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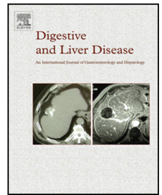

Oncology

Use of human papillomavirus genotyping and biomarkers for targeted screening of anal dysplasia in human immunodeficiency virus-infected patients

Clarisse Dupin [a,b], Laurent Siproudhis [b,c,*], Sébastien Henno [b,d], Sophie Minjolle [a,b], Cédric Arvieux [b,e], Pierre Tattevin [b,e]

[a] Department of Virology, Pontchaillou University Hospital, Rennes, France
[b] Université Rennes-I, Rennes, France
[c] Gastroenterology Unit, Pontchaillou University Hospital, Rennes, France
[d] Department of Pathology, Pontchaillou University Hospital, Rennes, France
[e] Infectious Diseases and Intensive Care Unit, Pontchaillou University Hospital, Rennes, France

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ABSTRACT

Background: Screening for anal dysplasia in human immunodeficiency virus (HIV)-infected patients is not standardized. High-resolution imaging is not adequate for mass screening, and anal cytology requires expertise. New biomarkers, selected because of their use in cervical cancer mass screening, have been originally tested for targeted and easy-to-perform screening.
Methods: 120 HIV-infected individuals (males 96.4%, mean age 47 ± 11 years) were referred for clinical examination, anoscopy, and cytological studies on anal swab. Dysplasia grading, Human Papilloma Virus genotyping, E6/E7mRNA detection and p16^{INK4A}/Ki-67 immunostaining were performed. High-grade lesions were histologically confirmed by anal biopsies after high-resolution anoscopy.
Results: Among the 120 anal swabs analyzed, 36 (30%) had low grade and 6 (5%) had high-grade lesions. Virus genotype was identified in 88 patients (73.3%), and 77 (64.2%) were positive for high-risk genotype(s). High-risk genotype was associated to low-grade or high-grade lesions with a sensitivity of 0.93 and a specificity of 0.51. For E6/E7mRNA, sensitivity and specificity for low-grade and high-grade lesions were, respectively, 0.88 and 0.78. Combination of genotyping, E6/E7mRNA and p16^{INK4A}/Ki-67 appropriately ruled out dysplasia in 55% of patients.
Conclusions: Three routine biomarkers may avoid unnecessary invasive procedures with the perspective of an improvement of patient compliance. A decision making algorithm, based on the combination of these three biomarkers, is proposed.
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1. Introduction

The incidence of anal cancer has dramatically increased over the last decade in high-risk groups, including men who have sex with men (MSM), women with a previous history of cervical or vulvar cancer, and immunocompromised patients, especially those who are Human Immunodeficiency Virus (HIV)-infected [1–4]. Several series estimated that the risk of anal cancer in HIV-infected MSM is comparable to the risk of cervical cancer in the general female population [5,6]. As for cervical cancers, intra-epithelial lesions induced by human papillomavirus (HPV) precede invasive anal cancer. Studies on the prevalence of HPV and its association to dysplasia in anal samples have demonstrated that both HPV and cytological abnormalities are common in high-risk populations. The overall prevalence of HPV infection in anal carcinoma was estimated at 85–97%, HPV-16 being the most frequent genotype, found in ~75% of cases [7,8]. Studies based on systematic cytology of anal swabs yield a much greater burden of abnormal findings than studies based on biopsies restricted to macroscopic lesions. In a large prospective study of HIV-infected MSM, the incidence of anal intra epithelial neoplasm was 37% over a 3-year period [9]. The

* Corresponding author at: Gastroenterology Unit, CHU Pontchaillou, 35033 Rennes Cedex 9, France. Tel.: +33 2 99284311; fax: +33 2 99282404.
E-mail address: laurent.siproudhis@chu-rennes.fr (L. Siproudhis).

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prevalence of abnormal cytology ranges from 30% to 67% in these populations, and high-grade squamous intra-epithelial lesions (H-SIL) are found in 5–24%.

These data advocate for the implementation of systematic screening for anal neoplasia in specific groups [4,10]. However, screening for anal dysplasia in high-risk populations is neither universally recommended, nor standardized. French guidelines recommend annual anoscopy in HIV-infected MSM, and in HIV-infected women with HPV-related genital lesions, and encourages systematic cytological studies of anal swabs during these screening [11], but these recommendations are poorly implemented for the following reasons: (i) the number of specialized clinics offering expert evaluation and anoscopy is not sufficient in most areas; (ii) this invasive procedure may be poorly tolerated by patients, and time-consuming for proctologists; (iii) most pathologists are not familiar with anal cytology. In addition, both methods (i.e. anoscopy and cytological studies on anal swab) have questionable reproducibility and sensitivity to detect dysplasia of the anal canal. Testing several biomarkers, Wentzensen et al. found that HPV DNA genotyping and p16/Ki-67 on anal swabs had the highest sensitivity (92–100%) for the detection of anal dysplasia in high-risk patients [12]. In order to improve the efficiency of screening programs, the aims of the present study were two-fold: (i) to evaluate the diagnostic accuracy of these biomarkers and (ii) to determine their potential use for targeted screening of anal dysplasia in HIV-infected patients.

