Prognostic Value of Human Papillomavirus in the Survival of Cervical Cancer Patients: An Overview of the Evidence

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Abstract
Some studies have suggested that the presence in tumors of nucleic acids from human papillomavirus (HPV) constitutes a prognostic marker of disease severity in cervical cancer. There are two conflicting lines of evidence in this regard. First, the presence of HPV 18 is equated to rapid progression through early disease stages, possibly resulting in a more aggressive clinical course. Although fragmentary, in terms of the clinical and epidemiological basis, this line of evidence has some experimental support. Second, the absence of HPV from the tumor would confer a worse prognosis than if any viral types were present. Unlike the former, the latter line of evidence is not bolstered by experimental data but emerged from persuasive clinical studies, which had adequate sample sizes, used survival end points, and controlled for confounders. The absence of HPV in some tumors could indicate that they originated through different oncogenic mechanisms, perhaps resulting in different cell proliferation rates and, consequently, distinct clinical behavior. On the other hand, HPV detectability could simply be a correlate of other genuine prognostic characteristics, which would explain its association with survival. Both the nature and the mechanism of the prognostic role for HPV in cervical cancer remain to be elucidated. The paucity of studies can be attributed to the labor-intensive nature of assays for HPV. It is hoped that the advent of the polymerase chain reaction method will facilitate the conduct of retrospective studies of archival histopathology specimens and survival information. Such studies would not only shed light on the important scientific aspects of the natural history of cervical cancer but would also generate information on what constitutes the biological markers influencing the clinical outcome of the disease.

Introduction
The survival expectancy for a woman diagnosed with invasive cervical cancer is determined both by characteristics of the tumor and by host-related factors. The most important treatment modalities for cervical neoplasia are surgery and radiotherapy, individually or in combination. Early-stage disease with no signs of lymphatic spread is usually managed by surgery alone, whereas extensive local and regional spread of the disease requires the combination of the two techniques. Chemotherapy plays only a secondary role in the management of cervical carcinoma.

Disease staging is based mostly on local and regional involvement. The most used classification is that of the International Federation of Gynecology and Obstetrics, which is based on pretreatment clinical evaluation. Although stages have strong predictive value with respect to survival probabilities, several anomalies have been reported, pointing to the need for a staging system based on additional prognostic factors. Several factors have been considered as prognostic for cervical cancer survival: depth of invasion; parametrical extension; pelvic (8) and paraaortic (9) lymph node involvement; vascular invasion and histological grade; steroid hormone receptors; oncogene C-myc expression; blood transfusions; and nuclear ploidy level. In addition, there is an ongoing controversy over the effect of age; in particular, whether it is associated with a tumor type of poor outlook or whether its apparent prognostic value is confounded by stage at presentation.

Some studies have suggested that the presence of nucleic acids from HPV of specific types in the tumor constitutes a prognostic marker of disease severity and outcome. The implication that HPV is an etiological agent of cervical cancer is plausible, with overwhelming evidence from both epidemiological and experimental studies. However, a prognostic role for these viruses in disease behavior cannot be easily explained in light of the available information on the biology of HPV and the pathology of cervical cancer. Nevertheless, considering the potential importance of HPV testing with respect to therapy and surveillance for recurrence, it is important that the existing evidence be examined. The present report analyzes the weight of the evidence provided by studies of HPV as a prognostic factor in cervical cancer and proposes some interpretations in light of their findings.

Prognostic Value of HPV
Role in Invasive Disease. Table 1 summarizes the main design characteristics and findings from the studies evaluating a prognostic role for HPV in invasive cervical
cancer. Meanwell et al. (21) compared tumors with and without HPV 16 DNA, as detected by SBH, and found that they did not differ significantly with respect to stage distribution and histological characteristics. The case series was small, comprising 47 patients (31 positive and 16 negative for HPV 16). There was, however, a possible hint that if any association were to emerge in analyses of larger case series, it would be that of the presence of HPV being correlated with poor prognosis. HPV-positive tumors tended to be more frequently of advanced stages than those that were negative for HPV. The proportion of HPV-positive cases who were younger than 40 years of age was significantly lower than that among HPV-negative patients.

