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Human papillomavirus viral load: a possible marker for cervical disease in HIV-infected women

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Laboratory markers of human papillomavirus infection have been recognized as relevant tools in programmes designed to reduce the burden of cervical cancer. The ongoing experience with these laboratory markers serves to confirm not only their negative predictive value (close to 100%) but also their positive association with developing or developed lesions. This aspect is particularly relevant in HIV-infected subjects who show an increased prevalence, incidence and severity of infections and lesions even in the era of efficacious control of their immunosuppression. Among the possible virus-related parameters proposed as relevant markers (viral persistence, load, expression, genomic integration capacity) we here analyse the informative value of human papillomavirus viral load measurement as a possible risk marker in this particular clinical setting.

Keywords: viral load, cervical cancer, prevention

Introduction

Human papillomavirus (HPV) is a known cause of cervical cancer,[1] but the high prevalence of transient infections, particularly in younger age groups, makes the detection of the presence of the virus alone an insufficient means to identify women at increased risk of developing high-grade squamous intraepithelial lesions (HGSILs) and cancer.

Further information, such as the viral type(s) involved in the infection, their persistence, load and oncogene expression, have been proposed to increase the positive predictive value of a virological parameter in the detection of developing lesions.

The modification of the above viral markers (generally towards an increment) may be interpreted, in fact, as the effect of a disequilibrium in the interaction between virus and host: an increased replicative capacity of the virus versus a reduced control activity of the immune system. In particular, the escape from the host control mechanisms over the whole process (from infection to lesion development) at both extra- and intra-cellular levels has been recognized as a critical step in the evolution of an otherwise benign and transient infection towards the transformation of infected cells.

In this context it is evident how, in HIV-infected women (and men), both viral and host factors conspire, as these patients are usually more exposed to HPV infection (through their sexual behaviour) and have an impaired immune system.

Since 1995 we have been studying HPV infection in a cohort of approximately 650 HIV-infected women, with the aim of identifying early markers of cervical disease.[2] In our studies we also considered the impact on cervical pathology of HIV disease care, mainly represented by the introduction of potent antiretroviral regimens (highly active antiretroviral therapy, HAART),[3–5] which through the substantial recovery of immune function has significantly changed the scenario regarding HIV-related pathologies such as opportunistic infections and cancers.

These studies include a strict gynaecological follow-up programme, with regular colposcopic examination, PAP test and biopsy (when needed), HPV testing and monitoring of HIV-related markers including CD4+ cell count, HIV viraemia and drug resistance pattern.

Among the HPV-related parameters that we have analysed (typing, persistence, mixed infections, load, mRNA expression), viral load has stimulated our interest as a clinically useful marker, mainly because of the relatively simple technical feasibility of its measurement and interpretation.[4] We here intend to discuss the possible use of HPV load as a predicator of cervical lesions, particularly in HIV-infected women.

Type-specific variability and HPV load

A preliminary consideration of the slightly different biological behaviours of single oncogenic HPV types, related to specific evolutionary patterns (α9 versus α7 groups), is required.[6]

HPV-16 (and its variants) is highly prevalent among HGSILs and cervical cancers, accounting for $\sim$50% of all invasive lesions. This, from the epidemiological point of view, suggests its higher replicative capacity, leading to an increased circulation

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and transmission rate. Moreover, in the cervical disease context, higher HPV-16 viral loads show a direct correlation with more severe lesions.[7–12] A similar association with high viral loads has been demonstrated for HPV-31, -33, -52 and -58, which belong to the same phylogenetic cluster (α9).[13,14] In contrast, HPV-18, -45 and -35 viral loads (α7), although associated with the development of a transformed phenotype, usually show lower replicative activity and a narrower dynamic range.[13,14]

As a first general comment, this observation points to the evaluation of HPV load as a type-dependent risk marker.

