The age-specific prevalence of human papillomavirus and risk of cytologic abnormalities in rural Nigeria: Implications for screen-and-treat strategies

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Cervical screening for carcinogenic human papillomavirus (HPV) infection is being considered for low-income countries. Effectiveness requires targeted screening in older women in whom prevalent infections are more likely to be persistent and predictive of precancer. Some studies in West Africa have found unusually high HPV prevalences across all adult ages, which may reduce the positive predictive value (PPV) of HPV-based screening, if positivity in older women does not sufficiently predict elevated risk. We conducted a population-based study in rural Nigeria to identify HPV prevalence and associated cervical abnormalities. Using stratified random sampling, we enrolled women age 15+. Nonvirgins had a cervical exam including liquid-based cytology and PCR HPV DNA testing from residual cytology specimens. Two-thirds of invited women participated, and 14.7% had detectable carcinogenic HPV, a proportion that did not decline with age (p-trend = 0.36) and showed slight peaks in the 15–29 and 60–69 age groups. Among women of the age typically considered for screen-and-treat programs (30–49 years), 12.8% were HPV positive, and the PPV for high-grade or worse cytology was 16.4%. Comparatively, women age < 30 were more likely to be HPV positive (18.9%, p = 0.03) with a lower PPV (4.2% p = 0.05). Among women age 50+ (typically excluded from screening in resource-poor settings because inexpensive treatment is not available), HPV positivity was 14.2% with a PPV of 13.9%. In Irun and similar settings where HPV does not decline with age, HPV-based screen-and-treat programs might be feasible for mid-adult women because prevalence is sufficiently low and positivity predicts elevated risk of more easily treated precancer.

Epidemiology

Virtually all cases of cervical cancer worldwide are caused by persistent infection with one or more of approximately a dozen carcinogenic genotypes of human papillomavirus (HPV).[1–3] Although effective HPV vaccines have been developed, none has immediate promise for low-income countries where the cancer burden is disproportionately high (>85% of total global burden),[4] because of relatively high cost and lack of feasibility of a three-dose regimen. With adequate screening and treatment, the vast majority of cervical cancer can be prevented during the typical 10–15+ year precancerous period.[5,6] Yet, the conventional model (Pap smear screening, followed by colposcopically directed biopsy of women with abnormal screening to determine who needs treatment) is neither sufficiently developed nor sustainable in most low-income countries.[7] In contrast, a one-visit screen-and-treat approach is promising where women who screen positive receive treatment with cryotherapy.[8] In particular, HPV-based screening is now being

proposed. Yet, effective application of an HPV-based screen-and-treat program requires a thorough understanding of the underlying natural history of HPV in a target population.

In most populations, the age-specific prevalence pattern of HPV infections observed in sexually active women resembles that of a typical sexually transmissible infection. Incidence and prevalence peak at young ages soon after the start of sexual activity, with a subsequent decline as infections clear, with fewer incident infections as patients age.[9] In these regions where HPV prevalence declines with age, the optimal age for HPV-based screening is in the ∼30- to 49-year age group—before the upturn in invasive cancer incidence. At this age in most places, carcinogenic-type HPV infection is detected in virtually all women with precancer or early-stage cancer but only 5–10% of the general population.[9] Therefore, HPV testing, when used properly at these correct ages, provides excellent risk stratification (acceptable positive predictive values (PPVs) and extremely good negative predictive values).[10,11] HPV-positive women (the group containing the women with cancer risk) can be treated immediately, using cryotherapy of the cervix in particular,[8] whereas HPV-negative women can be reassured that their cancer risk is minimal during the subsequent years.[12] One or two screening rounds per lifetime can reduce cancer risk substantially.[13]

Unfortunately, implementation of HPV-based screen-and-treat programs in some regions is complicated by unusual age-specific HPV prevalence patterns.[14–17] In particular, high HPV prevalence at all ages has been reported in some, but not all, population-based HPV studies conducted in sub-Saharan Africa, such as areas of West Africa.[16–18] We do not know the meaning of the elevated HPV prevalences at older ages in specific regions of Africa. Most of these HPV prevalence studies have not considered either concurrent age-specific cytologic or histologic status of the participants; thus, there is an unclear association with cervical neoplasia.

One such study was conducted in a highly mobile urban setting of Nigeria, the most populous country in Africa, with 150 million inhabitants and a high burden of cervical cancer (age-standardized incidence of 33 per 100,000 women). The high prevalence across all adult age groups (∼20% of women were carcinogenic HPV positive) would imply lower PPV and subsequently preclude the effective use of HPV screening if elevated HPV positivity at older ages was not associated with elevated risk of cervical precancer (n.b.: age-related cytology was not analyzed in this project).

