The Absolute Risk of Cervical Abnormalities in High-risk Human Papillomavirus–Positive, Cytologically Normal Women Over a 10-Year Period

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Abstract

In spite of the success of cervical cytology as a cancer-screening tool, it has important limitations, and human papillomavirus (HPV) testing may be valuable in future screening. The majority of women in screened populations, who test HPV positive, will have a concurrent normal smear, and we need more information about the risk for subsequent high-grade cervical lesions in these women. We examined 8,656 younger women (22–32 years old) and 1,578 older women (40–50 years old) who were followed for development of cervical neoplasia (cytology and/or histology) through the Danish Pathology Data Bank. We estimated the proportion of women developing cervical lesions of different types before a given time point as a function of time. Among women with normal cytology and positive high-risk Hybrid Capture 2 (HC2) test, 17.7% and 24.5% of younger and older women, respectively, had a subsequent abnormal Pap smear within 5 years. The risk of CIN3 or cancer within 10 years among younger women with positive HC2 test was 13.6% (10.9–16.2) and 21.2% (2.7–36.1) among older women. An analysis among younger women also being HC2-positive 2 years before baseline showed a subsequent 10-year risk of ≥CIN3 of 18% (14.6–21.5). Among older women where HPV may be added to general screening, the estimated absolute risk of ≥CIN3 in HC2-positive women was more than 20% within 10 years. These results indicate that even a single positive HPV test in cytologically negative women is substantially predictive of high-grade CIN and suggest that HC2 testing can help stratify women into different risk categories. (Cancer Res 2006; 66(21): 10630-6)

Introduction

Certain types of human papillomavirus (HPV) have now conclusively been shown as a necessary cause of cervical cancer (1). In addition, epidemiologic studies have shown a strong association between high-risk HPV types and the development of high-grade cervical intraepithelial neoplasias (CIN) (2–4). Cervical cancer screening based on the Papanicolaou (Pap) smear test has been credited with a significant reduction in the incidence of this disease. However, data currently available from organized cytology-based screening programs indicate that the declining trend in the disease incidence seen in the years following introduction of these programs has leveled off or may even be increasing in some populations (5). Moreover, no strong effect has been found on the incidence of adenocarcinomas, which constitute 5% to 10% of cervical cancers (6).

In spite of its long history of success as a cancer-screening tool, cervical cytology has important limitations. In a recent meta-analysis conducted by Nanda et al. (7) of 97 studies, it was concluded that cervical cytology, when used under optimal conditions, is only moderately accurate and that the average sensitivity to detect cervical cancer or precancerous lesions was considerably lower than generally believed.

HPV testing in relation to primary screening has been shown to usually have a higher sensitivity and negative predictive value for the detection of clinically relevant preinvasive disease than cervical cytology. However, the use of HPV testing as a screening tool is hampered by its relatively low specificity and the absence of an agreed protocol for handling women who are HPV positive but who do not have cytologic abnormalities.

As the majority of women in screened populations, who test HPV positive, will have a concurrent normal smear, it is important to assess the absolute risk for the subsequent development of abnormal cytology to establish appropriate clinical management and counseling. Only two recent studies have assessed the absolute risk of subsequent abnormal Pap in HPV-positive women with concurrent negative cytology, and found that 17% (8) and 20% (9) of the women were diagnosed with abnormal Pap or low-grade squamous intraepithelial lesion, respectively, during a follow-up period of ~4 to 5 years.

It is the aim of this study to estimate the absolute risk of different outcomes (≥atypia on cytology, ≥moderate dysplasia on cytology, histologic diagnosis of ≥CIN2, and ≥CIN3) during more than 10 years of follow-up of women from the general population who were cytologically negative and high-risk HPV DNA positive by Hybrid Capture 2 (HC2) test at enrollment in the study.

Materials and Methods

Study Population

The study population consists of women participating in two Danish population-based prospective cohort studies examined simultaneously and at the same study clinic.

Younger cohort. This cohort was based on a random sample of women (20-29 years of age) drawn from the general female population in

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Copenhagen, using the computerized Central Personal Registry (CPR). From May 1991 to January 1993, 11,088 women were included in the study after informed consent was obtained (approved by the Scientific Ethics Committee and the Data Protection Board). A detailed description of the study design has previously been provided (10). Briefly, at the enrollment, all 11,088 women had a gynecologic examination, where a Pap smear was taken, and endo-ectocervical cell material was obtained for HPV DNA detection. Two years later, from October 1993 to January 1995, the cohort was invited for a second examination in the same order as they were originally enrolled. Initially, the cohort was linked to the Central Personal Register using the CPR number as key identifier. All women in the cohort were traced using this register and information on vital status and current address was retrieved. During the following 1.5-year period, we included 8,656 (78%) women in this second examination. Again, Pap smears and cervical swabs for HPV testing (placed in PBS with 0.05% methiolate) were obtained and kept at −80°C until tested. In addition, all women went through a personal interview, conducted by female nurses especially trained for this task.