Technical considerations of HPV load measurement

Literature data are very heterogeneous on the technical aspects of HPV load measurement. In genital samples, which show a great variation in the cellular yield, a normalization of the result versus the amount of genomic DNA analysed has been suggested as well as screening for inhibitors of the detection/quantification method adopted.

Selecting only the most recent studies, the most relevant differences pertain to the choice of a quantitative approach (such as real-time PCR) versus a semi-quantitative approach and a 'total' or 'type-specific' HPV quantification method.[8,15–18]

With regard to methods for total high-risk HPV (HR-HPV) quantification, several authors (including ourselves) have observed a good correlation with the clinical evolution using the relative light units or index values obtained using the Hybrid Capture II test (HCII; Digene Corp.). Although highly imprecise, a semi-quantitative indication of low, intermediate or high oncogenic-HPV load may be extrapolated in the context of an assay intended for screening purposes. A procedure to count the cells obtained by cervical brushing could help to make HCII values more objective.

A type-specific approach is, of course, the most appropriate and accurate, taking into consideration the above-mentioned differences in type-specific replicative features. However from the practicality and cost-effectiveness points of view, reliable quantitative tests for any single HR-HPV type are difficult to handle in a diagnostic laboratory, while a total HR-HPV DNA test is more affordable.

A parallel test for viral typing could address the interpretation of the quantitative result according to the detected type, also providing information in a follow-up setting, about viral persistence: a recognized risk for the development of lesions. This approach could be particularly useful in HIV-positive patients, where the high prevalence of multiple infections[2] requires a 'generic' diagnostic tool able to select patients at increased risk of developing cervical lesions.

A possible answer to the 'total' versus 'type-specific' question may be provided by the recently proposed multiplex or consensus real-time PCR including pools of type-specific probes, each modified by fluorophores with different emission wavelengths. This approach could allow a reduction in the number of replicates, grouping of the different types according to their type-specific quantitative range and control for sample adequacy for amplification (by house-keeping gene testing).

A further critical point, as for any quantitative assay, is the definition of a clinically relevant cut-off value (e.g. an HPV load above which the patient is considered at risk of developing a cancer) as well as the need for a consensus on a standardized mode of expression of this marker (i.e. number of copies per cell equivalent or per amount of genomic DNA).

The topic of standardization points to the need for quality control procedures and to the availability of calibrated reference samples provided by accredited laboratories (i.e. plasmid cloned HPV genome preparations), to be used to standardize any quantitative results. To date, this aspect has been addressed by a WHO study, involving reference laboratories, that aimed to prepare and to distribute a reference panel, including international standards (IS), to verify the performance of the different diagnostic procedures nowadays applied. This reference panel, although calibrated, was intended to test only the sensitivity of qualitative tests to detect specific oncogenic types (www.who.int/entity/vaccine_research/documents/TechWorkshop_Oct04.pdf).

Epidemiological variables related to HPV load

The patient's age is the most general variable to be considered, as younger women's viral loads tend to be higher because of newly acquired infections and the absence of specific immune control. This consideration discourages the wide use of HPV testing in younger women.[13,19–21] In HIV-positive and immune-compromised patients, elevated HPV load could result from a generally higher shedding of infected cells and/or from enhanced HPV replication. However, the significantly higher persistence rate and HGSIL incidence in young HIV-infected women suggest that these considerations should be treated with caution.[22]

As regards HPV type distribution, HIV-positive women with HGSIL have a pattern of HPV types similar to that of HIV-negative women. In our experience, HPV-16 was detected in 38.3% of cases, alone or in combination with other types (excluding HPV-18), while HPV-18 was detected in 10%, alone or in combination with other types (excluding HPV-16), and both HPV-16 and -18 were detected in 9%. It is noteworthy that in 42.7% of cervical lesions neither HPV-16 nor HPV-18 was detected (F. B. Lillo, manuscript in preparation).