One study cannot be generalized to a huge and diverse population; we were concerned that the high HPV prevalences in older women from urban Ibadan might not be representative of a large part of the population (or West Africa) living in rural areas.[18] To further our understanding of the epidemiology of HPV infection and cervical neoplasia in Nigeria and clarify how screening might be optimized to fit the epidemiologic pattern, we conducted a population-based, cross-sectional screening study of age-specific HPV prevalence and cytology in another part of Yoruban Nigeria, among ∼1,500 women in the rural southwestern state of Ondo.

Material and Methods

Irun is a large rural village in which subsistence farming is the predominant occupation. The community is serviced by one local government health clinic, used mainly for primary care, and one general hospital. Residents have minimal education and a typical household earns 20,000–40,000 Naira per month (US\$ 170–340). About half of the residents of Irun are Christian, whereas the other half is Muslim, though most ascribe to traditional beliefs as well. The typical family consists of the head of a household with one or more wives and several children belonging to each wife, all living within the same house.

A census done by local health workers (in which household heads were surveyed) found that approximately two-thirds of 501 houses (roughly one-half of village) were home to more than one household and one-eighth of households contained more than one wife. Therefore, we chose the house as the sampling unit to avoid possible adverse community reaction if not all women in a house were invited. We selected all houses known to have a household with co-wives and a simple random sample of the remaining houses to reach ∼2,100 women (439 houses total). Local health workers visited women in their homes. Those meeting eligibility criteria (not pregnant and without hysterectomy, 15+ years old and living in the house for more than 3 months) were asked to enroll after providing informed consent; participants were assigned a clinic appointment. Unmarried women age 15–21 were coconsented by their legal parent/guardian as well.

Upon arrival at the clinic, women completed a second informed consent with more detailed information on clinic procedures. For nonvirgins, nurses conducted a cervical exam involving the collection of cervical cells using a broom-type device and an endocervical brush. Both specimens were placed into Preservcyt buffer (Hologic, Marlborough, MA) for liquid-based cytology and an HPV DNA test.

Cytology slides were prepared and read in the United States. The presence of high-risk HPV genotypes was determined from residual cytology specimens. HPV DNA was amplified using Gold Taq and a modified MY09-MY11 PCR-based method that included additional primers for HPV30, 35, 39, 51 and 68 as well as primers to amplify a cellular beta-globin fragment as a control for amplification.[19] PCR products were typed by dot-blot hybridization using type-specific probes as previously described.[19] We considered the 13 most carcinogenic types for HPV positivity: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. The numeric measure of signal strength (1–5) was used as a validated semiqualitative measure of viral load.[20]

The protocol was reviewed and approved by both Nigerian and NCI institutional review boards (NCT 00804466). Appropriate diagnosis and treatment among screen-positive women is now underway, using a combination of cryotherapy, LEEP and surgery.

