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False positive cervical HPV screening test results

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ABSTRACT

In cervical cancer screening, HPV testing is best at reassuring women when they are negative, but proper management of HPV positives is still evolving. Most HPV infections are benign, and over-reacting clinically to HPV positivity can cause psychological and possible iatrogenic physical (e.g., obstetrical) harm. We describe the built-in false positives in current tests, and the real harm that can result when the meaning of such false positive HPV tests is misunderstood. We suggest steps that could reduce harm being done by flawed tests and excessive clinical responses to positive HPV testing. We focus the discussion by presenting an illustrative case.

1. Introduction

The goal of cervical screening is to find and treat cervical precancer, in order to prevent cervical cancer. Testing for types of HPV that can cause cervical cancer is more sensitive, and a negative test is more reassuring, than cytology (Pap test) [1]. Consequently, HPV testing at extended screening intervals is justifiably replacing cytology as the preferred cervical screening method worldwide [2,3]. However, it is important to address the interpretation of the HPV testing, recognizing that the positivity rate is higher than cytology and that the majority of positive test results do not indicate a high absolute risk of cancer. These test results are, in effect, “false positive” cervical cancer screening results built into our HPV test-based screening methods and strategies. By “false positive” screening tests, we do not mean that HPV is not present, which has not been reported to be a problem for HPV nucleic acid detection methods. Rather, we are referring to the detection of HPV infections that are not destined to cause cervical cancer.

This brief commentary describes the kinds of false positives in current tests, and the real harm that can result when the meaning of such false positive HPV tests is misunderstood. The specific objective of the commentary is to urge the scientific community to take steps to reduce the harm being done by flawed tests and excessive clinical responses to positive HPV testing. Our major premise is that to tell a woman that she has a possibly carcinogenic, sexually transmitted cervical infection that can take years to clear can do real harm, even if unnecessary treatment is not undertaken. We should strive harder to reduce sources of false positive results that can affect very large numbers of women as HPV testing gains in global use.

2. Kinds of false positivity of HPV testing and their consequences

Factors that contribute to non-specificity that we will discuss are: 1) Testing for known non-carcinogenic HPV types; 2) Inadvertent cross-reactivity with marginally carcinogenic types; 3) Use of ultrasensitive assays; 4) Inclusion of a marginally carcinogenic types in screening tests; 5) Undervaluing large differences in carcinogenic potency; and 6) Treatment of all HPV-positives that represents overtreatment [4].

2.1. Testing for known non-carcinogenic HPV types

As a prime example of testing for non-carcinogenic types, we name a virtually useless product from a very reputable diagnostics company. The Digene Hybrid Capture 2 (HC2) HPV DNA test (QIAGEN®, Germany) has a well-established and valuable high-risk HPV probe set (which can be purchased separately). However, the company continues to offer, presumably because of continued demand, a “low-risk” probe set that targets HPV types 6, 11, 42, 43, and 44. HPV6 and HPV11 cause exophytic genital warts, which can be diagnosed clinically without need for molecular diagnostics. There are no important disease associations with HPV42, HPV43, and HPV44. This test was invented and approved 30 years ago while HPV phylogeny, natural history, and clinical utility were being clarified. Guidelines committees have called repeatedly for cessation of widespread testing with such tests [5]. One wonders why it is still offered and what it will take to get such a product off the market, given that it was approved in an earlier, less-informed era.

Clinical value is the ultimate goal of HPV testing. Thus, assay validation platforms such as VALGENT, should clearly define their recommendations on the basis of clinical/screening effectiveness and not simply analytical value.

2.2. Inadvertent cross-reactivity with marginally carcinogenic types

Certain HPV assays are well known to test positive when the sample contains a moderate to high viral load of HPV types not targeted by the probes in the assay. HC2 cross reacts with a sizable number of types related genetically to the 13 targeted types, which are classified as carcinogenic or probably carcinogenic [6]. This cross-reactivity leads to false positivity affecting a small percentage of tested women. Although the false positives are extremely unlikely to indicate risk of cancer, they tend to increase the sensitivity of the assay for detection of cervical high-grade lesions by a few percentage points. Types like HPV53 and HPV66, classified as possibly carcinogenic because they are only

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extremely rarely associated with cervical cancer, can cause high-grade appearing lesions. Thus, sensitivity for high-grade intraepithelial lesions is not a perfect indicator of carcinogenicity; the goal of screening is to prevent cancer, not to find all lesions that look high-grade.

2.3. Use of ultrasensitive assays

Some might argue that sensitivity is more important than specificity and that false positives are low-morbidity mistakes. However, the rarity of cancer caused by common borderline types cannot be overstated. To include all of them in an effort to maximize sensitivity would falsely label tens or hundreds of thousands of women as carcinogenic HPV-positive [13]. For at least HPV16-related HPV types, very low viral load implies lower risk of subsequent progression to high-grade lesions [14].

