
CIN3	179	1,389	12.89
AIS	49	94	52.13

Abbreviations: IHC, immunohistochemistry; HPV, human papillomavirus; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 4. Frequency of IHC Testing by Demographic Characteristics, HPV-IMPACT, 2015 - 2017

Characteristic	IHC Staining (n=1,412)	Total N (n=4,765)	%
Age			
18-24	154	523	29.45
25-29	488	1773	27.52
30-34	419	1527	27.44
35-39	282	852	33.10
Race/ethnicity			
White	763	2,605	29.29
Hispanic	209	719	29.07
Black	184	675	27.26
Asian	72	271	26.57
Other	23	65	35.38
Insurance status			
Private	765	2,657	28.79
Public	300	1,126	26.64
No insurance	24	98	24.49
Other	55	207	26.57
Site			
California	251	926	27.11
Connecticut	285	1,254	22.73
New York	292	607	48.11
Oregon	319	790	40.38
Tennessee	196	1,098	17.85
Year of diagnosis			
2015	411	1,524	26.97
2016	513	1,632	31.43
2017	419	1,519	27.58

Abbreviations: IHC, immunohistochemistry; HPV, human papillomavirus

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 5. Comparisons of Exposure Status and Clinical Characteristics by IHC Testing Status, HPV-IMPACT, 2015-2017

	IHC staining (n = 1,412)		No IHC staining (n= 3,408)		Significance	
Characteristic	**No.**	**%**	**No.**	**%**	**X^2(df)**	**p-value**
Pap results						
Normal	121	9.01	160	4.80	111.85(4)	<0.0001
ASCUS/ASC	434	32.32	906	27.19		
LSIL	443	32.99	909	27.28		
ASC-H/HSIL	320	23.82	1,301	39.05		
AGUS/AIS	25	1.86	56	1.68		
HPV testing						
Yes	954	71.03	2,538	76.17	13.36(1)	0.0003
No	389	28.97	794	23.83		
High-risk HPV type						
Positive	917	68.28	2,453	73.62	13.91(2)	0.0010
Negative	37	2.76	85	2.55		
Not tested	389	28.97	794	23.83		
Combined screening						
Normal, HPV+	110	8.19	147	4.41	152.16(9)	<0.0001
Normal, HPV-	11	0.82	13	0.39		
ASCUS/ASC, HPV+	402	29.93	850	25.51		
ASCUS/ASC, HPV-	32	2.38	56	1.68		
LSIL, HPV+	219	16.31	584	17.53		
LSIL, HPV-	224	16.68	325	9.75		
ASC-H/HSIL, HPV+	170	12.66	832	24.97		
ASC-H/HSIL, HPV-	150	11.17	469	14.08		
AGUS/AIS, HPV+	16	1.19	40	1.20		
AGUS/AIS, HPV-	9	0.67	16	0.48		
Vaccination status						
Vaccinated	278	20.67	593	17.80	16.42(3)	0.0009
Unvaccinated	142	10.57	397	11.91		
Ineligible	177	13.18	342	10.26		
Unknown	746	55.55	2,000	60.02		
Final dx						
CIN2	880	65.52	1484	44.54	278.78(3)	<0.0001
CIN2/3	235	17.50	593	17.80		
CIN3	179	13.33	1210	36.31		
AIS	49	3.65	45	1.35		

Abbreviations: CIN, cervical intraepithelial neoplasia; No., number; df, degrees of freedom; HPV, human papillomavirus; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 6. Comparisons of Demographic Characteristics by IHC Testing Status, HPV-IMPACT, 2015-2017