The study population in this article composes the 8,656 women participating in the second examination, and baseline in the present analysis is defined by the date of the second examination. We excluded 381 women who participated through a telephone interview only. We also excluded women with inadequate or missing baseline smear (n = 174), women with abnormal smear at baseline (n = 191; 73% of atypia, 84% of mild/moderate dysplasia, and 88% of ≥severe dysplasia were HPV positive), women being followed for an abnormal Pap smear diagnosed within 1 year before baseline (n = 45), and women who did not contribute a cervical sample at baseline (e.g., due to menstruation) or had a specimen that was inadequate for HPV testing (n = 336), leaving 7,529 women with normal cytology at baseline. Finally, only women with ≥1 smear during the follow-up period were included, leaving 7,218 women for study.

Older cohort. Simultaneously with the second examination of the younger cohort (October 1993–January 1995), we established a cohort of older women. A sample of 2,200 women (40–50 years of age) was drawn at random from the same study area as the younger cohort (Copenhagen) by means of the Central Personal Register. The older women were examined at the same study clinic and by the same study staff as in the younger cohort. A total of 1,578 (72%) women were enrolled in the study. Pap smears and cervical swabs for HPV testing were obtained and stored in PBS with 0.05% methiolate at −80°C until tested, and the women were interviewed using the same structured questionnaire as the younger women. We excluded 120 women only participating through a telephone interview, women with inadequate or missing smear at baseline (n = 64), women with abnormal baseline smear (n = 22), women followed for an abnormal Pap smear diagnosed within 1 year before baseline (n = 7), and women who did not contribute a cervical sample at baseline or had a specimen inadequate for HPV testing (n = 12). This left 1,353 women with normal cytology at baseline; of these, the 1,305 women with at least one smear during follow-up were included in the study.

Follow-up

The Central Personal Register. In Denmark, every citizen has a unique 10-digit identification number (CPR no.). These identification numbers, which comprise information on gender and date of birth, are registered in the Central Personal Register. The register is updated daily and contains information on, for example, vital status and migration including the current address. The existence of the unique personal identification numbers, which are used universally in the Danish society, including the public administration, secures correct linkage with different registers. In addition, it implies that in Denmark, follow-up studies can be done with virtually no loss of follow-up.

The Pathology Data Bank. This is a nationwide pathology register containing information on all cervical cytology (organized and opportunistic, normal and abnormal) and all cervical biopsies and cones (normal and abnormal histology) done in Denmark. The Pathology Data Bank also comprises historical data for the previous 20 to 30 years. The communication between the various pathology departments and the Pathology Data Bank operates through an online and real-time system. The abnormal cytologic diagnoses are mostly reported using the nomenclature atypia, mild dysplasia, moderate dysplasia, severe dysplasia, and carcinoma *in situ*. The histologic diagnoses were translated into the CIN nomenclature with moderate dysplasia categorized as CIN2 and severe dysplasia and carcinoma *in situ* categorized as CIN3. In Denmark, cervical cancer screening is recommended every 3 years from age 23 years.

Using the CPR number as key identifier, the cohorts were linked to The Pathology Data Bank and followed until November 2004 to obtain information on all cervical cytologic and histologic examinations and to identify all cervical pathology and treatments that had occurred. The women were unaware of the HPV results obtained in the study, and the study HPV testing was not used in any referrals for colposcopy, treatment, or management of the women. Likewise, HPV testing has not yet been introduced in Denmark, neither in the triage of women with minor cytologic cervical abnormalities nor in cervical cancer screening.