We calculated the age-stratified proportion of women infected with carcinogenic HPV and cytologic abnormalities. Bivariate analyses measured the risk of carcinogenic HPV infection among women with abnormal cytology as well as
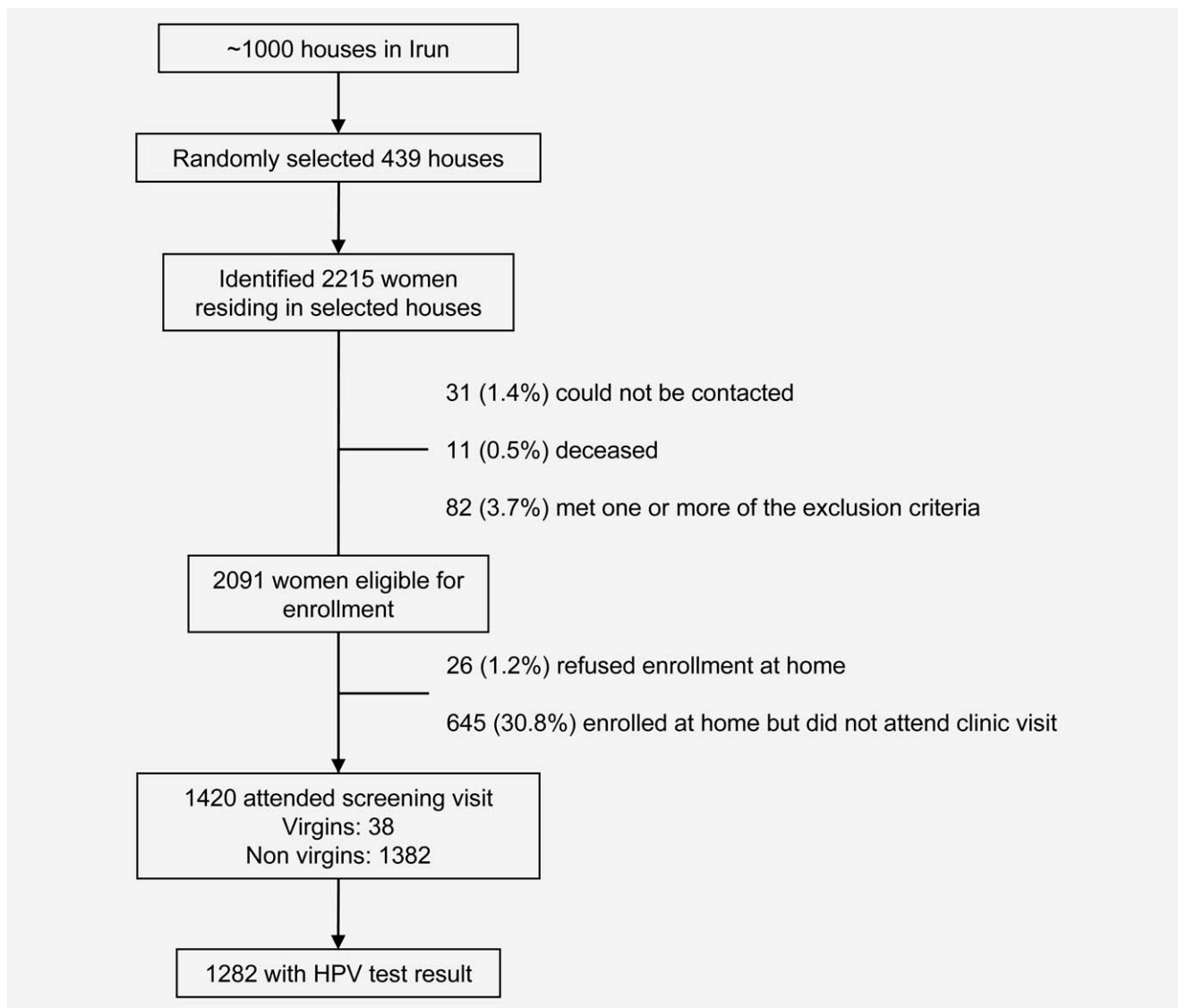
Figure 1. Consort diagram.

the risk of abnormal cytology given carcinogenic HPV infection. Differences by age were considered using standard contingency table analysis with Chi-square statistics, unless otherwise noted. To rule out the possibility that our findings were distorted by oversampling women who lived in houses containing a household with more than one co-wife, we stratified our results by co-wife status in the household (no co-wife *vs.* more than one co-wife); there were no notable differences. Analyses were performed using Stata 11.0 analytic software (Stata Corp LP, College Station, TX).

Results

Among 439 houses visited, we were able to contact 2,091 women who were eligible for enrollment (Fig. 1). Approximately one-third ($n = 671$) of these women either refused enrollment at home or did not attend the clinic visit. Participation varied by age: women 15–20 were less likely to enroll

and attend the clinic visit (43.1 *vs.* 74.3% among women over 20, $p < 0.01$).