	IHC staining (n = 1,412)		No IHC staining (n= 3,408)		Significance	
Characteristic	**No.**	**%**	**No.**	**%**	**X^2(df)**	**p-value**
Age						
18-24	154	11.47	369	11.07	10.57(3)	0.0143
25-29	488	36.34	1,285	38.57		
30-34	419	31.2	1,108	33.25		
35-39	282	21.00	570	17.11		
Race/ethnicity						
White	763	61.0	1,842	59.7	3.13(4)	0.5358
Hispanic	209	16.7	510	16.5		
Black	184	14.7	491	15.9		
Asian	72	5.8	199	6.5		
Other	23	1.8	42	1.4		
Insurance status						
Private	765	66.9	1,892	64.3	2.66(3)	0.4463
Public	300	26.2	826	28.1		
No insurance	24	2.1	74	2.5		
Other	55	4.8	152	5.2		
Site						
California	251	18.7	675	20.3	250.39(4)	<0.0001
Connecticut	285	21.2	969	29.1		
New York	292	21.7	315	9.5		
Oregon	319	23.75	471	14.14		
Tennessee	196	14.59	902	27.07		
Year of diagnosis						
2015	411	30.6	1,113	33.4	9.11(2)	0.0105
2016	513	38.2	1,119	33.58		
2017	419	31.2	1,100	33.01		

Abbreviations: CIN, cervical intraepithelial neoplasia; No., number; df, degrees of freedom; HPV, human papillomavirus; IHC, immunohistochemistry

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Table 7. Crude and multivariable logistic regression analysis of the association between IHC testing and clinical and demographic characteristics of CIN2+ cases, HPV-IMPACT, 2015-2017

	Unadjusted				Multivariable - Adjusted			
	OR	**95% CI**		**p-value**	**OR**	**95% CI**		**p-value**
Pap results								
ASCUS/ASC	1.00				1.00			
Normal	1.58	1.21	2.05	0.0006	1.42	1.07	1.88	0.01
LSIL	1.02	0.86	1.20	0.83	0.85	0.71	1.02	0.08
ASC-H, HSIL	0.51	0.43	0.61	<0.0001	0.54	0.44	0.65	<0.0001
AGUS/AGC, AIS	0.93	0.57	1.51	0.78	0.60	0.34	1.03	0.07
High-risk HPV type								
Not tested	1.00				1.00			
Positive	0.76	0.66	0.88	0.0002	0.75	0.63	0.88	0.0009
Negative	0.89	0.59	1.33	0.57	0.83	0.54	1.23	0.39
Final diagnosis								
CIN2	1.00				1.00			
CIN2/3	0.67	0.56	0.79	<0.0001	0.69	0.58	0.83	0.06
CIN3	0.25	0.21	0.30	<0.0001	0.27	0.23	0.33	<.0001
AIS	1.84	1.22	2.78	0.004	2.28	1.45	3.60	<.0001
Site								
TN	1.00				1.00			
CA	1.71	1.39	2.12	<0.0001	1.60	1.28	1.99	<0.0001
CT	1.35	1.11	1.66	0.004	1.15	0.93	1.42	0.19
NY	4.27	3.42	5.33	<0.0001	3.61	2.85	4.57	<0.0001
OR	3.12	2.53	3.85	<0.0001	3.07	2.47	3.83	<0.0001