In this population, the higher frequency of multiple HPV infections, as compared with HIV-negative subjects, may account for the increase of total HPV load not only as a summative effect but also as single-type increased relative amount. This aspect, however, is not confirmed by all authors.[13,18,23]

Sexual behaviour, such as multiple partners, and other variables, including parity, cigarette smoking, alcohol and drug abuse, although associated with an increased frequency of infections, do not appear to affect HPV load.

Correlation between HPV load and risk of developing cervical lesions

In the HIV-negative population it has been reported that high HR-HPV loads predict the risk both of an abnormal PAP test[6,8,14,21] and of HGSIL and cancer.[24,25] From these retrospective studies, conducted on archival samples (mostly PAP smears), the authors have demonstrated that, in patients with negative cytology, the probability of developing a histology-confirmed HGSIL or cancer is directly related to the highest percentile of HPV load (OR 58.7, CI 21.9–151.4 for HPV-16; OR 3.3, CI 1.5–7.2 for HPV-18).

In another interesting study, the longitudinal observation of HPV-16-positive women who develop (cases) or do not develop

(controls) HGSIL showed that in the cases, the disease progression was linked to an HPV-16 burden increasing over time, as compared with controls with similar HPV-16 load at baseline observation.[12] HPV load is in fact a measure of viral concentration and only its kinetics over time may give information about viral replication dynamics and disease implications. Our recent data on HIV-positive patients confirm this statement.[4]

Correlation between HPV load and presence of cervical lesions

Several authors have reported that women with higher viral load, both total and type specific, have a significantly greater probability of having SILs and cervical cancer.[7,11,13,17] Some authors have also demonstrated that high HR-HPV load is a predictor not only of histological severity but also of lesion size.[26] To act in accordance with these findings, these authors suggest a more careful diagnostic strategy in discrepant small lesions with high viral load (i.e. endocervical inspection for possible concealed lesions).

Other authors disagree on the predictive value of HR-HPV load for CIN 3 and cancer, pointing to the dominant impact of CIN 1 and ASCUS lesions (with more intense replicative activity especially in the case of multiple HPV infections) surrounding CIN 3 cells.[27]

In our experience in HIV-positive women, this consideration may be relevant, because the histological diagnosis of HGSIL in biopsies from patients with low-grade SIL (LGSIL) cytology is not infrequent. Moreover, among 89 HIV-positive women with HGSIL (cytology and/or histology), 66.5% showed a very high HR-HPV load, 22.4% an intermediate load and only 11.1% a low HPV load, while among 149 subjects with LGSIL, a very high load was detected in 36.7%, intermediate load in 38.3% and low or not detectable HR-HPV load in 25.0% (F. B. Lillo, manuscript in preparation).

We therefore suggest careful monitoring of any cytological or colposcopic abnormality in subjects with high or increasing HPV load to avoid delayed treatment of curable lesions.

Current recommendations to prevent cervical disease in HIV-positive women

In accordance with the recommendation of the Agency for Health Care Policy and Research, HIV-positive women should undergo PAP smear twice during the first year after diagnosis of HIV infection and, if the results are normal, annually thereafter. If the results of the PAP smear are abnormal, care should be provided according to the Interim Guidelines for Management of Abnormal Cervical Cytology published by a National Cancer Institute Consensus Panel.[28,29] Women who have low-grade cytological abnormalities should repeat PAP smear at a shorter time interval than those who are HIV negative and, if the lesion persists or HGSIL or squamous cell carcinoma is diagnosed, should undergo colposcopy, directed biopsy and therapy. These guidelines to prevent cervical disease have not been modified for women on HAART.

The introduction of routine testing for HR-HPV in non-HIV-infected women is likely to be cost-effective (approximately £4233 per life year saved) and will reduce the requirements for repeat smears by between 52 and 85%.[30] However, this could significantly increase the requirements for colposcopy. The cost-effectiveness of HR-HPV testing in HIV-infected women requires further research.