Note that one of the currently approved and marketed tests, Aptima, is an RNA assay, with increased specificity compared with DNA assays [8]. The false-positive problem is reduced somewhat but not eliminated, and long-term negative predictive value remains unproven.

2.4. Inclusion of a marginally carcinogenic types in screening tests

An ongoing source of false positive results is the inclusion of HPV66 in otherwise specific diagnostic assays. Planning for several of the currently approved and marketed HPV tests (Cervista, Hologic, San Diego, CA; Cobas, Roche, Pleasanton, CA; Aptima, Hologic; Onclarity, BD, Sparks, MD; Xpert HPV, Cepheid, Sunnyvale, CA) began after an IARC committee categorized 14 HPV types as carcinogenic [7]. The 14 types were categorized mainly from epidemiologic data based on HPV typing of approximately 10,000 cervical cancers, in multi-year cohort studies using cervical intraepithelial neoplasia grade 3 (CIN3) as the surrogate endpoint for cancer risk, and in phylogenetic studies indicating the close relationship of the carcinogenic types into species groups alpha-9 (HPV16, HPV31, HPV33, HPV35, HPV52, and HPV58), alpha-7 (HPV18, HPV39, HPV45, HPV59, and HPV68), alpha-6 (HPV56 and HPV66), and alpha-5 (HPV51) [9]. It is crucial to distinguish between the assessment of carcinogenic potential by authoritative groups like IARC, and decisions regarding which types to include in cervical screening. HPV66 was the most equivocal choice categorized at that time by IARC as carcinogenic. There are several types “at the borderline” of classification, with reports of occasional association with invasive cancer [4]. For example, a related type in alpha-6, HPV53, was not categorized as carcinogenic and much was made of the concern about overdiagnosis if common borderline-carcinogenic types were included incorrectly. One of us (MS) was centrally involved in what turned out to be a reasonable but incorrect assignment by IARC of HPV66 to the definitely carcinogenic group. The finding of an elevated risk of CIN3 following HPV66 infection factored heavily into the decision. Unfortunately, HPV66 was included in the design of most currently used HPV assays. The advancing knowledge that some HPV types that cause CIN3 only extremely rarely lead to cancer, and correction in classification of HPV66, came too late in the critical generation of designs and approvals. Specifically, by the time of the next IARC review [7,10], IARC and the Catalan Institute of Oncology had accumulated nearly 30,000 typed cervical cancers. It was clear then, and even clearer now based on 40,000 cancers [11,12], that HPV66 does not belong in the definitely carcinogenic category. It is not found alone in cancers to a greater extent than our benchmark of the non-carcinogenic type, HPV6. Although both types extremely rarely can cause cancer, they are much too prevalent in the general population to include in an HPV screening test. If the companies were to improve their tests by dropping the HPV66 probe, regulatory groups might require very expensive studies and documentation of performance, as is typically required for all major design changes. The economic incentive to correct the mistake is lacking.

2.5. Undervaluing large differences in carcinogenic potency

A subtler but important kind of false-positive interpretation is qualitative, i.e., failing to recognize that some carcinogenic types are much “weaker” than the major carcinogenic types. The IARC evaluation of human carcinogens does not consider potency and thereby forces a possibly false dichotomy of carcinogenic versus non-carcinogenic. At the extremes of low risk, for HPV at least, the division can be nearly arbitrary and prone to error. In re-examining how to handle borderline types, we have clarified that CIN3 is an imperfect surrogate for risk of cervical cancer. Increasingly, we have realized that the vast majority of cervical cancers are caused by HPV types in alpha-9 (HPV16-related) and alpha-7 (HPV18-related) species groups (i.e., HPV56 and HPV51 each cause only about one per cent of cancers) [15]. Thus, as an HPV research community, we have not sufficiently emphasized the profound differences between the most carcinogenic HPV type, HPV16, the other higher-risk types (alpha-9 HPV31, HPV33, HPV52, HPV58, and alpha-7 HPV18 and HPV 45) and the least potent ones (e.g., alpha-7 HPV39, HPV59, and HPV68, and HPV51 and HPV56) [16]. Of note, one type, HPV35, is particularly important among women in Africa or of African descent [17]. HPV16 causes half of cervical cancers while the five least carcinogenic contribute only about 5% of cases in aggregate. In settings where triage tests are unaffordable, or treatment resources including ablation and skilled loop electrosurgical excision procedure (LEEP) or large loop excision of the transformation zone (LLETZ) excision are in short supply, it is reasonable to question whether the lower-risk types merit inclusion in HPV screening assays. The excess positives can overwhelm the screening program and divert attention away from the most important HPV positives. The role of typing in HPV testing, as part of triage, is worth considering seriously; partial distinctions within groups might be useful to maintain sensitivity for precancer while minimizing overtreatment.