Of 1,282 nonvirgins attending the screening visit for whom HPV DNA test results were available, 14.7% were infected with one or more carcinogenic HPV genotypes. Figure 2 separately presents the independent results of HPV testing (line) and cytology (bar) by age. The proportion of HPV positive did not decline with age (Chi-square p-trend = 0.36) and had slight peaks in women 15–29 (18.9%) and 60–69 (17.6%) years old, compared to 12–14% in mid-adult women. Cytologic abnormalities were most common in the youngest women (ages 15–29) compared to women age 30 or older (15.0 *vs.* 8.0%, $p < 0.01$); no second peak of abnormal cytology results was observed in older women. When cytology was abnormal, severity varied by age: younger women were more likely than older women to have a low-grade abnormality (86.5% of abnormal results in women under 30 *vs.*
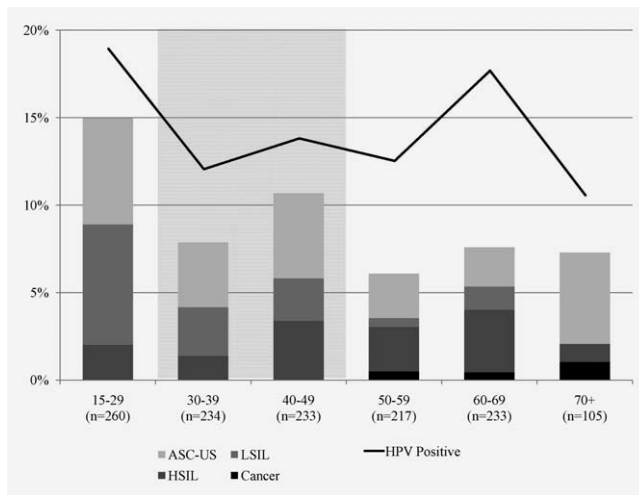
Figure 2. Percent of women with given cytologic abnormality[a] and PCR positive for one or more 13 carcinogenic HPV genotypes by age (mean and median age = 45 years). [a]Classification by severity: atypical squamous cells of undetermined significance (ASC-US), low-grade squamous intraepithelial lesion (LSIL) and high-grade intraepithelial lesion (HSIL) cancer. Shaded area highlights age considered optimal for screen-and-treat program: HPV prevalence is decreasing, risk of high-grade or worse cytology is increasing and women are not yet at elevated risk of cancer cytology.

64.0% in women 30 or older, $p = 0.01$), and older age was associated with higher risk of high-grade intraepithelial lesion (HSIL) or worse abnormality (36.0% of abnormal results in women 30 or older vs. 13.5% in women under 30, $p = 0.01$).

Overall, 10.0 and 4.1% of women were infected with one or more HPV genotypes in either the alpha-9 or alpha-7 species, respectively, with alpha-9 types HPV16, 31, 35, 52 and 58 being most common (2.0% or more) (Table 1). A total of 19.2% of women infected with a carcinogenic HPV genotype were concurrently infected with at least one other carcinogenic genotype. HSIL or worse cytology was most often associated with HPV16 and/or 35 (21.9 and 18.8%, respectively).

Among women with atypical squamous cells of undetermined significance (ASC-US) or low-grade squamous intraepithelial lesion (LSIL) vs. HSIL or worse cytology, the percentage concurrently testing positive for one or more of the 13 carcinogenic HPV genotypes was 51.3 and 65.6%, respectively (Table 1). This result was observed across all age groups (data not presented).

Among HPV-positive women, those with ASC-US/LSIL, HSIL or worse were more likely than those with normal cytology to have an HPV infection with a PCR signal strengths of 4 or 5 (80.5 and 76.2% vs. 50.9%, respectively, $p < 0.05$). Notably, however, in terms of absolute numbers, 53.8% of 106 women with an elevated HPV PCR signal strength had normal cytology (Table 1). Signal strength, a semiquantitative measure of viral load, was not associated with age (data not presented, one-way ANOVA $F[1, 1280] = 0.95$; $p = 0.33$).

In a complementary analysis, we considered the PPV of HPV testing, that is, how many HPV-infected women had cytologic abnormalities (versus the analysis above which considered how many women with cytologic abnormalities had HPV). Restricting our analysis to 188 HPV-positive women with cytology results (Table 2), most (64.6%) did not have a concurrent cytologic abnormality, especially over age 49. Age trends in this analysis were similar to those in the unpaired analysis shown in Figure 2; HPV infections in younger women were more likely than those in older women to be associated with ASC-US or LSIL (41.7% in women <30 vs. 16.5% in women 30 or older, $p < 0.01$). Conversely, HPV infections in older compared to younger women were more likely to be associated with HSIL or worse cytology, although this did not reach statistical significance (15.0% in women 30 or older vs. 4.2% in women under 30, $p = 0.05$). Among 55 HPV-positive women in the age range considered optimal for a screen-and-treat program (30–49 years), 16.4% had an HSIL or worse cytologic abnormality.

Discussion

Our population-based study in a rural Nigerian town achieved widespread participation among women over age 25 and found that, on an average, 14.7% of women were prevalently infected with carcinogenic HPV; rates were highest among the youngest and oldest women. Although most HPV infections were not linked to cytologic abnormalities, we did observe distinct trends across age groups that are important for planning screen-and-treat programs. HPV infections in the youngest women (<30 years) were more often associated with low-grade cytologic changes; these represent active, newly acquired infections too numerous and benign to treat immediately. For women in the age group typically considered for screen-and-treat programs (30–49 years), 12.8% (95% confidence interval: 9.8–15.9%) were infected with carcinogenic HPV, of whom 16.4% had concurrent high-grade or worse cytologic abnormalities. An unknown fraction of the remainder is at risk of developing high-grade abnormalities in the future, increasing the long-term PPV of carcinogenic HPV testing in this age group. We believe that screen-and-treat programs would most logically target this age group. Given the acceptable safety of cryotherapy, a PPV of at least 16.4% for the endpoint of high-grade or worse cytology might be judged high enough for immediate treatment in places without other cervical cancer prevention options.