Barnes et al. (22) used SBH to detect DNA of various HPV types in frozen biopsies from 30 patients with invasive cervical cancer. Specimens contained HPV DNA of one of the following types: HPV 16, 47%; HPV 18, 20%; HPV 31, 7%; uncharacterized, 27%. No association was found between any of the types and disease stage. Of all histopathological factors examined only grade seemed to be associated with HPV 18. Tumors harboring the latter viral type tended to be predominantly anaplastic. HPV 18 was also more frequently associated with regional lymph node involvement than HPV 16. An interesting observation was the lower age at diagnosis for HPV 18 patients: on average, 12 years younger than patients harboring HPV 16 in their tumors.

King et al. (23) studied cervical carcinomas from 85 patients by ISH using formalin-fixed, paraffin-embedded specimens. Only surgical cases were considered, i.e., those patients who underwent both hysterectomy and lymphadenectomy. In all, 38 (44.7%) specimens hybridized with the HPV probes: 18 with HPV 16 (21.2%), 20 with HPV 18 (23.5%), and none with HPV 6. HPV 16 was more frequently associated with squamous cell carcinomas, whereas HPV 18 was more common in adenocarcinomas. No associations were seen between HPV type, on the one hand, and patients’ age, vascular invasion, pelvic or paraaortic node metastases, and disease-free or overall survival times, on the other hand.

Walker et al. (24) used SBH to analyze the HPV genotype in frozen tumor biopsies from 100 patients with cervical carcinomas. Of the various HPV probes utilized, only types 16 (46%), 18 (16%), and 31 (2%) hybridized with the specimens. The predominant type in adenocarcinomas was HPV 18 (32%), whereas HPV 16 was more frequent in squamous cell carcinomas (63%). HPV 18 tumors tended also to be more anaplastic and nonkeratinizing than those harboring other HPV types or those that were HPV-negative. Likewise, HPV 18 tumors tended to exhibit signs of vascular invasion more frequently (45%) than those with HPV 16 (16%) or no HPV (26%). Nevertheless, no associations were found between HPV type and pelvic or paraaortic lymph node involvement. The authors observed higher recurrence (45%) and mortality (27%) rates among women with HPV 18 containing carcinomas than those with HPV 16 (16% recurrence and 16% mortality) or those with no HPV (15% recurrence and 7% mortality). Patients with HPV 18 tumors were on average 8 years younger than those with HPV 16 tumors or those with no HPV DNA. In addition, women with HPV 18 tumors reported histories of recent cytological screening (past 3 years prior to diagnosis) more frequently than the remaining patients. The authors concluded that HPV 18 could be a marker of rapidly progressing lesions, which would explain the association with undifferentiated tumors, the recent history of screening, and the poorer outcome experienced by patients with HPV 18 tumors. The latter study corroborated the preliminary findings that the authors had obtained earlier on a subset of the same patient population (25).

Table 1  Summary of findings from studies evaluating the association between HPV DNA in the tumor and clinical outcome or disease severity in cervical cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of cases</th>
<th>HPV detection</th>
<th>Associations with Age*</th>
<th>Histology*</th>
<th>Survival analysis findings (type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meanwell et al. (21)</td>
<td>47</td>
<td>SBH</td>
<td>Pos. (16)*</td>
<td>None</td>
<td>ND</td>
</tr>
<tr>
<td>Barnes et al. (22)</td>
<td>30</td>
<td>SBH</td>
<td>Neg. (18)</td>
<td>Anaplasia (18) Nodes (18)</td>
<td>ND</td>
</tr>
<tr>
<td>King et al. (23)</td>
<td>85</td>
<td>ISH</td>
<td>NS</td>
<td>SCC (16) Adeno. (18)</td>
<td>No association (univariate)</td>
</tr>
<tr>
<td>Walker et al. (24)</td>
<td>100</td>
<td>SBH</td>
<td>Neg. (18)</td>
<td>SCC (16) Adeno. (18) Anaplasia (18)</td>
<td>HPV 18 associated with poor prognosis (univariate)</td>
</tr>
<tr>
<td>Rou et al. (26)</td>
<td>106</td>
<td>SBH</td>
<td>NS</td>
<td>SCC (16) Adeno. (18)</td>
<td>Absence of HPV associated with poor prognosis (multivariate)</td>
</tr>
<tr>
<td>Rose et al. (27)</td>
<td>51</td>
<td>DBH</td>
<td>NS</td>
<td>None</td>
<td>No association (univariate)</td>
</tr>
<tr>
<td>Higgins et al. (28)</td>
<td>212</td>
<td>ISH (RNA)</td>
<td>Neg. (overall)</td>
<td>SCC (16) Adeno. (18)</td>
<td>Absence of HPV associated with poor prognosis (multivariate)</td>
</tr>
</tbody>
</table>

* HPV types shown in parentheses.

