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Cervical Cancer Screening in the Post-Vaccine Era – Confronting a New Clinical Reality

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Executive Summary

The American Society for Colposcopy and Cervical Pathology (ASCCP) cervical cancer screening management guidelines were updated in 2019 and utilize a new precision medicine, risk-based approach to patient management. The patient's immediate and 5-year risk for CIN3, high-grade or severe dysplasia, progression is calculated using the ASCCP Management Guidelines App & Web Application, and the results are used to determine if colposcopy or repeat HPV testing is recommended. The guiding principles of the prior 2012 guidelines remain and are centered around equal management for equal risk. In the 2012 guidelines, patients with HPV16 or HPV18 were referred for immediate colposcopy, regardless of cytology results, based on their CIN3 risk profile and the major contribution of these HPV types to cervical disease (which account for approximately 70% of all cancers in unvaccinated cohorts).[6] Over the last 10 years, our understanding of the CIN3 risks posed by the remaining 12 high-risk HPV types has changed considerably and coincided with the introduction of the 9-valent HPV vaccine in 2014. The HPV types covered by the 9-valent vaccine are responsible for approximately 90% of all cervical disease;[6] thus, we can better triage patient risk by identifying these 9 genotypes from other high-risk types. Another significant development has been the positive impact of the 4-valent HPV vaccine, which has dramatically reduced the prevalence of HPV16/18 and their associated contribution to cervical disease. This new clinical reality compels us to look beyond HPV16/18 to both identify those patients most at risk for disease and to inform patient management. The establishment of the new quantitative approach to CIN3 risk assessment in the 2019 guidelines and inclusion of the consensus clinical action thresholds for patient management were purposely designed to facilitate the incorporation of new technologies, such as the use of this type of extended (beyond HPV16/18) genotyping to improve patient care. The ASCCP New Technologies Committee was established to review novel evidence, and we are currently awaiting an update to the latest ASCCP Mobile app, which will enable extended genotyping to be incorporated into patient management decisions. This has been facilitated by the recent approval of by the US Food and Drug Administration the BD Onclarity™ HPV Assay (BD Onclarity HPV) with extended genotyping results output. BD Onclarity™ HPV Assay is now approved to identify all 14 high-risk types in its results output (most of the 9-valent vaccine types are identified individually (HPV16, HPV18, HPV31, HPV45, HPV52, and HPV33_58 as a paired type) with HPV51 and the remaining high-risk types, which pose a reduced risk of cervical pre-cancer and cancer, in two groups of three (HPV35_39_68, HPV56_59_66). The results from the BD Onclarity HPV PMA trial demonstrate the clinical utility of extended genotyping for identifying women at risk of cervical disease. Here we discuss the threat posed by HPV31, which has the second highest CIN3 risk after HPV16—one that is approximately twice the 4% colposcopy referral threshold and exceeds that of HPV18.[11] We confirm these results using real-world ≥CIN2 case studies from a regional reference laboratory in Utah. Finally, we discuss the future of cervical cancer screening and optimal patient management in a post-vaccination world.

Current U.S. Cervical Cancer Management Guidelines

The goal of cervical cancer screening is to identify pre-cancer (≥CIN2) before it advances to cancer or metastatic disease.[1] We have known for more than 20 years that persistent HPV infection is responsible for cervical disease in women who fail to clear the virus.[2] Research and development during this time period have resulted in numerous advances in both primary and secondary prevention. This includes the introduction of the 4- and 9-valent HPV vaccines[3,4] as well as the evolution of HPV tests that not only identify high-risk HPV but can simultaneously identify HPV16 and HPV18 (this is called limited or partial genotyping) or all of the major disease-causing 9-valent vaccine types (this is referred to as extended [beyond HPV16/18] genotyping).[5] Both of these advances have been driven by the evolution of our understanding of how individual high-risk HPV genotypes contribute to cervical disease, with the top 7 high-risk types accounting for approximately 90% of all cervical cancers.[6] Thus, identification of those genotypes provides immediate risk stratification in HPV-positive

