ONCOLOGY

Prior knowledge of HPV status improves detection of CIN2+ by cytology screening

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OBJECTIVE: The objective of the study was to investigate whether knowledge of human papillomavirus (HPV) deoxyribonucleic acid test results increases sensitivity of guided cytology screening for the detection of cervical intraepithelial neoplasia (CIN)-2 or higher-grade cervical lesions.

STUDY DESIGN: This was a prospective colposcopy-controlled study of 2905 BD SurePath samples to identify cases with CIN2+ within a 24 month follow-up period. Sensitivity and specificity to detect CIN2+ was evaluated, comparing guided cytology screening with and without prior knowledge of HPV status.

RESULTS: Prior knowledge of HPV status resulted in significantly higher detection rate of CIN2+ compared with screening blinded to HPV status ($P = .005$) with limited loss of specificity ($P = .026$). Gain in sensitivity is higher in older women (43.8%, $P = .008$) vs in younger women (10.2%, $P = .317$), whereas loss of specificity is more pronounced in younger women ($P < .001$) vs older women ($P = .729$).

CONCLUSION: Guided cytological screening performed with prior knowledge of HPV status results in an improved detection of CIN2 or higher-grade lesions.

Key words: cervical cancer, cervical cytology, human papillomavirus, human papillomavirus genotypes, real-time polymerase chain reaction

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The recognition of the strong causal relationship between persistent infection with high-risk (HR) human papillomavirus (HPV) (HR-HPV) types, and cervical cancer and its precursors, has resulted in the development assays that detect viral nucleic acids as an alternative for or as an adjunct to cervical cytology.[1] One can distinguish assays that detect all HR-HPV types as a group and genotyping tests that distinguish individual HPV types.[2] Liquid-based cytology is now gradually replacing conventional cytological testing, because of practical advantages (quicker interpretation, easy ancillary molecular testing, and possibility of computerized guided screening), in spite of the lack of evidence that it increases the detection of cervical intraepithelial neoplasia (CIN)-2 or higher-grade cervical abnormalities.[3-5]

Cytological screening combined HR-HPV testing and HPV-based screening followed by cytology triage have been evaluated as more sensitive than cytology screening alone.[6] Compared with cytology alone, this screening strategy improves detection of precancerous growths but with a certain increase in the number of false-positive tests.[6-8] Recent randomized trials have confirmed that HPV-based screening, in women older than 30-35 years followed by cytology triage results in detection of more CIN2 or worse lesions compared with cytology screening. Moreover, longitudinal results of these trials have demonstrated that women with a negative HPV test have a lower risk of CIN3 and even invasive cancer.[9,10]

This study aimed to evaluate the influence of knowing the different HR-HPV genotypes present in cervical specimens before performing guided cytological screening.

Materials and Methods

Study population

In this prospective, colposcopy-controlled study, we enrolled 3126 voluntary participants from August 2005 until February 2007 (Figure 1). Samples were collected during opportunistic routine health checks by 11 selected gynecologists in Flanders

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The first 2 authors contributed equally to the study and manuscript.