However, if an HPV test is selected as a supplementary test, the use of a quantitative test, instead of a qualitative one, would in addition to the optimal predictive value of a negative test provide information on low or high titre load.

Further efforts should be made to clarify the role of these indicators in cervical disease of HIV-positive patients and their management[4,7,8,22] and to create a consensus approach to their appropriate use and interpretation.

Conclusions

Record linkage studies of population-based registries of people with AIDS and cancer have estimated excess cancer risk for people with AIDS, particularly as regards the AIDS-defining tumours linked to oncogenic viruses, including Kaposi's sarcoma, non-Hodgkin lymphoma and, to a lesser extent, Hodgkin lymphoma and invasive cervical and anal cancer.[31,32]

In recent years (since 1996) the immune recovery obtained with the introduction of HAART has resulted in a marked reduction in morbidity and mortality of HIV-positive individuals mainly because of the control of opportunistic infections. The impact of HIV therapy on some AIDS-defining events such as cervical and anal invasive cancers is, in contrast, still undefined: the prevalence of persistent HR-HPV infection and of dysplastic phenomena has not shown relevant variations even in subjects with a significant recovery in CD4+ T cell count.

Overall, these findings suggest some conclusions:

(i) The natural evolution of cervical cancer and of HIV disease cover different time spans; thus a clear effect of HAART in this setting might be expected in decades, not years.
(ii) We can conjecture a model where, once a dysplastic process is triggered by a long-lasting HR-HPV infection, acquired during a phase of lower immune control, the progression to a more severe neoplastic disease may evolve independent of immune reconstitution.
(iii) At present, HAART schemes tend to be more conservative owing to serious side effects (lipodystrophy, metabolic disorders, drug resistance) and time to start therapy, according to CDC 2005 guidelines, should be delayed until the CD4 count decreases to 200 cells/mm^3, in asymptomatic patients, or between 200 and 350 cells/mm^3 depending on other parameters (HIV load, symptoms).

However, it is also possible to offer some speculations, as the anti-HIV drug market is in continuous evolution and a future positive impact cannot be a priori excluded as novel molecules are under investigation.

There are currently no antiviral agents available with activity against HPV that can be used for the treatment of cervical infections. However, as new therapeutic strategies tend to target non-viral mechanisms such as non-specific cellular binding, metabolic patterns, protein–protein interactions and immune system modulation, a positive interference with HPV-specific mechanisms cannot be excluded.

On the other hand, if the tendency to defer HIV treatment until strictly required is confirmed, its 'ancillary' effect on HPV-related diseases will remain uncertain.

Generally speaking, if a high viral load is associated with the presence of CIN, the ablation of the lesion for diagnostic or therapeutic purposes, contributing to a drastic reduction of viral DNA concentration, offers a better chance for the

immune-mediated clearance of the infection. From this perspective, the specific immune stimulation induced by the incoming HPV vaccines could represent a new tool to control some HR-HPV infections (namely HPV-16 and -18).

However, in the present scenario, the use of HPV vaccines in HIV-positive women is still questionable. To date no data have been presented about the safety and efficacy of vaccination in adult HIV-infected women, but it is reasonable to speculate, as demonstrated by other vaccinations, that an impaired immune system may not guarantee a satisfactory response, requiring a revision of schedule and doses. In the HIV-positive population, the frequency of high-risk infections (up to 60–70%), also owing to multiple and non-HPV-16/-18 types, could hamper the utility of the available products, even if a cross-protective effect towards related types is posited. Moreover a high level of discussion is ongoing regarding the scheme and costs of HPV vaccination in the HIV-negative population. Waiting for the conclusion of Phase III and IV trials, only HPV DNA-negative women are thought to gain advantage from vaccination; HPV DNA negativity is a rare situation in HIV-positive women. In the future, according to individual national health programmes, vaccination should be given to girls (and probably boys) before puberty. If this choice is selected, young girls will be protected from HPV-16/-18 infections prior to their exposure to the risk of acquiring both HPV and HIV infections, but other HR-HPV infections could fill the gap created by HPV-16 and HPV-18 eradication.

