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A daunting challenge: Human Papillomavirus assays and cytology in primary cervical screening of women below age 30 years

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Abstract We compared cytology with Hybrid Capture 2 (HC2), cobas, CLART and APTIMA Human Papillomavirus (HPV) assays in primary cervical screening at age 23–29 years based on data from the Danish Horizon study. SurePath samples were collected from 1278 women undergoing routine cytology-based screening. Abnormal cytology was managed according to the routine recommendations, and women with cytology-normal/HPV-positive samples were invited for repeated cytology and HPV testing in 1.5 years. Loss to follow-up was similar between HPV assays. ⩾CIN3 was detected in 44 women. The sensitivity of HC2 for ⩾CIN3 was 95% (95% confidence interval (CI): 85–99), of cobas 98% (95% CI: 88–100), of CLART 100% (95% CI: 92–100), of APTIMA 82% (95% CI: 67–92), and of cytology 59% (95% CI: 43–74). Specificity for ⩾CIN3 varied between 61% (95% CI: 59–64) for cobas and 75% (95% CI: 73–78) for APTIMA, and was 94% (95% CI: 93–96) for cytology. Similar results were observed for ⩾CIN2 ($N = 68$). HPV screening with cytological triage doubled the number of colposcopies compared to cytology screening, and increased the frequency of repeated testing by four (APTIMA) to seven (cobas) times. The positive predictive value of a referral for colposcopy was relatively high for all screening tests (⩾30% for ⩾CIN3, and ⩾50% for ⩾CIN2). CIN1 was detected by cytology in ~1% of women, and in ~2% by any of the four HPV assays. Although highly sensitive, HPV-based screening of

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young Danish women should be approached cautiously, as it resulted in large reductions in specificity, and increased the demand for additional testing.

1. Introduction

Human Papillomavirus (HPV) infections are frequent in young women, but the majority clears spontaneously. To avoid false-positive test results, HPV-based primary cervical screening has been considered primarily for women aged ⩾30 years [1–4]. Nevertheless, some studies suggested that certain HPV assays, particularly those based on detection of HPV mRNA rather than DNA, might be suitable for screening at younger ages. In the French FASE (French APTIMA Screening Evaluation) study, for example, 1109 women aged 20–29 years attending routine cervical screening were tested with ThinPrep liquid-based cytology (LBC), Hybrid Capture 2 (HC2) HPV DNA assay, and APTIMA HPV mRNA assay [5]. In that study, APTIMA detected as many cases of high-grade cervical intraepithelial neoplasia (CIN) as HC2, whereas its specificity for high-grade CIN was similar to that of LBC. However encouraging, these findings should ideally be confirmed by data from other studies. Unfortunately, other studies comparing APTIMA with HPV DNA assays in screening populations did not present data specifically for this age group [6] or did not ascertain histological results in women with positive HPV tests and normal cytology [7].

Here, we presented data from the Danish Horizon study using samples from 1278 women aged 23–29 years attending primary cervical screening. All samples were tested with SurePath LBC and four HPV assays (APTIMA HPV Test (APTIMA; Hologic, San Diego, CA), HC2 (QIAGEN, Gaithersburg, MD), cobas HPV Test (cobas; Roche Diagnostics, Pleasanton, CA), and CLART HPV2 Assay (CLART; Genomica, Madrid, Spain)). We used these data to study the impact of the five tests on screening sensitivity, specificity, proportions of women with false-positive tests, and on colposcopy referral rates in primary screening of young women.

2. Materials and methods

2.1. Study design

The design of the Horizon study was described in detail previously [8–12]. Consecutive SurePath samples from 5034 women arriving for routine LBC analysis at the Department of Pathology of Copenhagen University Hospital, Hvidovre, in June–August 2011 were tested with the four HPV assays. By linkage to the national Pathology Data Bank (Patobank) [13], primary screening samples were defined as those without a: previous cervical cancer, CIN in ⩽3 years, atypical squamous cells of undetermined significance (ASCUS) or non-CIN cervical biopsy in ⩽15 months, or a more severe cytological abnormality, inadequate cytology, or a positive HPV test in ⩽12 months. Approximately 10% of women aged 23–29 years living in the then-catchment area of the laboratory were vaccinated against HPV [14].

Danish women are recommended for routine cervical screening every 3 years from age 23 onwards. Women included in the Horizon study were managed in line with their cytology and HPV test results, so that this setup where cytology was the basis for routine clinical management also mimicked primary screening with HPV testing and cytology triage. Women with abnormal cytology, regardless of their HPV status, were managed according to the routine Danish guidelines (repeated cytology if ASCUS or low-grade squamous intraepithelial lesions (LSIL), referral for colposcopy otherwise). Women with a positive test result on at least one of the four HPV assays at baseline and normal cytology (i.e. triage-negative) were invited, for study purposes, for repeated cytology and HPV testing in November 2012, approximately 1.5 years after the baseline. A reminder was sent in March 2013.

Women who responded to the study follow-up invitation had two SurePath samples taken. Those with abnormal cytology or a positive HC2 test result (corresponding to the routine HPV testing in the laboratory at the time of the study) were recommended for colposcopy. Histology in women with follow-up outside of the study was included in the analysis. All follow-up outcomes were retrieved from the Patobank in December 2013. This means that all histology was ascertained in approximately 2.5 years after the baseline testing, i.e. throughout most of the recommended 3-year screening interval. Colposcopies were performed following routine protocols recommending biopsies from all suspicious areas, or random biopsies from the four quadrants if lesions were not visible.

2.2. Cytology

Routine cytological evaluation was undertaken first by FocalPoint Slide Profiler (BD, Burlington, NC). Blinded to the outcomes of HPV testing, samples were thereafter evaluated by cytoscreeners using FocalPoint GS Imaging System (BD), and abnormal findings were adjudicated by pathologists.