HPV Detection

The laboratory personnel and the investigators were fully blinded to cytology and clinical diagnosis. Due to the fact that the specimens were collected into a medium (PBS) that is not recommended for the HC2 test, a conversion protocol was done to allow HC2 testing. We used the HPV16-positive cervical carcinoma cell line SiHa as a positive control and HPV-negative C33A cells as a negative control. An aliquot of cervical swab specimens stored at −80°C in PBS was transferred into a Digene specimen collection tube and adjusted to a final concentration of 1 mol/L guanidine hydrochloride. After addition of 50% of the sample volume of denaturation reagent (HC2 HPV DNA Test Kit), the tubes were placed in a specimen collection tube rack, rigorously vortexed for 10 to 20 seconds, and incubated in a water bath at 65°C for 45 minutes, followed by another round of vortexing for 5 seconds. A 75-μL aliquot of the samples was then tested with the high-risk probe of the HC2 assay using the robot platform device (RCS1) that can simultaneously process four microtiter plates at the same time. In addition to the three negative and positive controls per HC2 microplate assay, we included as a control two samples of 10^4 C33A cells and two samples (10^4 and 10^5 cells) of the SiHa cell lines per four microplates tested to monitor the performance of the HC2 assay. The assay was done with the Food and Drug Administration–recommended cutoff of 1.0 pg/mL using only the high-risk probe that detects at least 13 oncogenic types (11). HPV testing on the older cohort was done as indicated above except that HPV detection using HC2 with the high-risk probe was done manually. Replicate assays were done on a number of plates on both cohorts with virtually identical results.

Statistical Analysis

The percentages of women developing ≥atypia during follow-up were compared between high-risk HPV-negative and high-risk HPV-positive women within age group (younger or older) using Fisher's exact test.

The women were followed for incident abnormal cervical cytology/histology from baseline until November 2004. We estimated the proportion of women developing cervical lesions of different types before a given time point as a function of time. The estimation was carried out assuming a model of piecewise constant intensity, taking into account that the exact failure times are unknown (12); the only information available being either that an event occurred between two dates of testing or that no event had occurred by the last test date (interval censored observations). For the younger cohort, the intensity was assumed constant within the following intervals of follow-up time: <3, 3 to 4, 4 to 5, 5 to 6, 6 to 7, 7 to 8, and ≥8 years, whereas for the smaller, older cohort, we assumed a constant intensity in the intervals <3, 3 to 6, and ≥6 years.

Results

Risk of abnormal cytology following one positive HR-HPV test. Figure 1 presents a description of the two cohorts. At baseline, the 7,218 younger women with normal cytology were 22 to 32 years

