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Time to viral clearance after successful conservative treatment for high-risk HPV–infected high-grade cervical intraepithelial neoplasia and early invasive squamous cervical carcinoma

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ABSTRACT

Two-thirds of 152 patients treated for high-grade cervical disease, free of persistence/recurrence, and followed-up both with human papillomavirus (HPV) DNA testing and HPV genotyping cleared their high-risk HPV infection within 1 year. Viral clearance continued at diminishing rates during the second and the third year, at the end of which it was virtually complete.

The rationale for human papillomavirus (HPV) DNA testing in the follow-up of patients conservatively treated for high-grade cervical intraepithelial neoplasia (CIN) (Mariani et al., 2016) and microinvasive squamous carcinoma (Costa et al., 2015) relies primarily on its high or full (Costa et al., 2015) negative predictive value, which would permit to rapidly determine whether treatment has eradicated the disease and to discharge virus-free patients from clinical surveillance (Cuzick et al., 1999). Theoretically, this could enormously reduce the burden of follow-up visits, and the physicians' efforts could concentrate on the subset of HPV-positive patients.

Despite the key importance of the elimination of the virus in follow-up protocols, however, the overwhelming majority of follow-up studies have focused on the persistence or recurrence of infection (Rositch et al., 2014) and on the value of HPV status in predicting the persistence or recurrence of CIN (Cuschieri et al., 2016). As a consequence, the natural history of HPV infection after successful treatment of cervical disease is still poorly understood (Kim et al., 2010). In particular, sparse data have suggested that the time to viral clearance varies greatly, from a few weeks to more than 2 years (Elfgren et al., 1996; Elfgren et al., 2002; Kim et al., 2010; Costa et al., 2015), the causes of which are still unexplained. This would deserve more research because a prolonged time to elimination of the virus weakens the rationale and utility of HPV DNA testing for follow-up purposes and has service and human costs.

We briefly present a study aimed, first, at evaluating the cumulative probability of elimination of high-risk HPV types during follow-up, as assessed both with HPV DNA testing and HPV genotyping, and, second, at identifying the patient and disease characteristics associated with the time to viral clearance. HPV genotyping enabled us to validate the results of HPV DNA testing and to evaluate the type-specific viral clearance. The study took place in 2 third-level clinics in northern Italy. We studied a consecutive series of patients with CIN2–3 treated between 2008 and 2010 and a consecutive series of patients with stage IA1 cervical squamous carcinoma treated between 1997 and 2010 in 1 of the 2 clinics. The eligibility criteria were: no diagnosis of CIN or invasive cervical carcinoma before the diagnostic episode under investigation; positive preoperative HPV DNA test result; detection of at least 1 high-risk/probably high-risk HPV type (i.e., 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53,

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56, 58, 59, 66, 68, 73, and 82); treatment by electrosurgical excisional procedure; histologically confirmed diagnosis of CIN 2–3 and stage IA1 squamous cervical carcinoma; at least 1 follow-up visit; and no diagnosis of CIN2-3 or invasive cervical carcinoma during follow-up, assumed to indicate disease persistence.

At the time of treatment, exocervical and endocervical cytological samples for molecular tests were taken and stored in PreservCytTM medium (Thin Prep®; Hologic, Marlborough, MA, USA). HPV DNA detection was performed using the commercially available Digene Hybrid Capture 2 DNA test (Qiagen [formerly Digene Corporation], Gaithersburg, MD, USA). HPV DNA was extracted with the QIAamp DNA Mini® kit (Qiagen, Hilden, Germany). Extracted DNA was genotyped using the INNO-LiPA HPV Genotyping Extra® assay (Innogenetics, Ghent, Belgium [currently Fujirebio, Pomezia, Italy]) that detects the 18 high-risk/probably high-risk HPVs listed above, 6 low-risk HPVs, and 3 undetermined risk HPVs, targeting a 65-bp fragment of the viral gene L1. Paraffin-embedded tissue blocks of stage IA1 carcinomas treated between 1997 and 2007, originally genotyped for HPV with a homemade protocol (Musiani et al., 2007), were retrieved from the pathology archives, processed, and genotyped using the same techniques as above.

Follow-up procedures were scheduled at 6, 12, 18, 24, 30, and 36 months after treatment. All patients were examined by Pap smear and, in the case of abnormal result, by colposcopy. Directed punch biopsies were taken from colposcopically abnormal areas. In patients with unsatisfactory colposcopy, endocervical curettage was performed. Repeated samples for HPV DNA detection and genotyping were taken at each follow-up visit and were analyzed according to the same protocol as above. Pap smears and histologic specimens collected at baseline and during follow-up were evaluated by dedicated cytologists and pathologists with extensive experience in cervical disease.

Viral clearance was defined as the elimination of the high-risk/probably high-risk HPV type(s) detected in the excision specimens. The viral clearance rates were calculated as cumulative probabilities using the Kaplan–Meier method (StataCorp, 2007). Patients undergoing retreatment during follow-up were censored. Distributions by time to viral clearance were compared using the log-rank test.

The study population was composed of 152 patients. Table 1 shows their characteristics. Fig. 1 shows the cumulative probability of viral clearance during follow-up by type of assay. The 2 curves were almost overlapping, with mutual corroboration of the 2 series of results. The median time to viral clearance was nonsignificantly shorter for the Hybrid Capture 2 DNA test (7 versus 9 months). For both assays, the increase in the probability of viral clearance was steeper in the first year of follow-up and then continued at diminishing rates during the second and the third year, at the end of which the complete elimination of the virus was virtually achieved.