Abbreviations: CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Figures

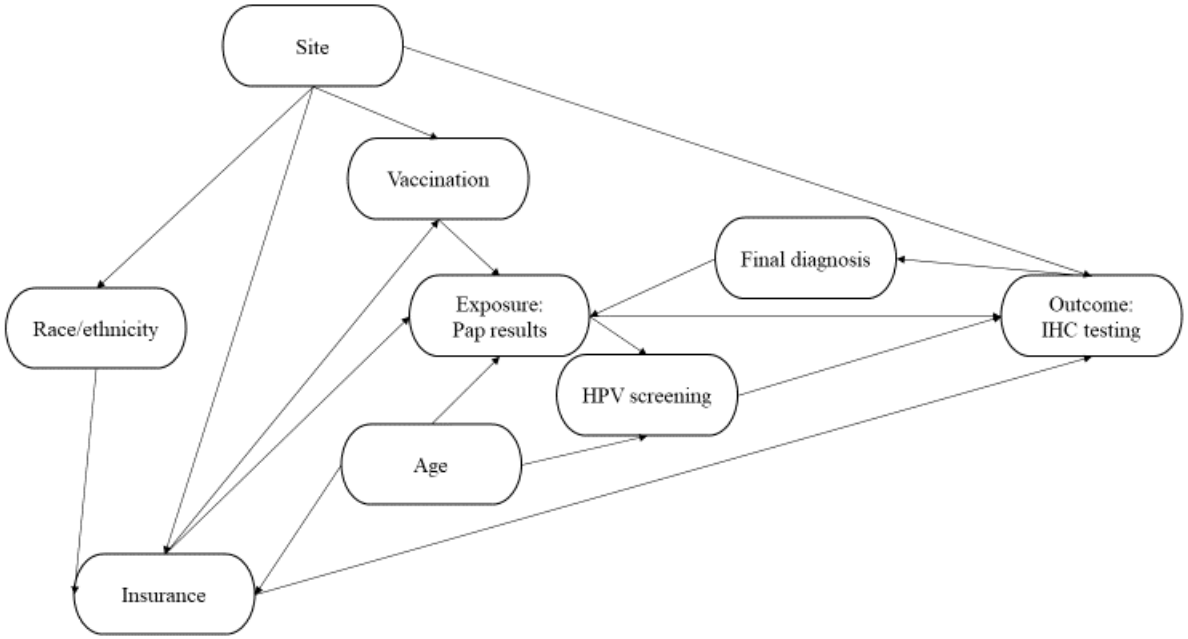

Figure 1. Directed Acyclic Graph (DAG) of the Association Between Papanicolaou (Pap) Test Results and Immunohistochemistry (IHC) Testing