2.6. Treatment of all HPV-positives represents overtreatment

In some resource-limited settings, because triage methods can be expensive, programs mandate treatment of all HPV-positive women using ablation or excision [18,19,20]. Even the more carcinogenic types of HPV typically do not lead to cancer; rather, they clear without consequence. Hopefully, it is evident how an unfocused strategy leads many women to undergo unnecessary procedures, with attendant risk that can lead to serious adverse reproductive events such as premature delivery [21].

Non-specific diagnosis of precancer ultimately leads to some degree of excessive treatment. Diagnosis of a true precancer implies that, if untreated, the woman faces a high risk of cancer and death. Time-honored histopathologic proxies are over-diagnosing true precancer by an order of magnitude. Guidelines successfully recommended against treatment of low-grade lesions (e.g., CIN1) decades ago. CIN2, CIN3, and adenocarcinoma in situ (AIS) are often combined as high-grade intraepithelial lesions or precancer, but the prevalence, especially of the squamous lesions, relative to cancer is 10–20 to 1 [11,12,14]. In other words, we cannot consider these histopathologic diagnoses to represent de facto cervical “disease”. Precancers produce no symptoms, are found only by screening, and largely would not harm women if left untouched. We are beginning to better define the subset representing true risk, via improved understanding of HPV-host interactions (e.g., high numbers of dual stain positive cells [22], strong cellular gene [23] or HPV L1 and L2 methylation [24], viral integration [25,26], overexpression of oncoproteins E6 and E7,[26] which all suggest a transforming infection equivalent to precancer). Targeting more accurately defined precancer will in turn lead to better diagnostic assays with fewer false positives and higher positive predictive values (risk of cancer when testing is positive).

3. Conclusion

The critical point underlying this entire discussion is that current HPV tests and testing strategies can lead to over-reaction to HPV positivity, causing psychological and possible iatrogenic physical (e.g., obstetrical) harm. It is impossible to weigh exactly the cost of missing a cancer against the harm done by hundreds or thousands of false-positive HPV tests. Nonetheless, we are forced to consider such trade-offs, and need to better minimize harm to untold numbers of women as we increase cervical-screening sensitivity by use of HPV testing.

4. Sidebar

A real-life example of morbidity from false positive HPV testing. We present a case report as an example that is well in line with the observed increased anxiety when a woman is given an abnormal pap result [27].

A young, married Spanish woman approached us in emotional distress because a routine cytologic screen in the fall of 2017 indicated low-grade squamous intraepithelial lesion (LSIL). The clinician opted to test for HPV using Inno-Lipa (Innogenetics, Ghent), and HPV66 was found. HPV66 is labeled as a high-risk type; the clinician expressed that impression to the woman and recommended colposcopy and biopsy. The woman, who was a teacher on maternity leave at that time, reacted with great concern to the finding of a carcinogenic, sexually transmissible HPV infection, but was also reluctant to undergo colposcopy. Over the ensuing months, she had difficulty overcoming a feeling of being infected and felt at risk to herself and her husband. She ceased sexual intercourse and became increasingly depressed. She found one of us (MS) in mid-2018 by internet search. She admitted to severe anxiety and depression. While stressing that we could not be her doctor long-distance, we explained the extremely low risk posed by HPV66, the original mistaken classification of HPV66 that persists in the kit labels, and the down-classification of its carcinogenic potential at the time of the IARC re-evaluation. The information, conveyed by her from us to her gynecologist, failed to convince her clinician to revise the statement that the infection represented a high-risk type, as the label clearly stated that it was a high-risk type. At the patient's request further analysis was performed. A newly-validated next-generation sequencing-based method that detects 51 HPV types [28] was performed on self-samples (collected on five sequential days by Evalyn Brush, Rovers, Denmark and tested in triplicate). All 15 assays confirmed HPV66 was still present. No other HPV type was consistently found. Presentation of the IARC manuscript downgrading HPV66 and an explanatory publication did convince her that the infection, even if still persistent for nine months, was likely to clear and highly unlikely to lead to cancer. She is currently feeling better about the test result, and with her clinician is monitoring the infection with cytology and repeat HPV testing in anticipation of clearance. She consented to sharing her story to help other women ultimately to avoid the scare she has experienced.

Declarations of interest

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COI statement

Silvia de Sanjose reports no conflict of interest with regard to this article.

Mark Schiffman reports that NCI has received HPV and cytology test results at reduced or no cost from Roche, BD, and Qiagen for independent research conducted by NCI.

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