In summary, in HIV-positive women, the prevention of HPV-related cervical disease requires particular care as antiretroviral therapies continue to progress in terms of efficacy and reduced toxicity and patient life expectancy and quality continue to improve. The risk of cervical dysplasia and the high frequency of recurrence of SILs and cervical cancer after conventional therapy are, however, increased among HIV-infected women in any clinical stage. Preventing illness associated with serious cervical lesions depends on careful follow-up of patients; in this context, any diagnostic tool capable of reducing the risk of developing any grade of potentially evolving lesion should be welcome.

Transparency declarations

The authors of this article have not made a declaration.

References

1. zur Hausen H. Papillomavirus infections—a major cause of human cancers. *Biochim Biophys Acta* 1996; **1288**: F55–78.

2. Uberti-Foppa C, Origoni M, Maillard M *et al.* Evaluation of the detection of human papillomavirus genotypes in cervical specimens by hybrid capture as screening for precancerous lesions in HIV-positive women. *J Med Virol* 1998; **56**: 133–7.

3. Uberti-Foppa C, Ferrari D, Lodini S *et al.* Long-term effect of highly active antiretroviral therapy on cervical lesions in HIV-positive women. *AIDS* 2003; **17**: 2136–8.

4. Lillo FB, Lodini S, Ferrari D *et al.* Determination of human papillomavirus (HPV) load and type in high-grade cervical lesions surgically resected from HIV-infected women during follow-up of HPV infection. *Clin Infect Dis* 2005; **40**: 451–7.

5. Lillo FB, Ferrari D, Veglia F *et al.* Human papillomavirus infection and associated cervical disease in human immunodeficiency virus-infected women: effect of highly active antiretroviral therapy. *J Infect Dis* 2001; **184**: 547–51.

6. Schiffman M, Herrero R, Desalle R *et al.* The carcinogenicity of human papillomavirus types reflects viral evolution. *Virology* 2005; **337**: 76–84.

7. Fontaine J, Hankins C, Mayrand MH *et al.* High levels of HPV-16 DNA are associated with high-grade cervical lesions in women at risk or infected with HIV. *AIDS* 2005; **19**: 785–94.

8. Josefsson AM, Magnusson PK, Ylitalo N *et al.* Viral load of human papilloma virus 16 as a determinant for development of cervical carcinoma *in situ*: a nested case-control study. *Lancet* 2000; **355**: 2189–93.

9. Lefevre J, Hankins C, Money D *et al.* Human papillomavirus type 16 viral load is higher in human immunodeficiency virus-seropositive women with high-grade squamous intraepithelial lesions than in those with normal cytology smears. *J Clin Microbiol* 2004; **42**: 2212–15.

10. van Duin M, Snijders PJ, Schrijnemakers HF *et al.* Human papillomavirus 16 load in normal and abnormal cervical scrapes: an indicator of CIN II/III and viral clearance. *Int J Cancer* 2002; **98**: 590–5.

11. Ylitalo N, Sorensen P, Josefsson AM *et al.* Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma *in situ*: a nested case-control study. *Lancet* 2000; **355**: 2194–8.

12. Monnier-Benoit S, Dalstein V, Riethmuller D. Dynamics of HPV16 DNA load reflect the natural history of cervical HPV-associated lesions. *J Clin Virol* 2006; **35**: 270–7.

13. Flores R, Papenfuss M, Klimecki WT *et al.* Cross-sectional analysis of oncogenic HPV viral load and cervical intraepithelial neoplasia. *Int J Cancer* 2006; **118**: 1187–93.