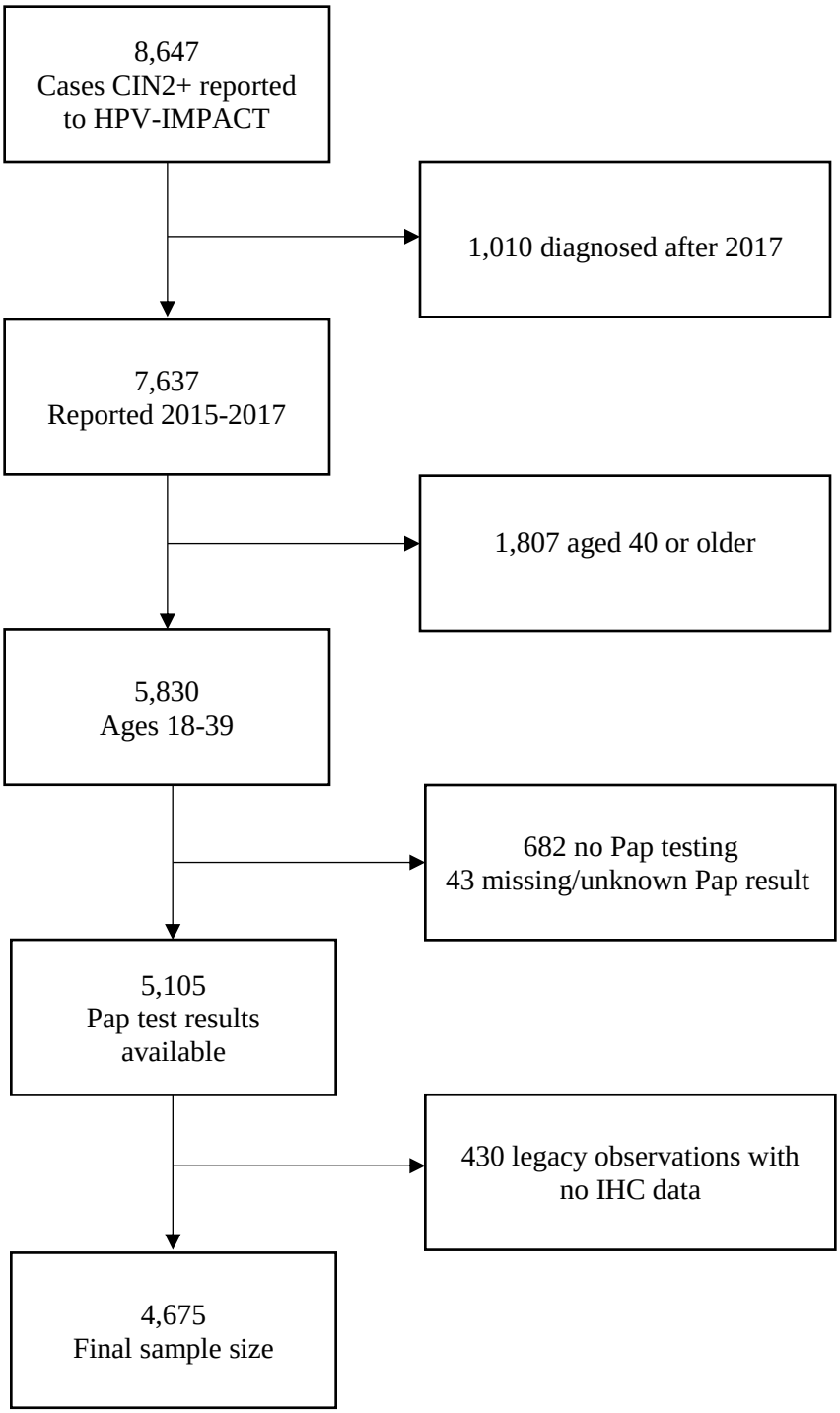

Figure 2. Flow Chart of Exclusion Criteria of CIN2+ Cases Reported to HPV-IMPACT

Appendix: Supplemental Tables

Supplemental Table 1. Comparisons of Combined Screening Results by Final Diagnosis, HPV-IMPACT, 2015-2017

	CIN2 (n = 2,364)		CIN2/3 (n= 828)		CIN3 (n = 1,389)		AIS (n = 94)		Significance	
	No.	%	No.	%	No.	%	No.	%	X^2(df)	p-value
Combined screening										
Normal, HPV+	128	5.41	44	5.31	72	5.18	13	13.83	737.21(27)	<0.001
Normal, HPV-	11	0.47	6	0.72	7	0.5	0			
ASCUS/ASC, HPV	709	29.99	213	25.72	311	22.39	19	20.21		
ASCUS/ASC, HPV	51	2.16	14	1.69	22	1.58	1	1.06		
LSIL, HPV+	492	20.81	144	17.39	163	11.74	4	4.26		
LSIL, HPV-	380	16.07	75	9.06	92	6.62	2	2.13		
ASC-H/HSIL, HPV	337	14.26	184	22.22	458	32.97	23	24.47		
ASC-H/HSIL, HPV	232	9.81	135	16.3	244	17.57	8	8.51		
AGUS/AIS, HPV+	16	0.68	5	0.6	14	1.01	21	22.34		
AGUS/AIS, HPV-	8	0.34	8	0.97	6	0.43	3	3.19		

Abbreviations: CIN, cervical intraepithelial neoplasia; No., number; df, degrees of freedom; HPV, human papillomavirus; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Supplemental Table 2. Comparisons of Clinical Characteristics by Exposure Status, HPV-IMPACT, 2015-2017

	Normal (n = 281)		ASCUS/ASC (n= 1,340)		LSIL (n = 1,352)		ASC-H/HSIL (n = 1,621)		AGUS/AIS (n = 81)		Significance	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**X^2(df)**	**p-value**
HPV testing												
Yes	260	92.53	1,282	95.67	832	61.54	1,060	65.39	58	71.60	557.67(4)	<0.0001
No	21	7.47	58	4.33	520	38.46	561	34.61	23	28.40		
High-risk HPV type												
Positive	257	91.46	1252	93.43	803	23.83	1,002	61.81	56	69.14	580.15(8)	<0.0001
Negative	3	1.07	30	2.24	29	2.14	58	3.58	2	2.47		
Not tested	21	7.47	58	4.33	520	38.46	561	34.61	23	28.40		
Vaccination status												
Vaccinated	37	13.17	220	16.42	300	22.19	299	18.45	15	18.52	41.39(12)	<0.0001
Unvaccinated	32	11.39	146	10.90	167	12.35	182	11.23	12	14.81		
Ineligible	45	16.01	163	12.16	129	9.54	166	10.24	16	19.75		
Unknown	167	59.43	811	60.52	756	55.92	974	60.09	38	46.91		
Final dx												
CIN2	139	49.47	760	56.72	872	64.50	569	35.10	24	29.63	652.76(12)	<0.0001
CIN2/3	50	17.79	227	16.94	219	16.20	319	19.68	13	16.05		
CIN3	79	28.11	333	24.85	255	18.86	702	43.31	20	25.69		
AIS	13	4.63	20	1.49	6	0.44	31	1.91	24	29.64		