Patient age; severity of the baseline lesion; visibility of the squamocolumnar junction; involvement of the resection margins; mixed-type HPV infection; infection with HPV 16; and infection with HPV 16, 18, 31, 33, and 45 were not significantly associated with the time to viral clearance as assessed through the INNO-LiPA HPV Genotyping Extra assay ($P > 0.1$).

Table 1
Characteristics of patients.

Total number	152
Median patient age in years (range)	35 (23–62)
Patients with baseline diagnosis of CIN2–3	118 (78%)
Patients with baseline diagnosis of stage IA1 cervical squamous carcinoma	34 (22%)
Patients with visible squamocolumnar junction	77 (51%)
Patients with mixed-type HPV infection	53 (35%)
Patients with high-risk HPV infection	152 (100%)
Patients with type 16, 18, 31, 33, and 45 HPV infections	124 (82%)
Median duration of follow-up in months (range)	8 (1–39)
Median number of follow-up visits	1 (1–5)
Median time interval between follow-up visits in months[a] (range)	6 (1–36)

[a] Including the interval to the first visit.

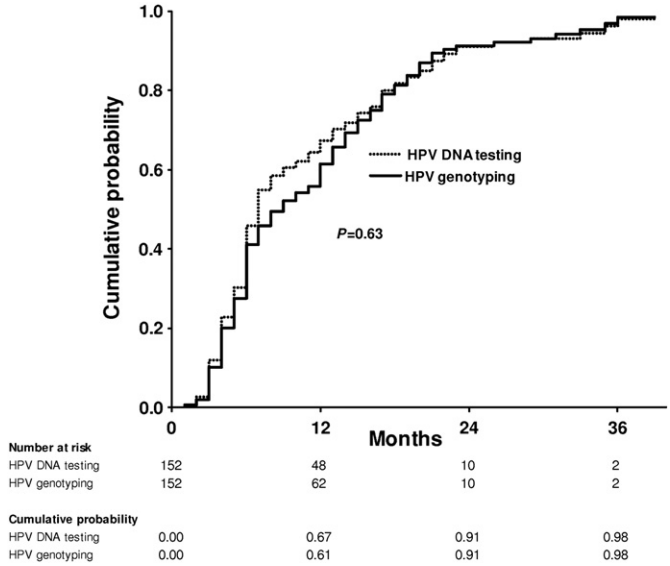

Fig. 1. Kaplan–Meier curve of the cumulative probability of viral clearance, as assessed using HPV DNA testing and HPV genotyping, after conservative treatment for high-risk HPV–infected CIN grades 2–3 and stage IA1 squamous cervical carcinoma. The *P* value is for the log-rank test. The number of patients with cleared infection was 132 with the HPV DNA testing and 134 with the HPV genotyping.

Previous comparable studies have yielded contradictory results. The elimination of the virus has been reported to occur within 3 months of treatment (Elfgren et al., 2002), within 6 months in most patients (Kim et al., 2010), within 1 year (Cuzick et al., 2000), or between 16 and 27 months of follow-up (Elfgren et al., 1996). This variation is explained, in part, by the inclusion of patients treated for low-grade CIN in some studies (Elfgren et al., 2002; Kim et al., 2010) and by differences in computational methods. We focused on high-grade diseases and analyzed our data using formal time-to-event methods. The elimination of high-risk virus types, which would enable the discharge of patients from clinical surveillance, was completed only 3 years after treatment. However, approximately two-thirds of patients cleared their infection within 1 year, which means that HPV DNA testing would quickly make a substantial impact on the load of follow-up appointments.

In previous studies, patient age, parity, and severity of the lesion were not associated with the time to viral clearance (Kim et al., 2010; Du et al., 2013). According to an unconfirmed observation, the elimination of the virus is slower in patients with HPV 16 infection and with high HPV DNA loads (Kim et al., 2010). We took into consideration several clinical and viral factors, including infection with HPV 16, but none was found to influence the time to viral clearance. The persistent lack of knowledge on the determinants of the time to high-risk HPV clearance and on the underlying mechanisms prevents further improvements in the current approach to early ascertainment of cure. The proposed explanations for delays in the elimination of the virus include the transient presence of an adjacent latent high-risk HPV infection with late antiviral immune response (Distéfano et al., 1998) and the transient presence of residual foci of the treated lesion with late antitumor immune response (Costa et al., 2015). Our finding of the absence of association between the involvement of the resection margins and the time to viral clearance seems not to support a role for residual disease in delaying the elimination of the virus.

In conclusion, although the complete elimination of high-risk HPV types after successful conservative treatment of high-grade cervical disease takes 3 years, the impact of HPV DNA testing on follow-up care is already substantial in the first year. Follow-up services can be planned

assuming that approximately two-thirds of cured patients—the large majority of those treated (Costa et al., 2002, 2015)—will be discharged within this time interval. However, the persistent absence of knowledge on the determinants of the time to viral clearance does not allow to further improve the use of HPV DNA testing as a test of cure of high-grade cervical disease.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the review boards of the 2 institutions where patients were treated.

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