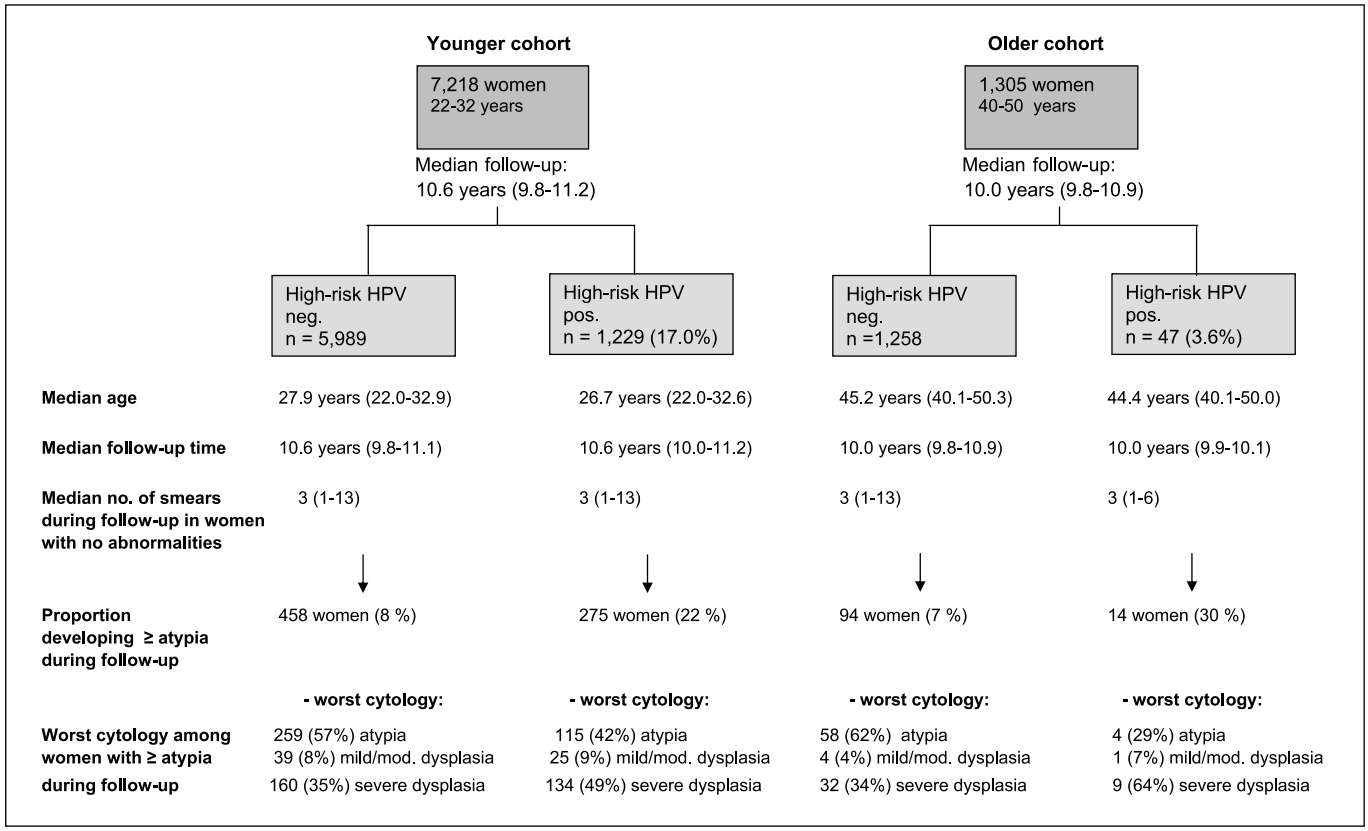

Figure 1. Overview of the two study cohorts.

of age, the median age being 27.7 years. The women were followed for a median of 10.6 years (range, 9.8–11.2 years). Overall, women with no cervical abnormalities in the follow-up period had a median of 3 (range, 1–13) follow-up Pap smears, whereas the median number of Pap smears in women with a diagnosis of atypia or worse during follow-up was 6 (range 1–21; data not shown).

A total of 1,229 (17.0%) younger women were positive for a high-risk HPV type at baseline. The follow-up time was similar for HPV-positive women and HPV-negative women. The median number of smears during follow-up among women who stayed cytologically negative was 3 in both the HPV-negative group (range, 1–13) and the HPV-positive group (range, 1–13). When considering the entire follow-up period (until November 2004), 458 (8%) of the HPV-negative women and 275 (22%) of the HPV-positive women experienced cervical abnormalities (≥atypia) during follow-up. In these groups of women, the distribution of the worst cytologic diagnosis was different, with more atypias in the HPV-negative group and more ≥severe dysplasias in the HPV-positive group.

The 1,305 women in the older cohort had a median age of 45.2 years at baseline and the median follow-up time was 10.0 years (range, 9.8–10.9 years). Women staying cytologically normal during follow-up had a median of 3 (range, 1–12) follow-up smears, whereas women developing atypia or worse had a median of 6 (range, 2–20) Pap smears during follow-up (data not shown).

Among the 1,305 older women, 47 (3.6%) were high-risk HPV positive at baseline. There was no difference in follow-up time between HPV-negative women and HPV-positive women. Among HPV-negative women, as well as HPV-positive women, staying cytologically normal during follow-up, the median number of smears in the follow-up period was 3. During the entire follow-up period, 30% among the HPV-positive women had an abnormal smear. In contrast, only 7% of the HPV-negative women had ≥atypia on cytology. The worst cytologic diagnosis among those who developed cervical abnormalities during follow-up differed between HPV-negative and HPV-positive older women. The difference was more pronounced among older women than seen in the younger cohort; e.g. 64% of the HPV-positive older women had a worst cytologic diagnosis of severe dysplasia or worse compared with only 34% of the HPV-negative older women.

Absolute risks of subsequent cytologic cervical abnormalities in younger and older women are shown in Figs. 2 and 3. For younger women with normal cytology and a concurrent positive test for high-risk HPV DNA, it was estimated that 17.7% [95% confidence interval (95% CI), 15.4–20.0] experienced a cytologic diagnosis of atypia or worse (Fig. 2*A*) and 8.2% (95% CI, 6.6–9.9) a moderate dysplasia or worse within 5 years (Fig. 2*B*). The corresponding 10-year estimates were respectively 25.4% (95% CI, 22.0–28.6) and 13.4% (95% CI, 10.8–15.0). In contrast, women who were both cytologically negative and tested negative for high-risk HPV had much lower risks of subsequent cervical abnormalities, respectively 4.7% (95% CI, 4.1–5.3; ≥atypia) and 1.6% (95% CI, 1.3–1.9; ≥moderate dysplasia) after 5 years, and 9.1% (95% CI, 8.1–10.2; ≥atypia) and 3.4% (95% CI, 2.8–4.0; ≥moderate dysplasia) after 10 years (Fig. 2*A* and *B*).

For comparison, estimated absolute risks of subsequent cervical abnormalities in older women (40–50 years old) are shown (Fig. 3).