14. Moberg M, Gustavsson I, Gyllensten U. Type-specific associations of human papillomavirus load with risk of developing cervical carcinoma *in situ*. *Int J Cancer* 2004; **112**: 854–9.

15. Dalstein V, Riethmuller D, Pretet JL *et al.* Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: a longitudinal French cohort study. *Int J Cancer* 2003; **106**: 396–403.

16. Hernandez-Hernandez DM, Ornelas-Bernal L, Guido-Jimenez Mrgas M *et al.* Association between high-risk human papillomavirus DNA load and precursor lesions of cervical cancer in Mexican women. *Gynecol Oncol* 2003; **90**: 310–17.

17. Sun CA, Liu JF, Wu DM *et al.* Viral load of high-risk human papillomavirus in cervical squamous intraepithelial lesions. *Int J Gynaecol Obstet* 2002; **76**: 41–7.

18. Weissenborn SJ, Funke AM, Hellmich M *et al.* Oncogenic human papillomavirus DNA loads in human immunodeficiency virus-positive women with high-grade cervical lesions are strongly elevated. *J Clin Microbiol* 2003; **41**: 2763–7.

19. Sherman ME, Schiffman M, Cox JT *et al.* Effects of age and human papilloma viral load on colposcopy triage: data from the randomized atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion triage study (ALTS). *J Natl Cancer Inst* 2002; **94**: 102–7.

20. Ordi J, Alonso I, Torne A *et al.* Human papillomavirus load in Hybrid Capture II assay: does increasing the cutoff improve the test? *Gynecol Oncol* 2005; **99**: 313–9.

21. Bigras G, de Marval F. The probability for a PAP test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13,842 women. *Br J Cancer* 2005; **93**: 575–81.

22. Moscicki AB, Ellenberg JH, Crowley-Nowick P. Risk of high-grade squamous intraepithelial lesion in HIV-infected adolescents. *J Infect Dis* 2004; **190**: 1413–21.

23. Heard I, Tassie JM, Schmitz V *et al.* Increased risk of cervical disease among human immunodeficiency virus-infected women with severe immunosuppression and high human papillomavirus load(1). *Obstet Gynecol* 2000; **96**: 403–9.

24. Moberg M, Gustavsson I, Wilander E *et al.* High viral loads of human papillomavirus predict risk of invasive cervical carcinoma. *Br J Cancer* 2005; **92**: 891–4.

25. Andersson S, Safari H, Mints M *et al*. Type distribution, viral load and integration status of high-risk human papillomaviruses in pre-stages of cervical cancer (CIN). *Br J Cancer* 2005; **92**: 2195–200.

26. Sun CA, Lai HC, Chang CC *et al*. The significance of human papillomavirus viral load in prediction of histologic severity and size of squamous intraepithelial lesions of uterine cervix. *Gynecol Oncol* 2001; **83**: 95–9.

27. Sherman ME, Wang SS, Wheeler CM *et al*. Determinants of human papillomavirus load among women with histological cervical intraepithelial neoplasia 3: dominant impact of surrounding low-grade lesions. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1038–44.

28. Wright TC Jr, Cox JT, Massad LS *et al*. 2001 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002; **287**: 2120–9.

29. Wright TC Jr, Cox JT, Massad LS *et al*. 2001 consensus guidelines for the management of women with cervical intraepithelial neoplasia. *Am J Obstet Gynecol* 2003; **189**: 295–304.

30. Legood R, Gray A, Wolstenholme J. Lifetime effects, costs, and cost effectiveness of testing for human papillomavirus to manage low grade cytological abnormalities: results of the NHS pilot studies. *BMJ* 2006; **332**: 79–85.

31. Clifford GM, Polesel J, Rickenbach M *et al*. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. *J Natl Cancer Inst* 2005; **97**: 425–32.

32. Frisch M, Biggar RJ, Engels EA *et al*. Association of cancer with AIDS-related immunosuppression in adults. *JAMA* 2001; **285**: 1736–45.