Detection of DNA of high–oncogenic-risk human papillomaviruses (HPVs) in cervical cells is increasingly gaining acceptance in primary screening for cervical cancer (1). HPV testing is not only more accurate than Papnicolaou cytology for revealing preexisting cervical precancerous lesions (2) but also more prognostic than the latter test with respect to the subsequent risk of lesions in women who are lesion free at first screening (3). It thus stands to reason that the extent of the predictive value of HPV testing, both cross-sectionally and prospectively, can be augmented by taking into account ancillary measurements of viral load in cervical specimens. A high viral load may indicate a productive HPV infection, which is common in low-grade squamous intraepithelial lesions. Productive viral replication may also result from a primary infection in which host immune control has not yet been developed. In the absence of cytologic abnormalities, a high HPV load may be indicative of an infection that is likely to persist and thus be more prone to develop into a dysplastic lesion. Alternatively, a high HPV DNA load may result from testing a cervical sample that has an abundance of dysplastic cells relative to the background of normal cells that are coexfoliated during specimen taking, thus revealing a high-volume lesion. Therefore, regardless of mechanism, there is plausibility for the expectation that measuring HPV DNA load in cervical samples may be associated with clinical outcomes and thus serve as an additional biomarker in cervical carcinogenesis.

Quantification of HPV DNA load is still restricted to research laboratories. The two most common techniques that can be used for this purpose are real-time polymerase chain reaction (PCR) and low-stringency PCR (4,5), which permit measuring the number of viral copies per amount of DNA or per cell. By design, real-time PCR is HPV type specific, and thus, results are expressed relative to the target HPV type, usually HPV-16, the most common oncogenic type in cervical cancers, precancerous cervical lesions, and in cytologically normal cervical specimens (6,7). Several studies have indicated that HPV16 DNA load is positively associated with the likelihood of existing cervical intraepithelial neoplasia (CIN) of grades 2 or 3 and, among those who are cytologically normal, of a greater likelihood of CIN2/3 detection during follow-up (8–13). However, despite this association, a clinically useful HPV16 DNA load cutoff has yet to be defined.

The course of an HPV18 infection as it progresses to squamous or glandular lesions is not as well understood as that of HPV16-initiated carcinogenesis. Because of its rarity in precancerous lesions, only a few studies have focused on HPV18, which is the second most important oncogenic HPV type in terms of etiologic fraction in cervical cancer (14). Their results have been largely inconsistent (8,10,15–18). Part of the reason for the discrepant findings may be due to the nature of HPV18-induced cervical neoplasia, which preferentially begins above the squamocolumnar junction of the cervix, where HPV18 exhibits tropism for the columnar cells in the endocervical canal.

The study by Xi et al. (19) in this issue of the Journal advances our understanding of HPV18 cervical carcinogenesis. It represents another innovative incursion into the wealth of data and specimens collected as part of the US National Cancer Institute–sponsored “Atypical Squamous Cells of Undetermined Significance (ASC-US) and Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study,” known as ALTS (20). On first inspection, the findings defy logic; contrary to the authors' own ALTS results with HPV16 load (13), the cumulative risk of CIN2/3 in ALTS was not associated with HPV18 DNA load by E7 real-time PCR (19). Interestingly, viral load was strongly associated with an existing cervical cytologic abnormality, with the magnitude of risks increasing with cytologic grade, which favors the expectation of clinical utility. However, when the cumulative history of CIN2/3 was used as a stratifier for this relationship, the HPV18 copy burden increased with cytologic grade among those without CIN2/3 but was fairly constant at intermediate levels among those diagnosed with high-grade lesions. The latter lack of an association with entry cytological grade may be due to the nature of HPV18 infection, which is common in low-grade squamous intraepithelial lesions but preferentially begins above the squamocolumnar junction of the cervix, where HPV18 exhibits tropism for the columnar cells in the endocervical canal.
status was also true for CIN3 lesions. Their findings did not seem to be confounded by age, coinfection with HPV16 or other oncogenic HPV types, and other potential confounders. The authors’ judicious statistical analysis approach further restricting analyses to women with early opportunity for having lesions detected in ALTS (ie, those in the immediate colposcopy and HPV triage arms) indicated that having CIN2/3 was associated with relatively lower HPV18 copy numbers than in those without such lesions, although all women included in the comparison had squamous intraepithelial lesions at enrolment.

Why is it that the association between HPV18 load and cytological grade severity, which was so clear for those without CIN2/3, was nonexistent for those with such lesions? Xi et al. (19) reason along both mechanistic and biological arguments that involve sampling opportunity for lesion detection, site of the lesion, and the degree of viral proliferation in the cervix. The relatively greater propensity for HPV18 to initiate lesions inside the endocervical canal and in the glandular cells within the transformation zone perimeter makes it difficult to capture the full extent of cytological abnormalities in smears in which endocervical cells are underrepresented. As such, detecting HPV18 and, by extension, measuring its copy number may augment the value of cytology in detecting glandular precursor lesions that can eventually develop into adenocarcinomas or mixed squamous/glandular lesions later. The above reasoning implies an artifactual effect of tissue sampling for detecting disease. The fact that HPV18 is underrepresented in the preinvasive stages relative to all other oncogenic types, while being so prominent in squamous cell cancers and adenocarcinomas of the cervix, is consistent with this argument. HPV18 being underrepresented in CIN2/3 may come from the inability to account for the fraction of lesions that were missed because the underlying cytology was of insufficient grade to trigger histopathologic assessment (a form of verification bias). Perhaps because the numbers were too small to justify inclusion, Xi et al. (19) do not provide additional information on cytological or histologic diagnoses that would be relevant to our understanding of HPV18-associated disease, such as atypical glandular cells on the smears or adenocarcinoma in situ.

Another argument by Xi et al. (19) refers to the widely accepted tenet that the physical state of the HPV genome gradually shifts from episomal to integrated as the lesion becomes high grade (21). Viral replication may decrease as a consequence. It is possible that this decrease in viral replication may have occurred for women with documented CIN2/3 lesions in ALTS, although their smears indicated a lesser abnormality or were normal. It is likely that a CIN2/3 detected on follow-up may have been present at enrolment but missed. The fact that there was histological confirmation may indicate that it was of sufficient volume to permit a large enough sample of HPV18-containing malignant cells that went for viral load measurement, without having the sample being diluted by surrounding HPV18-positive cells in earlier and more productive stages of viral proliferation.

As far as clinical practice is concerned, the obvious conclusion from the study of Xi et al. (19) is that quantifying the HPV18 DNA load may not have the same value as for HPV16. Issues related to the accessibility to sampling of the cellular targets of HPV18 carcinogenesis and events related to the physical state of the virus as lesions progress are central to this conclusion. That said, the findings from this study considerably extend our appreciation for the heterogeneity of molecular events and their cellular targets in cervical carcinogenesis. Our ability to understand these events and conceive preventive strategies will rely on conducting natural history studies of HPV infection and cervical lesions at the level of viral type that can circumvent issues related to cervical sampling and disease ascertainment. ALTS has set an important new standard for such studies.

**References**