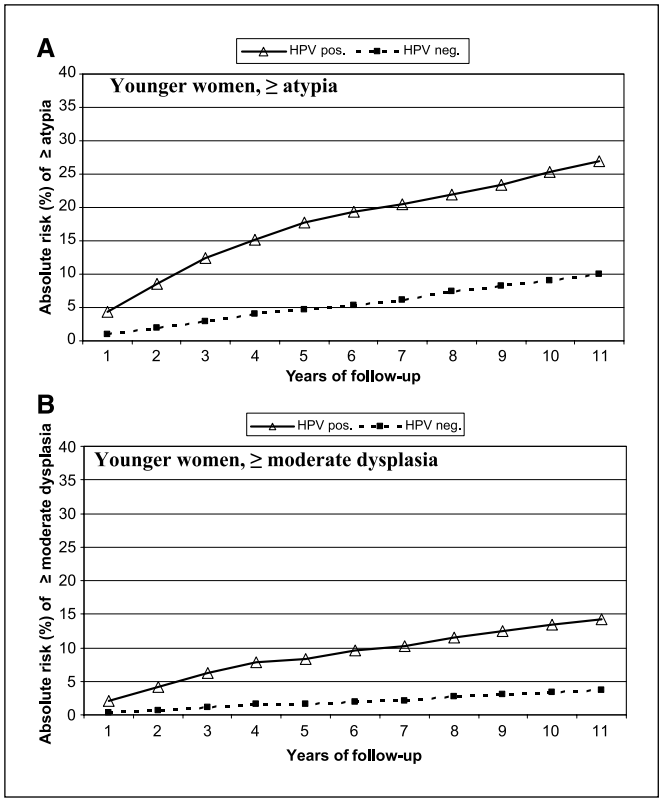

Figure 2. Absolute risks of respectively ≥atypia and ≥moderate dysplasia in younger women with a normal baseline cytology in relation to concurrent HPV status.

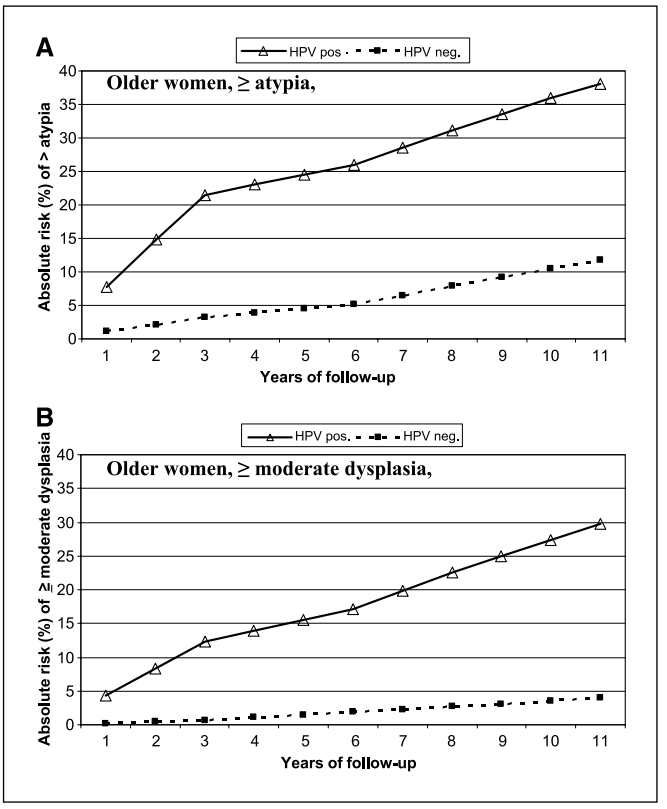

Figure 3. Absolute risks of respectively ≥atypia and ≥moderate dysplasia in older women with a normal baseline cytology in relation to concurrent HPV status.

Women with normal cytology at baseline and a concurrent positive high-risk HPV test had risks of 24.5% (95% CI, 10.6–36.3) for a subsequent cytologic diagnosis of atypia or more severe and 15.6% (95% CI, 4.0–25.8) for moderate dysplasia or worse after 5 years, and the corresponding absolute risks after 10 years were 35.9% (95% CI, 14.6–51.9; ≥atypia) and 27.4% (95% CI, 7.0–43.3; ≥moderate dysplasia). After 5 years of follow-up, older women with both negative baseline cytology and a negative high-risk HPV test had an absolute risk of respectively 4.5% (95% CI, 3.3–5.7) for atypia or more severe and 1.4% (95% CI, 0.7–2.1) for moderate dysplasia or worse (Fig. 3*A* and *B*). At 10 years, the absolute risk was respectively 10.5% (95% CI, 8.0–12.9; ≥atypia) and 3.6% (95% CI, 2.1–5.1; ≥moderate dysplasia; Fig. 3*A* and *B*).