patients. Identification of individual high-risk genotypes with the highest risk of cervical disease can help triage women more effectively for colposcopy and improve the sensitivity for ≥CIN2 detection.[7] Conversely, knowing that a patient is harboring one or more high-risk HPV types that are less likely to result in pre-cancer or cancer also allows these patients to be treated more conservatively, returning for a repeat test rather than referring them for colposcopic biopsy.[8] ASCCP management guidelines reflect the current standard of care and will be periodically updated to reflect advances in screening technologies or in response to the impact of HPV vaccination on HPV prevalence and disease.[8] The 2012 ASCCP management guidelines advanced patient care by using limited HPV16/18 genotyping to identify highest-risk patients and referring them directly to colposcopy.[9,10] This strategy was subsequently validated in two large US registrational trials, where it was found to be more effective than cytology, resulting in primary HPV screening claims (the ability to use HPV alone as the primary test) for manufacturers of the tests, including the BD Onclarity™ HPV Assay.[10,11] The field continues to advance with the recent extension of the original BD Onclarity HPV approval to include extended genotyping and a biomarker test from Roche.[12,13] Both technologies are now candidates for inclusion in additional updates to the 2019 ASCCP Management Guidelines to improve the triage of HPV-positive women.[14] Here, we focus on extended genotyping and why this is important for cervical cancer screening.

Identifying Genotypes Beyond the Current HPV16/18 Types is Critical

There are a number of pressing clinical reasons to expand our ability to detect individual high-risk genotypes beyond the current HPV16/18 partial genotyping standard of care:

I. The current paradigm is incomplete and does not reflect our current understanding of the natural history of high-risk infection

The 2012 management guidelines were driven by our then understanding of the large contribution of HPV16/18 to overall disease and a seminal study that showed that the risk posed by the remaining 12-other pooled types was low.[6,15] Subsequent research in both the United States and other countries established that this was an over-simplification and that the true underlying risk of the 9-valent vaccine types in the 12-other pool was being masked by the prevalence of other high-risk types with low disease attribution.[11,16,17] Several studies, including those by the original US authors, went on to show that HPV31, HPV33, and HPV52 posed similar risks to that of HPV18.[18] In particular, HPV31 has been shown to have a higher pre-cancer risk than HPV18 and is, in fact about twice the recently adopted colposcopy referral threshold of 4% established by ASCCP in the updated 2019 guidelines.[8,11] Thus, under the principle of equal management for equal risk, all HPV31-positive women should be referred to colposcopy, even those with negative cytology.[8,9]

II. The positive impact of the 4-valent vaccine on routinely screened women

The 4-valent vaccine was introduced in 2006 and after an initial slow start, national vaccine levels have risen to over 50%.[19] Cohorts of vaccinated younger women are now entering the screening population, and National Health and Nutrition Examination Survey (NHANES) data provides strong evidence for an approximately 90% reduction in the prevalence of HPV16/18 genotypes, in addition to herd immunity in unvaccinated women in these communities.[19] These results mimic those seen in other countries with high vaccine coverage, and this has led to an associated large reduction in the prevalence of ≥CIN2 associated with HPV16/18.[20,21] The extension of catch-up vaccination to age 45 and the extension of vaccination to males will likely further accelerate this decline.[22] Thus, the current clinical practice of only referring HPV16/18-positive women to colposcopy is outmoded and is increasingly less relevant to the US patient population with each passing year. We also note that the additional impact of the 9-valent vaccine, which was introduced in 2014 and has now replaced the 4-valent vaccine, will not be reflected in the clinic until about 2030, as younger women age into the screening program.