* Pos., positive association; Neg., negative association; DBH, dot blot hybridization; ND, not done; NS, not significant; SCC, squamous cell carcinoma; Adeno., adenocarcinoma.
Riou et al. (26) studied 106 patients with cervical cancer of stages IIB and earlier. HPV DNA testing was performed by SBH and PCR amplification (subset of 38 specimens). HPV 16 was the predominant type, being detected in 55% of the tumors. HPVs 18, 33, 35, and other uncharacterized types were detected in 16%, 6%, 1%, and 10% of the specimens, respectively. Overall or type-specific HPV DNA positivity was not associated with geographical origin, menopausal status, parity, tumor size, stage, or age. HPV 16 was more frequently associated with well-differentiated squamous cell carcinomas, whereas HPV 18 was more frequently associated with adenocarcinomas. Patients with HPV-negative tumors had almost twice the rate of lymph node involvement as compared to those with HPV-positive tumors. The authors also analyzed the risk of recurrence as a function of the different HPV types and of other prognostic factors. Patients with HPV-negative tumors experienced a recurrence rate that was 2.6 times that of all other patients with detectable HPV of any type in their tumors. When the outcome was restricted only to distant metastases the overall rate ratio was 4.5. In both analyses the authors controlled for the confounding effect of important prognostic factors, such as tumor size and nodal status.

The latter results were not corroborated by Rose et al. (27), who did not find a difference in recurrence rates by HPV status determined by dot blot and SBH in 51 cervical cancer patients. The authors found that younger women were more likely to have relapses than older patients, but there was no association between age and HPV positivity. The analysis was based on crude proportions of recurrences in each group of patients without use of the survival time. Only patients who had been followed-up for 22 months or longer were included, which may have presumably biased the case series with exclusion of all recurrences occurring before that period.

A recent study by Higgins et al. (28) used survival time information as the end point to analyze the prognostic value of HPV in 212 women with cervical carcinomas. Unlike previous studies, the authors approached the problem of HPV detection by demonstrating the presence of viral messenger RNA. They found HPV RNA in 83% of squamous cell carcinomas and in 71% of adenosquamous and adenocarcinomas using the ISH technique with RNA probes for the main genital types. HPV 16 RNA transcripts were most common in squamous tumors (63%), whereas HPV 18 was most frequently found (44%) in specimens with glandular components. A strong association was seen with age at diagnosis. Patients with HPV-RNA-positive tumors were on average 12 years younger than those with negative tumors. The death rate among HPV-positive patients was only one-third of that among HPV-negative patients. Confounding by age and stage was largely ruled out, since the adjusted analysis indicated that the hazard ratio by presence of HPV was not materially different from that seen in the univariate analysis. Histological variables did not seem to influence the survival of patients.

Role in Preinvasive Lesions. The aforementioned studies examined the association between presence of HPV and severity or outcome of overt, invasive cervical cancer. It is important also to consider studies analyzing the influence of the type of HPV on the progression through preinvasive stages of cervical cancer, i.e., cervical intraepithelial neoplasia.

Kurman et al. (29) used biopsy specimens from 156 cases of CIN and 58 invasive cervical carcinomas to analyze the correlation of histological grade with presence of HPV. The authors found that HPV 18 was predominantly associated with invasive tumors and high grade CIN (CIN III), whereas the distribution of HPV types 16, 31, and others was more evenly dispersed across the spectrum of severity for the lesions. The expected pattern of association of types 6 and 11 with low-grade lesions was also observed. The authors suggested that the relative deficit of HPV 18 in CIN as compared to invasive disease could be ascribed to a shorter duration of the earlier stages of disease caused by this viral type.

In a follow-up study of 532 women with genital HPV lesions characterized by SBH, Kataja et al. (30) documented regression, persistence, and progression of lesions according to HPV type. Overall HPV positivity was equated to a progression rate across the CIN spectrum more than 3 times that of HPV-negative lesions. HPV 16 was associated with the highest progression rate among all types (35.2% over 50 months). The lowest progression rates were seen for lesions associated with HPV types 6, 11, and 18 (11–14%), as well as for HPV-negative lesions (6%).