Risk of ≥CIN2 and ≥CIN3 following one positive high-risk HPV test. We also wanted to assess differences in absolute risk of a histologic diagnosis of ≥CIN2 and ≥CIN3 among younger and older high-risk HPV-negative and HPV-positive women, respectively, during 3-, 5-, and 10-year follow-up periods (Table 1). Among the younger women with negative baseline cytology and a positive high-risk HPV test, 6.8% developed CIN2 or worse up till 5 years, whereas this only applied to 1% of the women with a negative test for high-risk HPV. Among the older women, this difference was bigger as 11.3% of women with a positive high-risk HPV test at baseline developed ≥CIN2, in contrast to only 0.4% among high-risk HPV-negative women. The estimated absolute risk of ≥CIN2 after 10 years was 3.7% (95% CI, 3.0–4.4) among younger high-risk HPV-negative women and 16.2% (95% CI, 13.4–19.0) among younger high-risk HPV-positive women. Again, the difference in risk between the high-risk HPV-negative and HPV-positive groups was more pronounced among older women where the estimated risk within 10 years was 1.9% (95% CI, 0.8–3.0; HPV-negative group) and 22.9% (95% CI, 4.4–37.8; HPV-positive group). This was also reflected in the corresponding relative risks after 3, 5, and 10 years, which were 9.3, 6.8, and 4.4 among younger women, and 78.8, 28.3, and 12.1 among older women, respectively. Finally, looking at the risk of developing CIN3 or cancer within 10 years, the risk among initially cytologic negative women with a positive high-risk HPV test was nearly two times higher in older women (21.2%) than in younger women (13.6%; Table 1).

Risk of CIN3 and cancer following two high-risk HPV-positive tests. Finally, in the younger cohort, we assessed the absolute risk of CIN3+ following two times positive high-risk HPV tests [i.e., baseline high-risk HPV-positive women who also tested high-risk HPV positive 2 years before baseline in the current study (at the initial examination of the younger cohort)]. Similarly, we also assessed the risk of ≥CIN3 following two times negative high-risk HPV tests (baseline high-risk HPV-negative women who also tested high-risk HPV-negative 2 years before baseline). A total of 414 women tested positive at both examinations whereas 5,165 women were HPV-negative at both occasions (data not shown). The absolute risk for ≥CIN3 at 3, 5, and 10 years following two times positivity for high-risk HPV types was 3.9% (95% CI, 2.0–5.8), 8.5% (95% CI, 5.7–11.3), and 20.0% (95% CI, 14.7–24.9), respectively. The absolute risk in younger women with two negative high-risk HPV tests with 2 years in between was, for ≥CIN3+, 0.1% (95% CI, 0.02–0.2) within 3 years, 0.5% (95% CI, 0.3–0.6) within 5 years, and 2.3% (95% CI, 1.7–2.9) within 10 years (Fig. 4).

Table 1. Absolute risk of a subsequent histologic diagnosis of ≥CIN2 and ≥CIN3 among women with normal baseline cytology and with or without concurrent positive high-risk HPV test

Follow-up time (y)	≥CIN2 during follow-up			≥CIN3 during follow-up		
	HPV status at baseline		Relative risk	HPV status at baseline		Relative risk
	HPV negative	HPV positive		HPV negative	HPV positive	
	Absolute risk % (95% CI)	Absolute risk % (95% CI)		Absolute risk % (95% CI)	Absolute risk % (95% CI)	
Younger women						
3 y	0.3 (0.1-0.4)	2.8 (1.9-3.8)	9.3	0.2 (0.08-0.3)	2.2 (1.3-3.0)	11.0
5 y	1.0 (0.8-1.3)	6.8 (5.4-8.3)	6.8	0.8 (0.6-1.0)	5.5 (4.2-6.8)	6.9
10 y	3.7 (3.0-4.4)	16.2 (13.4-19.0)	4.4	3.1 (2.4-3.7)	13.6 (10.9-16.2)	4.4
Older women						
3 y	0.8 (0-0.2)	6.3 (0-13.0)	78.8	0.08 (0-0.2)	4.3 (0-9.9)	53.8
5 y	0.4 (0.04-0.7)	11.3 (1.6-20.0)	28.3	0.4 (0.4-0.7)	9.3 (0.4-17.3)	23.3
10 y	1.9 (0.8-3.0)	22.9 (4.4-37.8)	12.1	1.7 (0.6-2.8)	21.2 (2.7-36.1)	12.5

Discussion

Results from this prospective cohort study with a long-term follow-up of women from the general population through the routine screening system showed that among women 40 to 50 years old, who were cytologically negative with a concurrent positive HPV DNA test (high-risk types), nearly 25% developed cytologic abnormalities (≥atypia) within 5 years, and after 10 years, more than 35% had had an abnormal Pap test. These risk estimates were higher than those observed among women with both negative cytology and negative HPV DNA test (high-risk types), which were as low as 4% and 10% after 5 and 10 years, respectively. Also among younger HPV-positive women (22-32 years old at enrollment), we found a high absolute risk of subsequent cervical abnormalities (≥atypia), 18% after 5 years and 24% after 10 years, and a low risk among HPV-negative women, 5% after 5 years.