III. Genotype-specific persistence is a key driver of patient outcomes

We have known for over 20 years that persistent high-risk HPV infection is responsible for virtually all cervical pre-cancer and cancer.[2] Most women clear the virus, but in a small number of patients, the virus persists and goes on to cause disease which, if left untreated, may lead to cancer. This knowledge has recently been updated with the understanding that there is a significantly larger risk for disease posed by genotype-specific persistence (repeatedly testing positive for the same genotype) than for a patient who is persistently positive with a pooled high-risk type (repeatedly testing positive, but the genotype changes between testing periods).[23,24] This is an important distinction and one that is aligned with our fundamental understanding of HPV biology.[25] First, we know that cervical disease is slow-moving and that the different high-risk types are immunologically distinct. Thus, when one type is eliminated, even if it is immediately replaced by another high-risk type, one is essentially resetting the clock on disease progression, and the risk posed by the new infection is no higher than that of a *de novo* infection of a previously HPV-negative patient.[24] Second, high-risk HPV viruses that persist tend to integrate into the host genome, setting up conditions for advanced disease progression and cancer development.[26] Thus, the inability to discern whether a high-risk positive result in the 12-other pool is actually a type-specific persistent infection or just a type-switch persistent pool positive result significantly reduces our ability to predict who is most at risk for future disease.

IV. Key Findings of the BD Onclarity HPV PMA Trial

The BD Onclarity HPV trial was a large US national registrational trial that enrolled 33,858 participants with more than 6,000 receiving colposcopic biopsy to diagnose ≥CIN2 disease using central pathology review. This resulted in the diagnosis of 224 ≥CIN2 cases, 173 CIN3 cases, and 14 cases of adenocarcinoma-in-situ or cancer at baseline.[27] Enrolled participants were followed for an additional 3 years with annual colposcopy and biopsy for women with abnormal cytology or those positive for high-risk HPV infection. The trial design allowed baseline and 3-year CIN3 risks being calculated for BD Onclarity HPV results. The baseline results

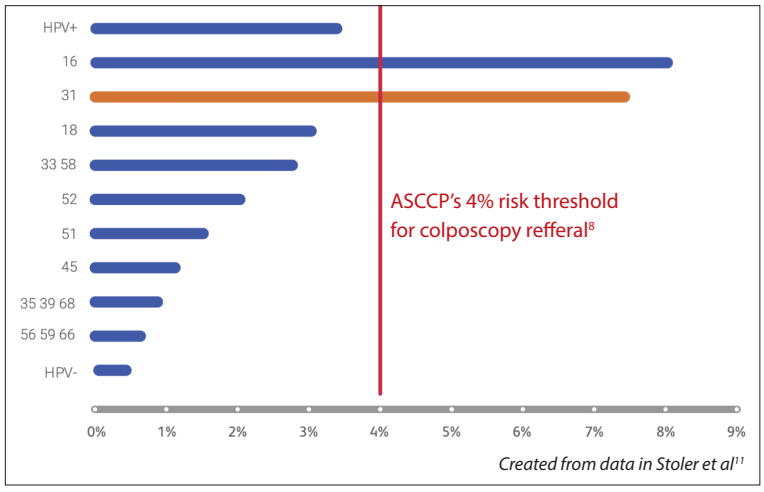

FIGURE Risk of CIN3+ by HPV genotype in women aged > 25 years with normal cytology

(**Figure**) confirmed our new understanding of the relative risk contribution of the non-HPV16/18 genotypes, in particular that HPV31 poses a higher pre-cancer risk than HPV18 and is approximately twice the ASCCP 4% threshold for referral to colposcopy.[8,11] Thus, under the principle of equal management for equal risk, all HPV31-positive individuals (including those with NILM cytology) should be referred to colposcopy. The results also confirmed the contribution to overall disease of the 9-valent vaccine types and the reduced importance of HPV51 and the pooled BD Onclarity HPV types (HPV35_39_68; HPV56_59_66).[28] Finally, masking of CIN3 risk for genotypes, when pooled, is also evident, as the pooled high-risk positive result ("HPV+") falls well below that of the two highest-risk types, HPV16 and HPV31.

Real-World-Evidence from a population in Utah

Associates of Pathology is an anatomic pathology services group serving the Ogden, Utah area, and has been offering the BD Onclarity™ HPV Assay with extended genotyping to its clients for over a year. Routine screening of more than 25,000 women resulted in 2,502 HPV-positive cases, yielding 104 diagnosed cases of CIN2/3[29]. HPV16 contributed 30 (28.8%) of these cases, and HPV31 had 20 attributed CIN2/3 diagnoses (19.2%), confirming the findings in the BD Onclarity HPV US clinical trial. Thus, HPV31 is responsible for approximately 1/5 of all pre-cancer cases in the Ogden, Utah, area.