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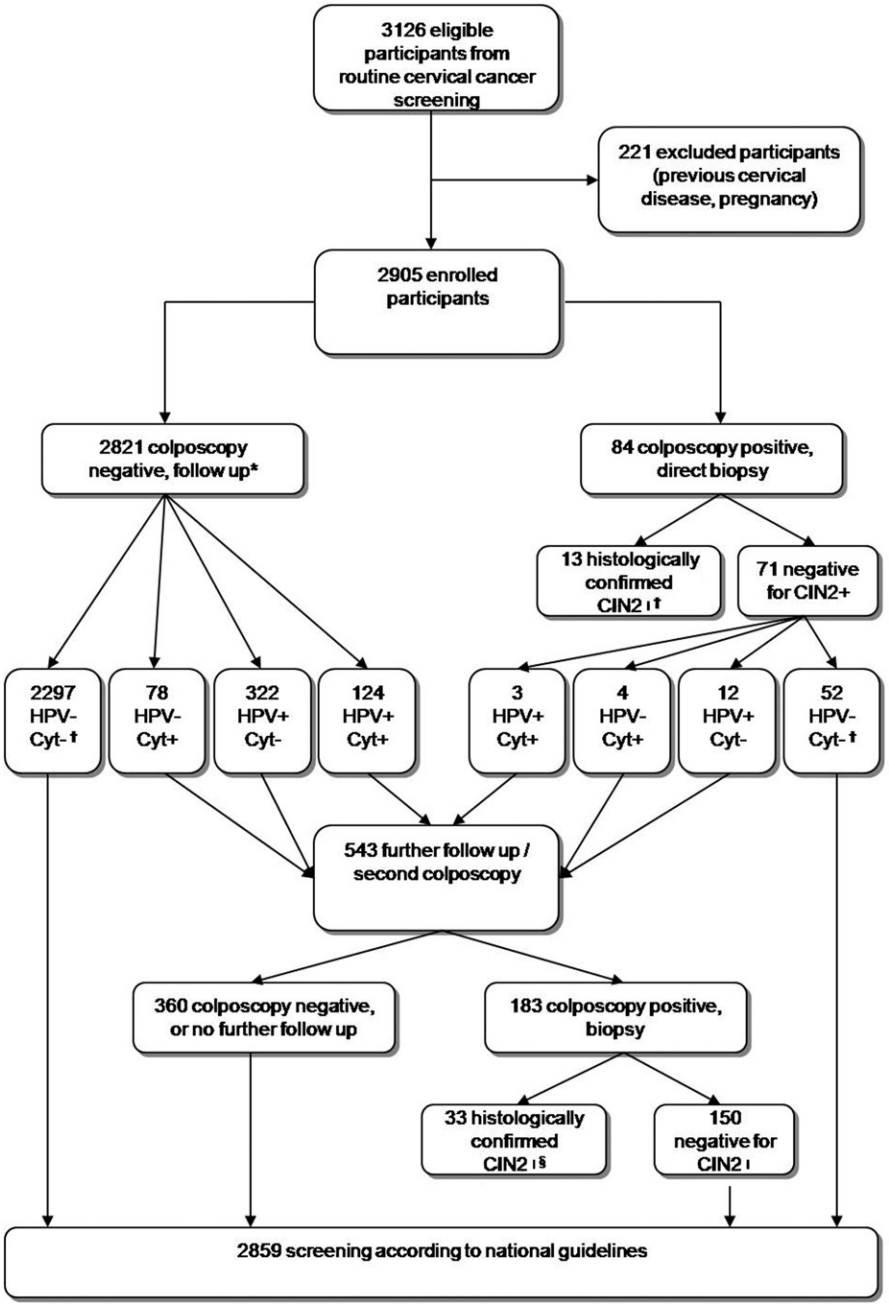
FIGURE 1
Enrolled women classified by colposcopy test results, screening results, and detection of CIN 2 or higher



Asterisk indicates follow-up defined as HPV genotyping by real-time PCR analysis and cytology screening (with and without prior knowledge). *Dagger* indicates HPV DNA test negative and cytology screening test negative defined as no further referral for study procedures; screening was according to national guidelines applicable. *Double dagger* indicates that of which 5 women had CIN2 lesions and 8 women had CIN3+ lesions. *Section mark* indicates that this includes 16 women with CIN2 and 17 women with CIN3+ lesions.

CIN, cervical intraepithelial neoplasia; *Cyt-,* cytology negative; *Cyt+,* cytology positive; *HPV,* human papillomavirus; *PCR,* polymerase chain reaction.

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(Belgium). All women gave written informed consent.

Exclusion criteria included pregnancy and history of cervical disease (previous history of CIN2+); 221 women were excluded. At enrollment, participants underwent a colposcopy after smear taking. Colposcopy was performed in the framework of this study to obtain a gold standard. Study-specific patient identification codes were assigned and transmitted in such a manner that patient confidentiality was preserved and linked with follow-up and histology results. This study was approved by the local ethical committee (Ziekenhuis Oost Limburg, Genk, Belgium).

Cervical sample processing

Slide preparation

Cervical cells were collected using the Cervex-Brush (Rovers, Oss, The Netherlands).

After collection, brush heads were transferred directly into alcohol-based preservative (SurePath; Tripath Imaging Inc, Burlington, NC), and the vials were transported to the Laboratory for Clinical Pathology (labo RIATOL, Antwerp, Belgium). Thin-layer slide preparations were made with the fully robotic AutoCyte PREP System (AutoCyte; Tripath Imaging) and were prepared as described elsewhere.[11]

High-risk HPV testing

All specimens from the screening visit were tested for HPV deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR) amplification.DNA isolation from liquid-based cytology was performed as previously described.[12,13] Briefly, HPV DNA was extracted from cervical cells using standard proteinase K-based digestion according to the manufacturers' protocol. Washed cell pellets were incubated with proteinase K solution (100 μg/mL) for 3 hours at 55°C. First, each sample was subjected to quantitative PCR (qPCR) amplification for the detection of β-globin to confirm that the DNA quality was suitable for PCR analysis.

All samples were tested for the presence of 14 different HR-HPV genotypes using TaqMan-based real-time qPCR, targeting type-specific sequences of viral genes: 16 E7, 18 E7, 31 E6, 33 E6, 35 E6,