Gynecologic Oncology 117 (2010) 77–81

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Original Research Article

Triage of women with atypical squamous cells of undetermined significance (ASC-US): Results of an Italian multicentric study ☆

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ARTICLE INFO

Article history:
Received 14 October 2009
Available online 8 February 2010

Keywords:
HPV
Screening
Triage
Cervical cancer

ABSTRACT

Objectives. To compare the performance of immediate colposcopy, repeat Pap test and HPV test as triage options for women diagnosed as having atypical squamous cells of undetermined significance (ASC-US) while attending organised screening for cervical carcinoma in five centres of the Veneto region.

Methods. Women consecutively diagnosed as having ASC-US were included in a prospective study, and underwent colposcopy and collection of cervico-vaginal cells for conventional Pap test and HPV test (Hybrid Capture 2, High-risk probe set, Digene). Repetition of all three tests was scheduled for 12 months later. DNA was subsequently extracted from residual cells of positive samples, and analysed by polymerase chain reaction with several primers for typing of HPV sequences. Sensitivity, specificity and positive predictive value (PPV) of the different triage options for histology-confirmed cervical intraepithelial neoplasia, grade 2 or worse (CIN2+) were calculated among all women and by age (under and above 35 years).

Results. Seven hundred forty-nine women 25–64 years old (median age 42 years) were enrolled in the study. Pap smears at enrolment were read as ASC-US or more severe in 211 (29.4%) cases, colposcopy disclosed an atypical transformation zone in 254 (34.2%) women, and HPV test was positive in 181 (24.2%). High-grade cervical lesions developed in 29/749 (3.9%) women. HPV typing was possible in 163 (90%) of the samples, and carcinogenic types were present in 123.

Conclusions. HPV test showed the best performance; overall, it had the highest sensitivity (92.3%), specificity (78.6%) and PPV (14.9%).

Introduction

Cervical cancer is etiologically linked to persistent Papillomavirus (HPV) infection with high-risk types, mainly HPV 16 [1], and can be prevented by early detection and treatment of pre-neoplastic high grade lesions (cervical intraepithelial neoplasia grade 2 or higher—CIN2+), or by prophylactic vaccination [2].

The screening process has been relying so far on identification of abnormal cervical cells by Pap test, followed by colposcopy (with directed biopsy when indicated) in case of detection of abnormal squamous cells. According to the Bethesda 2001 classification [3], these abnormal cells can be diagnosed as high-grade squamous intraepithelial lesion (HSIL), low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells, probably high-grade (ASC-H), or atypical squamous cells of undetermined significance (ASC-US). The proportion of CIN2+ is very low among women with an ASC-US diagnosis [4], so identification of those at higher risk would be clinically useful [5]. Three different options can be used for triage of women with an ASC-US diagnosis: repeat cytology, immediate colposcopy, or HPV search for high risk types (from now onward referred to as HPV test). HPV testing has been shown to be more accurate than repeat Pap testing for detection of high grade lesions [6], but the moderate to poor reproducibility of the cytologic ASC-US diagnosis [7] warrants local investigations for assessing the best triage option. Moreover, since in most women, and particularly in those under 35 years of age, HPV infection, even with high risk types, is

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doi:10.1016/j.ygyno.2010.01.003

transient in nature, it is important to evaluate the results of the different tests in relation to women's age.

Differences in carcinogenicity among HPV types, with type 16 infection carrying a risk of cancer development substantially higher than for the other eleven carcinogenic types [8], constitute the rational for investigating the clinical usefulness of HPV type definition.

Here we present the results of a prospective study involving women with an ASC-US diagnosis enrolled in five screening centres in Northeast Italy.

Materials and methods

Patients

Women attending the organised screening program for cervical carcinoma prevention in five centres (ULSS7, ULSS8, ULSS13, ULSS16, ULSS18, hereafter indicated as A, B, C, D, E) located in the Veneto Region (Northeast Italy) were invited to participate to the study if their Pap test was diagnosed as ASC-US, according to the 2001 Bethesda classification system, less than 12 months before. The five screening centres in the central year of study enrolment had frequencies of ASC-US diagnosis ranging from 0.6 to 2.5% (median 1.5%).

At enrolment, after informed consent, the participating women underwent colposcopy (with punch biopsy when indicated) and collection of cervico-vaginal cells for repeat Pap test (conventional slide) and HPV test, and were advised that the same procedures were to be repeated 12 months later. Women with any positive finding were invited for an additional visit after 6 months to repeat Pap and HPV tests. Women with CIN2+ underwent excisional therapy and follow-up according to the regional guidelines.

HPV search

Cervico-vaginal cells were initially analysed by Hybrid Capture 2 (HC2, Digene, Qiagen) with the High Risk probe set, according to the manufacturer's instructions. All samples with a Relative Light Units/Positive Control (RLU/PC) ratio>1 were considered positive. An aliquot of each sample was kept at −40 °C for further analyses.