HPV31 Case study examples

What is the significance of HPV31 infection at the patient level?

Case #1: A 28-year-old whose last Pap was negative in 2018 was routinely screened in 2022 and was diagnosed with HSIL cytology and HPV31 infection. The high-grade cytology result was confirmed as CIN3 following colposcopy and biopsy.

Case #2: A 31-year-old with a reactive Pap in June 2021 with multiple HPV infections (HPV18, HPV31, HPV35_39_68) was retested in August 2022 and found to have a high-grade Pap. Repeat HPV testing revealed only HPV31-positivity, and the high-grade cytology result was diagnosed as CIN3 by biopsy-confirmed histopathology.

Case #3: A 37-year-old with unknown history was routinely screened, and the Pap results indicated ASC-US cytology, positive for HPV31. Colposcopy and biopsy resulted in a CIN3 diagnosis.

These case studies confirm the clinical importance of HPV31 in the US population and its ability to quickly lead to high-grade disease in younger women due to its elevated risk for CIN3 progression. The ability to identify HPV31 individually is an important addition to cervical cancer screening because while it would still lead to an HPV-positive result in a pooled assay output, there is no way to identify the underlying risk and thus alert the clinician to a potentially serious persistent infection. For example, the 12-other high-risk positive result could be the result of an HPV31 infection (a candidate for referral to colposcopy) or HPV66 (a genotype no longer considered to cause cervical cancer[30]).

Summary and Perspectives

Our understanding of the different risks posed by high-risk HPV types has advanced considerably in recent years and is reflected in the high-risk types present in the 9-valent vaccine. This knowledge also clarifies our understanding of HPV16/18 triage as outdated and incomplete and one that needs to be updated. Other non-HPV16/18 types in the 9-valent vaccine contribute significantly to disease, with HPV31 being second only to HPV16 in the US population in terms of contribution.[11] Our original understanding of the contribution of non-HPV16/18 types to disease was literally masked by the pooling of the 12-other high-risk types, which resulted in an underestimate of some individual high-risk types such as HPV31.[15,18] We now also better appreciate the increased risk posed by genotype-specific persistent infection as a sign that the immune system is not clearing the virus and that this patient is at elevated risk for disease development.[24] This is underscored by the reduced risk of a type-switch infection, whereby the new infection has to re-establish itself, similar to that of a *de novo* infection. Finally, the introduction of the 4-valent vaccine in 2006 is now resulting in positive gains in the clinic, with HPV16/18 substantially reduced in both prevalence and disease contribution.[19,20] While welcome news, it does mean that we now need to refocus our attention to non-HPV16/18 types, even in unvaccinated women where herd immunity has also reduced their incidence.[19] Fortunately, the 2019 ASCCP management guidelines have arrived just in time to address this changing cervical cancer screening landscape. By applying the equal management of equal risk principle, and the new understanding that genotype-specific persistent infection substantially increases a patient's risk for future disease, we can leverage extended genotype information to inform patient management, focusing more precisely on those who require immediate follow-up and allowing those with reduced risk to return at an interval of 1-, 3- or 5- years (based on their 5-year risk profile). It is also important to note that the COVID-19 pandemic has had a devasting impact on both cervical cancer vaccination and screening rates.[31,32] Modeling

predicts that this will result in an increase in both cervical pre-cancer and cancer rates.[33,34] In the post-pandemic era, extended genotyping provides an immediate triage of a patient's risk, thus allowing physicians to focus strained colposcopic resources on those women with highest immediate risk for pre-cancer and cancer.[31] For all these reasons, we look forward to the next update of the ASCCP Mobile App, which will expand coverage to extended high-risk types, enabling clinicians to utilize risk-based management for their patients and provide more personalized care.[35]

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