2. Materials and methods

2.1. Study design

This cross-sectional study was conducted in the Rennes University Hospital, a tertiary care teaching hospital which serves as the referral centre for HIV-infected patients, and for anal pathology in the area (Western France, catchment population estimated at one million inhabitants). All HIV-infected patients were invited for anal neoplasia screening at the department of proctology, with special emphasis in MSM and women with HPV-related genital lesions, according to national guidelines. The first visit consisted in clinical examination, digital rectal examination, anoscopy, and collection of anal swabs for cytological studies. Data about life style and HIV disease were collected using a standardized questionnaire: gender, age, sexual activity, past history of sexually transmitted infection(s), and HIV-related parameters, including CDC stage, antiretroviral treatment (ART), CD4 cell counts, and HIV viral load. Before anoscopy, two Dacron swabs were collected from the anal canal and immediately suspended into ThinPrep PreservCyt medium (Hologic Inc., Bedford, MA): one was sent for cytology and immunohistochemistry studies, the other one for virological analysis. Specimens were maintained at +4 °C before processing for analyses or aliquoted and stored at −80 °C. All patients diagnosed with abnormal cytology through this first step underwent high-resolution anoscopy (HRA), and guided-biopsies were performed for histological studies if lesions were suspected, using acetic acid and Lugol's solution for better visualization. No biopsy was obtained from patient with normal cytology.

2.2. Cytology and histology

Two slides were prepared with the same medium. One was stained for cytology and the other one for immunochemistry. The pathologist who analyzed samples has a long-standing experience in the analysis of anal samples, and was blinded to the results of biomarkers (HPV genotype, HPV E6/E7 mRNA and p16/Ki-67). Cytology grades were reported according to the Bethesda classification: no intraepithelial lesion (NIL), atypical squamous cell of undetermined significance (ASC-US), low-grade or high-grade squamous intraepithelial lesions (L-SIL or H-SIL) [13]. The same pathologist classified histology as normal, low- or high-grade intraepithelial lesions.

2.3. p16^{INK4A}/Ki-67 dual staining

All specimens were tested for dual immunostaining p16^{INK4A}/Ki-67 CINtec® PLUS (Roche, MTM Laboratories AG, Heidelberg, Germany), according to the manufacturer's instructions. Protein p16 ^{INK4A} is a cell cycle regulatory protein overexpressed in high risk (hr) HPV persistent infection, and Ki-67 is a proliferation marker. The kit is a combination of two antibodies against p16^{INK4A} and Ki-67 used in the same slide, with different chromogens. The presence of at least one double-stained cell is interpreted as a positive test.

2.4. HPV DNA detection and genotyping

DNA testing was performed using the PreservCyt medium: 200 μL were used for DNA extraction on MagNA Pure LC (Roche, Bâle, Switzerland). Anal specimens were tested for 35 HPV genotypes with CLART Human Papillomavirus 2 (Genomica, Madrid, Spain), a commercial kit for the detection of 15 low risk (lr) HPV (6, 11, 40, 42, 43, 44, 54, 61, 62, 71, 72, 81, 83, 84, 89), and 20 high risk (hr) HPV (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82, 85). The tests included a cellular control and an internal control. Microarray studies were analyzed using Clinical Arrays Reader (Genomica). A sample of 2 mL of each liquid-based medium was then frozen at −80 °C. A hr-HPV DNA test was considered positive if at least one hr-HPV genotype was detected.

2.5. High-risk (hr) HPV E6/E7 mRNA detection

Specimens were tested for E6/E7 mRNA using HPV APTIMA assay (Hologic) on the PANTHER System, which detects E6/E7 viral mRNA of 14 hr-HPV types collectively by transcription-mediated amplification (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68). An aliquot of 1 mL of each sample was transferred into the APTIMA transfer tube and introduced into the PANTHER system without any other manipulation. According to the manufacturer's instructions, a cervix sample specimen is considered positive when the value of the signal-to-cut-off ratio (S/CO) is >1. Because there is no validated S/CO threshold for anal samples, we reported the quantitative result of the hr-HPV E6/E7 mRNA test, and empirically used a conservative S/CO ratio threshold of 6. To validate this threshold, we tested HPV DNA negative samples and found that they all were either negative for hr-HPV E6/E7 mRNA, or had a S/CO ratio < 6.

2.6. Ethics

The database was authorized by the national regulatory institution, Commission Nationale Informatique et Liberté (CNIL n°1412467), and the study was approved by our institutional review board (N09-14). All participants provided written informed consent.

2.7. Statistical analysis

Mean and standard deviation were used to describe quantitative variables; number and percentage were used to describe qualitative variables. Differences between groups were measured using non-parametric tests where appropriate (Wilcoxon, Pearson, Chi2 or MacNemar). A P value <0.05 was considered significant. The performance of p16^{INK4A}/Ki-67 dual staining, hr-HPV DNA, and hr-HPV

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