HPV typing

DNA was extracted from frozen aliquots of HC2-positive samples, and evaluated for amplificability by using the primers GH20/PC04 which amplify a 268 basepair (bp) fragment from the beta-globin gene. HPV sequences were searched for by using MY09/MY11 consensus primers; the 450 bp amplicons were digested by Restriction Enzymes (RE) *Rsa*I, *Hae*III, *Dde*I (and eventually *Pst*I) (Roche Diagnostics, Basel, Germany) to define the specific type(s) [9,10]. Negative and untypable samples were further amplified by GP5+/GP6+ primers, and the 150 bp fragments sequenced for type identification, as previously described [11]. A few samples were also analysed by the INNOLipa (INNOGenetics) kit, according to the manufacturer's instructions. All samples were also amplified by HPV 16 specific H16L1/H16R3 primers, which amplify a 323 bp fragment of the E6 gene; the amplicons were sequenced to investigate the viral variants [11]. According to the evaluation [8] by the International Agency for Research on Cancer (IARC), HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 were classified as high-risk types (carcinogenic).

Statistical analysis

Sensitivity, specificity and positive predictive value (PPV) of the different triage options for histology-confirmed CIN2+ were calculated among all women and among two age groups (under and above 35 years). We also computed the receiver operating characteristic (ROC) curves, as an overall measure of test accuracy. The association between independent variables and viral clearance was evaluated by a multiple regression test using the STATA 10.0 software; $P<0.05$ was considered statistically significant. For viral persistence, women surgically treated in the time interval evaluated were excluded, and in the case of multiple infections, the presence of at least one of the types detected at enrolment was considered as persistent infection.

Results

Study population and results at enrolment

Between March 2005 and July 2006, 749 women 25–64 years old were enrolled in the study. Follow-up ended in December 2007. Median age was 42 years; 198 (26.4%) were under 35 years of age, and 551 (73.6%) were above 35 years of age. In Italy, women are invited to screening every three years, according to geographical or to age criteria. As a consequence, age distribution of women screened in a given period of time can vary among centres; in our study, the proportion of women aged<35 was similar for centres A, B, and C, and differed in centres D and E (Table 1).

The reference ASC-US diagnosis, for most of the enrolled women, had occurred in the screening episode attended during the 3 months before enrolment (median 72.2 days).

Pap smears at enrolment (available for 717 women) were read, blindly to the results of HPV test, as normal in 490 women (68% of the cases), ASC-US or higher (ASC-US+) in 211 (29.4%) (ASC-US in 99, 13.8%), and inadequate in 16 (2.2%).

Colposcopy (available for 742 women) was within normal limits in 488 (65.8%), while an atypical transformation zone was observed in 254 (34.2%) women, 237 of which with grading 1 (G1), and 17 with grading 2 (G2).

Search for HPV DNA sequences by means of HC2 (High Risk probe set) gave a positive result (RLU/PC>1) in 181/749 (24.2%) women. Prevalence varied by age (<35 years: 86/198, 43.4%;>35 years: 95/551, 17.2%) and by screening centre, as shown in Fig. 1.

Performance of the tests as triage options

Colposcopy-directed cervical and/or vaginal biopsies were taken in 338 women, either at enrolment or during follow-up. In 150 samples, no morphological alterations were recorded and 8 resulted inadequate, while low grade lesions (HPV, CIN1, VAIN1) were diagnosed in 149 women, and high grade lesions in the remaining 31 (9.2%) cases (14 CIN2, 15 CIN3, 2 VAIN2). In relation to age, high grade lesions were diagnosed in 16/198 (8.1%; 9 CIN2 and 7 CIN3) women <35 years, and in 13/551 (2.4%; 5 CIN2 and 8 CIN3) women >35 years.

Overall, CIN2+ lesions were detected in 3.9% (29/749) of the women; in 14.9% (27/181) of HPV-positive cases, and in 0.35% (2/566) of HPV-negative women.

Sensitivity, specificity and positive predictive value (PPV) of repeat Pap test, colposcopy, and HPV test in identifying women with or at

Table 1
Main characteristics of enrolled women and findings by centre.

Centre	Frequency of ASC-US diagnosis (%)	Study subjects (*N*)	Subjects >35 years (%)	HPV positivity (%)	Frequency of CIN2+ (%)
A	2.0	90	72.2	25.5	4.44
B	2.5	239	72.4	20.5	1.67
C	0.6	103	71.8	40.8	7.76
D	1.4	181	84.5	18.9	5.52
E	1.5	136	63.2	23.5	2.20
Heterogeneity (*p*-value)	–	–	0.001	<0.001	0.045
Overall	1.6	749	75.6	24.2	3.9