The results from our younger cohort are nearly identical to those observed in a recent study by Castle et al. (7). They found that ~17% of women (median age at enrollment, 28 years) attending a screening clinic, having negative cervical cytology and positive high-risk HPV DNA test, developed atypical squamous cells or worse within 57 months (~4.8 years), whereas among cytologically negative women who also tested negative for high-risk HPV types, 4.2% had atypical squamous cells within 57 months.

In the present study, there was not a great difference in the risk of cytologic abnormalities (atypia or more severe) between younger and older cytologically negative women without high-risk HPV DNA detected. In younger women, the majority of subsequent abnormalities, especially the milder ones, most likely were caused by transient HPV infection, whereas some lesions in the older women may actually have been nonneoplastic lesions originating from atrophic epithelial changes resembling cellular atypia.

Defining cytologic abnormalities at the level of ≥atypia may obviously cover very heterogeneous cytologic categories when applied to different groups. Thus, although we observed a high risk of cervical abnormalities both in younger and older high-risk HPV DNA–positive women, the importance and implications of these results may be different. In this study, the group of older HPV-positive women developing ≥atypia covered a cytologic diagnosis of no worse than atypia in only 29% of the cases and severe dysplasia or worse in as much as 64%, whereas among the younger HPV-positive women developing ≥atypia during follow-up, atypia was the worst diagnosis in 42%, and 49% had ≥severe dysplasia. This reflects the well-known HPV natural history phenomenon that, especially among younger women, HPV infection is mostly of transient nature and therefore causes mostly mild cytologic changes, whereas in older women, a higher proportion of HPV infections are persistent and are thus more likely to induce more severe cellular changes. In line with this, and taken together with the fact that HPV infection is more common in younger women (17% in the present study) than in older women (3.6% in the present study), HPV DNA testing in cervical cancer screening has been suggested for women older than 30 years. It should be emphasized that the absolute risks seen in the present study are a result of the underlying screening pattern in Danish women, and as reported by Castle et al. (7), the existing screening intensity will influence the estimates of absolute risk for abnormal Pap tests.

When looking at more severe outcomes like a histologic diagnosis of ≥CIN2 and ≥CIN3, we observed substantial differences in the subsequent absolute risks between baseline cytologically negative women with and without a concurrent positive high-risk HPV DNA test. Within 5 years, 11% had developed CIN2 or worse among the older HPV-positive women compared with only 0.4% among the older HPV-negative women, and after 10 years, nearly one fourth of the older HPV-positive women had a diagnosis of ≥CIN2, whereas the absolute risk was more than 10 times lower (1.9%) in the high-risk HPV-negative women. As expected, the risk of ≥CIN2 among younger HPV-positive women was lower, although not negligible (16% after 10 years), and the risk in HPV-negative women was higher (3.7% after 10 years) than among the older women, which suggests a faster kinetic of HPV acquisition and subsequent CIN development in younger women. This is also reflected in the differences between the 10-year positive predictive values of a single positive HPV DNA test (high-risk types) for the cytologic detection of ≥moderate dysplasia, which was 21% in older women and only 13% to 14% in younger women Likewise, the 5-year positive predictive value for the detection of ≥CIN2 was 11% in older women and ~7% in younger women.