Abbreviations: CIN, cervical intraepithelial neoplasia; No., number; df, degrees of freedom; HPV, human papillomavirus; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Supplemental Table 3. Comparisons of Select Demographic Variables by Exposure Status, HPV-IMPACT, 2015-2017

	Normal (n = 281)		ASCUS/ASC (n= 1,340)		LSIL (n = 1,352)		ASC-H/HSIL (n = 1,621)		AGUS/AIS (n = 81)		Significance	
Characteristic	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**X^2(df)**	**p-value**
Age												
18-24	6	2.14	131	9.78	172	12.72	209	12.89	5	6.17	135.19(12)	<0.0001
25-29	54	19.22	524	39.1	581	42.97	593	36.58	21	25.93		
30-34	146	51.96	433	32.31	387	28.62	532	32.82	29	35.8		
35-39	75	26.69	252	18.81	212	15.68	287	17.71	26	32.1		
Race/ethnicity												
White	156	59.77	749	61.44	747	59.62	897	28.82	56	72.73	50.84(16)	<0.0001
Hispanic	38	14.56	203	16.65	209	16.68	263	17.25	6	7.79		
Black	29	11.11	160	13.13	212	16.92	266	17.44	8	10.39		
Asian	34	13.03	88	7.22	65	5.19	77	5.05	7	9.09		
Other	4	1.53	19	1.56	20	1.6	22	1.44	0			
Insurance status												
Private	185	74.6	818	69.68	810	67.39	798	57.29	46	64.79	66.82(12)	<0.0001
Public	53	21.37	285	24.28	308	25.62	459	32.95	21	29.58		
No insurance	4	1.61	20	1.7	29	2.41	42	3.02	3	4.23		
Other	6	2.42	51	4.34	55	4.58	94	6.75	1	1.41		
Site												
California	89	31.67	286	21.34	266	19.67	273	16.84	12	14.81	119.29(16)	<0.0001
Connecticut	54	19.22	390	29.1	400	29.59	382	23.57	28	34.57		
New York	54	19.22	152	11.34	168	12.43	215	13.26	18	22.22		
Oregon	54	19.22	223	16.64	228	16.86	275	16.96	10	12.35		
Tennessee	30	10.68	289	21.57	290	21.45	476	29.36	13	16.05		
Year of diagnosis												
2015	87	30.96	474	35.37	428	31.66	515	31.77	20	24.69	11.4(8)	0.18
2016	105	37.37	455	33.96	478	35.36	568	35.04	26	32.1		
2017	89	31.67	411	30.67	446	32.99	538	33.19	35	43.21		

Abbreviations: CIN, cervical intraepithelial neoplasia; No., number; df, degrees of freedom; HPV, human papillomavirus; IHC, immunohistochemistry; ASCUS, atypical squamous cells of unknown significance; ASC, atypical squamous cells; LSIL, low-grade squamous intraepithelial neoplasia; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion; AGUS, atypical glandular cells of undetermined significance; AIS, adenocarcinoma in-situ

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

Supplemental Table 4. Frequency of IHC Testing Types, HPV-IMPACT, 2015 - 2017

IHC Test[a]	IHC Staining (n=1,412)	%
p16	1319	93.41
Ki-67	275	19.48
BD Pro Ex C	0	
Other	25	1.77
p16 and Ki-67	270	19.12
p16 and other	7	0.50
Ki-67 and other	2	0.14

Abbreviations: IHC, immunohistochemistry; HPV, human papillomavirus

All data are from the Human Papillomavirus Vaccine Impact Monitoring Project

[a] Values are not mutually exclusive