HPV in Lymph Node Metastases. HPV detection may also have an important ancillary role in the characterization of metastases of primary cervical cancers. Fuchs et al. (31) analyzed frozen biopsies of primary tumors and lymph nodes from 14 patients with invasive cervical cancer for the presence of DNA of HPV 16 by SBH. They found that the majority (13/16) of metastatic lymph nodes also contained HPV 16 DNA. The authors also correlated the physical state of the viral DNA in the primary tumor with that found in the lymph nodes. The pattern of integration of viral DNA into the host chromosomes tended to be similar between primary tumors and node metastases. A striking finding was the detection of HPV 16 in 18 out of 59 lymph nodes considered histologically negative for metastasis. The authors suggested that HPV DNA hybridization could be a more sensitive indicator of the presence of micrometastasis in nodes but that the potential prognostic value of the presence of viral DNA in histologically negative nodes remained to be proved. Other studies have also demonstrated HPV DNA in lymph node metastases of cervical carcinomas using ISH (32) or PCR (33, 34).

Discussion
As shown, some important contradictory findings have emerged in recent studies examining the association between HPV types and markers of cervical cancer severity and clinical outcome. In the first line of evidence, presence of HPV 18 was considered to be correlated with rapidly progressing lesions (29) and mostly anaplastic histologies (22), features which are characteristic of poor prognosis in cervical cancer. Direct corroboration of this evidence came from the observation of greater recurrence or death rates among women with tumors harboring HPV 18 DNA as compared to other patients (24). Experimental evidence for the more aggressive course of HPV-18-related disease was given by Barnes et al. (35). These authors transfected human epithelial cells with
HPVs 16 and 18 obtaining immortalized cell lines, which were then implanted s.c. in nude (athymic) mice. Dysplastic transformation of the implanted keratinocytes was more frequent and appeared sooner when DNA from HPV 18 was used for transformation, as compared to HPV 16.

On the other hand, the alleged poor clinical course of cervical cancer related to HPV 18 was not confirmed by other studies, two of which had adequate sample sizes and used the proper survival end points for the analysis of disease outcome (26, 28). In addition, the notion that HPV 18 could be a marker of rapid progression through preinvasive stages was challenged by a follow-up study (30).

Only three studies examined the clinical course of overt cervical cancer using actuarial methods to analyze survival time information (23, 26, 28). One of these studies did not find a prognostic effect for HPV positivity (23), whereas the other two presented strong evidence for a negative association between presence of HPV and the probabilities of recurrence (26) and death (28) from cervical cancer. Additional evidence for a negative association has also emerged from a survival study of a subset of cervical cancer cases admitted to a large epidemiological investigation in Latin America, where HPV-negative patients experienced almost twice the risk of death as compared to those who were HPV-positive. The latter studies were the only ones that analyzed survival by multivariate methods, thereby estimating prognostic effects for HPV positivity after adjusting for the confounding effects of other variables.

In addition to the nature of the prognostic relation itself, some important inconsistencies have also emerged from the clinical studies of HPV. The association between the patient's age and likelihood of the tumor being positive for HPV has been said to be negative (particularly for HPV 18) in some studies (24, 28), nonexistent in others (26, 27), or even positive in one study (21). The evidence for a negative association between HPV positivity and age predominates in the cervical cancer literature and is supported by findings for other anogenital sites, such as the vulva (36) and the anus (37). The association may reflect a cohort effect in etiological terms, i.e., cervical cancer acquired by younger women may be predominantly HPV related, as opposed to that affecting older women. Since age itself could be correlated with stage and directly influences length of survival, it becomes crucial to rule out the confounding effect of age when analyzing the prognostic value of the presence of HPV. Although one would feel reassured that in the study by Higgins et al. (28) the prognostic effect for HPV persisted after adjustment by age, there is some concern for residual confounding, since only three relatively wide strata for age were used in the analysis.

One must also consider the relation between HPV type and histology. Most studies have found that HPV 16 tends to be associated with squamous cell carcinomas, whereas HPV 18 affects predominantly glandular epithelium, i.e., adenocarcinomas (23, 28). Since these two types of cervical neoplasms may behave differently in clinical terms, it is important that one eliminates the mutual confounding between viral type and histology by performing stratified analysis or restricting the prognostic evaluation of the virus to one histological type or the other.