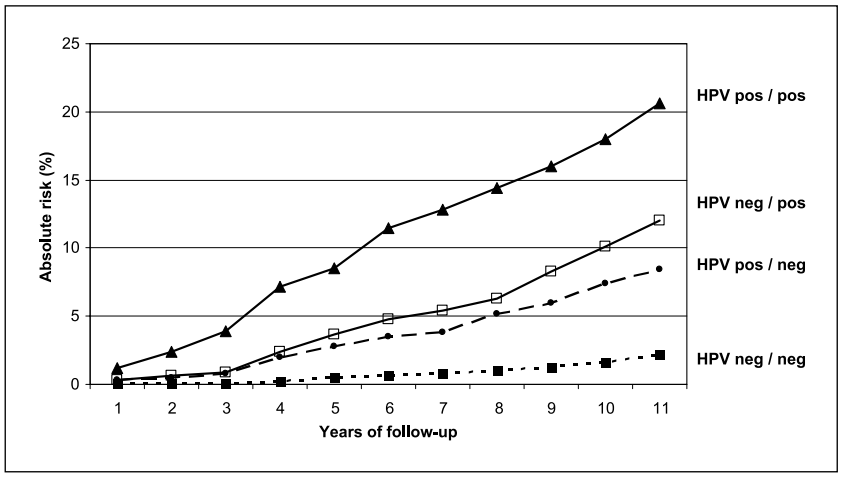

Figure 4. Absolute risks of subsequent ≥CIN3 in younger women with normal baseline cytology and two high-risk HPV tests, one negative and one positive, or two high-risk HPV negative tests.

It has previously been shown that adding HPV testing as a reflex to mildly abnormal cytology adds to both sensitivity and specificity for detection of underlying clinically relevant lesions (13). In the present study, we show that in a screening context, a concurrent high-risk HPV negative test in cytologically normal women carries a high long-term negative predictive value. The 5-year negative predictive value for ≥CIN2 was 99% for the younger cohort and 99.6% for the older cohort, suggesting that the screening interval might be safely increased.

In the younger cohort, we were also able to assess the absolute risk of CIN3+ following two HPV tests (HC2 high-risk HPV types) taken at an interval of 2 years. The negative predictive value of two negative tests was better than for one negative test, although this was already very high. An additional positive test significantly increased the absolute risk and positive predictive value. This of course reflects that two positive tests include a higher proportion of women with persistent infection compared with only one positive test. Even with this better predictive value in younger women, the clinical value may be limited as compliance is likely to decrease with number of visits needed. Instead, we ideally need one test constituting a better marker for progression. Recently, it has been reported that an HPV test distinguishing HPV16 and HPV18 infections from the other types may better identify women at risk for developing ≥CIN3 (14, 15).

Our study has the strength that there was virtually no loss to follow-up, which may be a problem in some other studies. This is due to the existence of the unique personal identification numbers in Denmark and the nationwide coverage of the registries used for the follow-up procedure. It should be emphasized that our results are based on a follow-up of routine screening and management, where the clinicians had no knowledge of the HPV status. The study may be limited by being based on everyday clinical management rather than being a randomized trial. This may play a role especially when looking at outcomes like ≥CIN3 because a very intensive screening (as in some study trials) and management of milder lesions may change the natural history and thereby underestimate the cumulative incidence rates of CIN3 or worse. However, in Denmark, cervical cancer screening is recommended from age 23 years and every 3 years, and screening and management of cervical abnormalities is less aggressive than in, e.g., Germany and the United States. To assess whether there were different biopsy rates between younger and older or HPV-positive and HPV-negative women, which might affect comparisons, we assessed the proportion of women who had a biopsy taken following a cytologic diagnosis of milder abnormalities (atypia or mild dysplasia), and we found that there was no significant difference in biopsy taking among older and younger women, and no difference between HPV-positive and negative women as the percentage of women with a biopsy following atypia/mild dysplasia was relatively low (~30%) in all groups (data not shown). In addition, we found that the screening intensity was also very similar in younger and older women and in HPV-positive and HPV-negative women (Fig. 1). Another limitation may be that the cytologic and histologic diagnoses originated from routine examinations and were not subjected to an expert review, and thus some misclassification may occur.

In summary, in this relatively large-scale prospective study where we have followed a younger and an older cohort with negative baseline cytology for more than 10 years, we found a high long-term (5 years) negative predictive value (≥99%) of a single HPV negative test for both ≥CIN2 and ≥CIN3. We also showed that a single high-risk HPV positive test (HC2) at baseline is associated with a high absolute risk of subsequent development of cytologic abnormalities (≥atypia), and that respectively 1 of 10 within 5 years and 1 of 5 within 10 years of the women ages 40 to 50 years developed ≥CIN3. These results indicate that even a single positive HPV test in cytologically negative women is substantially predictive of high-grade CIN, and suggest that HPV testing by HC2 can help to stratify women into different risk categories for high-grade cervical abnormalities. One repeated round of HPV testing further increased the negative and the positive predictive value of a negative or positive test result, respectively. However, an exact algorithm for follow-up of cytologically negative women with a concurrent high-risk HPV DNA positive test will have to be decided on the basis of a formal medical technological analysis, including cost-effectiveness modeling. Finally, HC2 positivity may constitute too crude a measure so that we have to add analyses for specific HPV types, such as HPV16 and HPV18, to differentiate between high-risk HPV infections with a higher oncogenic potential and those that can be managed less intensively.

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