Some of the discrepancies among results can be ascribed to the heterogeneity of clinical markers to infer prognosis. Most studies were based on the correlation of HPV genotype with putative markers of disease severity or unfavorable histology found at diagnosis. Since these markers are predictors of cervical cancer survival, the main assumption of these studies is that if a correlation with HPV is identified, then it is possible that indirectly the HPV finding may eventually affect survival as well. Although useful for identifying possible prognostic factors for further analysis, such studies are inherently flawed because of the inability to distinguish genuine prognostic effects from mere secondary associations of prognostic factors with other markers.

The inability to demonstrate prognostic effects can also be attributed to the insufficient statistical power of most studies. The seven studies analyzing the prognostic value of HPV in invasive cancer had case series ranging from 30 to 212 patients. Five of these seven studies included 100 or fewer patients. Only very large to moderately large effects (in terms of hazard ratios) can be detected with such sample sizes. If the confounding effects of tumor size, nodal status, and age are to be adequately controlled for, then subset analyses may be necessary, thereby reducing even further the overall study power. Moreover, the tendency for HPVs 16 and 18 to be associated with distinct histology types (squamous cell for HPV 16 and adenocarcinomas for HPV 18) also highlights the importance of performing subset analyses or covariate adjustment by histology, which further restricts the ability to detect small prognostic effects.

Another source of bias in the estimation of prognostic effects of HPV positivity may be related to the case selection process for inclusion in the analysis. Only a few of the studies reviewed above attempted to describe the selection process so as to ensure that no obvious biases would have occurred. Selection of patients in some studies may have been dictated by the availability of biopsy material or the type of treatment.

An important source of variation may be represented by the method used for detection of HPV. In analogy to the situation in etiological studies, even moderate levels of misclassification of HPV status may cause a marked loss of the ability to detect prognostic associations (38). Most studies reviewed above used the SBH, which has been widely accepted as the gold standard for the detection of HPV DNA. However, variations in specimen quality and tumor sampling areas may have conceivably introduced spurious results. The SBH method requires sizable amounts of fresh or frozen tumor tissue, which restricts its use to prospective studies only. The SBH technique is not adaptable to the examination of formalin-fixed, paraffin-embedded histopathology specimens because of the low recovery of high-molecular-weight DNA. The latter problem may also affect HPV detection by PCR, since protocols based on primers flanking large HPV genome segments are likely to yield false-negative results in specimens the DNA of which is largely fragmented. Degraded DNA may also result from improper fixation (due to inadequate fixative formulation or to the use of excessively large tissue fragments), which
will represent another source of variability in the study of archival specimens.

Without additional experimental, clinical, and epidemiological evidence, hypotheses can be only tentatively formulated to explain a possible prognostic role for HPV in cervical cancer. It is possible that HPV-negative tumors may contain new, not yet identified viral types. Tumors initiated by these new types may follow a different clinical course as compared to those associated with the more common genital types. On the other hand, the absence of HPV DNA in some cervical tumors could indicate that they originated through different mechanisms, e.g., tobacco, hormones, or even other viruses, perhaps resulting in different cell proliferation rates (as a consequence of distinct genomic abnormalities) and, consequently, distinct clinical behavior.

A plausible oncogenic mechanism for HPV that has gained recent acceptance assumes that the products of the E6 and E7 viral genome regions bind to the proteins encoded by two important tumor suppressor genes (p53 and Rb), mimicking loss of the alleles or dysfunctional mutations on those cellular genes (39, 40). To explain the negative relation of HPV with survival, we would have to assume that cervical cancer initiated by HPV-independent carcinogens (or by those hypothetical, not yet identified HPV types) follows a more aggressive course. Conceivably, this model could also accommodate the particular nature of HPV-18 disease. The lower prevalence of this viral type in tumors and the tendency to be found in younger patients could indicate that HPV 18 may be lost after the initiation of the malignant phenotype, perhaps because of a more pronounced genetic instability in the initiated host cell. The fact that HPV 18 is more frequently associated with undifferentiated, anaplastic tumors (24, 35) may be a reflection of such genetic instability. It could be postulated that HPV-18-related lesions that are biopsied after the disappearance of the viral genome would be mistaken as HPV-negative tumors. This would explain why studies of overt disease may fail to detect a worsening of the prognosis for HPV 18 and are more sensitive to the prognostic distinction between HPV-negative and HPV-positive tumors.

One must also entertain the possibility of a secondary association between the presence of HPV in the tumor and prognosis. Since HPV is an epitheliotropic virus it may attain higher proliferation rates in lesions that virus it may attain higher proliferation rates in lesions that

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