Among women age 50 or greater (an age not typically considered for screen-and-treat programs), HPV infection was also associated with high-grade cytologic abnormality. This elevated risk of high-grade abnormalities likely reflects the lack of previous screening and treatment in this population and subsequent accumulation of high-grade lesions. The lack of low-grade lesions could reflect atrophy at that age. Unfortunately, no widely accepted treatment option is available for low-cost screening and treatment of older women, because cryotherapy is commonly believed to be ineffective

Table 1. Cytology results and risk of concurrent type-specific carcinogenic HPV infection for 1,282 women

| | Total (col %) | | Cytology (col %)[1] | | | | | |
| | | | Normal | | ASC-US/LSIL[2] | | HSIL+[3] | |
	n	%	n	%	n	%	n	%
HPV negative for all 13 types[4]	1094	85.3	962	89.5	39	48.8	11	34.4
Any of 13 carcinogenic types	188	14.7	113	10.5	41	51.3	21	65.6
HPV16	27	2.1	16	1.5	3	3.8	7	21.9
HPV18	17	1.3	13	1.2	1	1.3	2	6.3
HPV31	32	2.5	21	2.0	6	7.5	3	9.4
HPV33	5	0.4	2	0.2	0	0.0	1	3.1
HPV35	31	2.4	16	1.5	7	8.8	6	18.8
HPV39	6	0.5	3	0.3	2	2.5	0	0.0
HPV45	7	0.6	4	0.4	0	0.0	2	6.3
HPV51	20	1.6	9	0.8	9	11.3	1	3.1
HPV52	29	2.3	20	1.9	7	8.8	2	6.3
HPV56	9	0.7	6	0.6	3	3.8	0	0.0
HPV58	31	2.4	18	1.7	7	8.8	1	3.1
HPV59	6	0.5	4	0.4	2	2.5	0	0.0
HPV68	10	0.8	8	0.7	2	2.5	0	0.0
All types in alpha-7 species[5]	52	4.1	37	3.4	8	10.0	4	12.5
All types in alpha-9 species[6]	130	10.1	76	7.1	26	32.5	17	53.1
Infected with 2+ carcinogenic types[7]	36	2.8[5]	24	2.2	6	7.5	3	9.4
PCR signal strength[8]								
1	9	4.8	7	6.3	1	2.4	0	0.0
2–3	67	35.8	48	42.9	7	17.1	5	23.8
4–5	111	59.4	57	50.9	33	80.5	16	76.2
TOTAL (row %)	1,282	100.0	1,075	90.6	80	6.7	32	2.7

[1]Cytology results are not available for 95 women. [2]Atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion (ASC-US/LSIL) include eight diagnoses of ASC, rule out HSIL (ASC-H) and six diagnoses of atypical glandular cells (AGC), not otherwise specified. [3]High-grade intraepithelial lesion (HSIL) includes three diagnoses of carcinoma, one diagnosis of AGC, favor neoplasia (AGC-FN) and one woman with both AGC-FN and ASC-H cytologic diagnoses. [4]HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. [5]HPV genotypes 18, 39, 45, 59, 68, 70 and 85. [6]HPV genotypes 16, 31, 33, 35, 52, 58 and 67. [7]Among 188 women with any carcinogenic HPV infection, 19.2% were infected with more than one genotype. [8]If woman was infected with more than one carcinogenic HPV genotype, the highest signal strength is reported. Column percents are among HPV-positive women.

treating lesions likely inside the endocervical canal and unreachable by the cryoprobe (n.b.: A few studies have suggested that cryotherapy is perhaps 50–90% efficacious in women with lesions that extend into the endocervical canal,[21–24] but no definitive study has shown the efficacy of cryotherapy in older women.). In any case, although this oldest age group was at greatest risk of high-grade cytology, their disease would be caught and treated years earlier through HPV-based screening, once a screen-and-treat program targeting younger women had been in operation for a sufficient length of time.

Our findings are similar to those previously reported in nearby urban Ibadan, Nigeria.[16] Although the overall prevalence of carcinogenic HPV was statistically higher in Ibadan than our findings (18.3 *vs.* 14.7%, Chi-square $p = 0.02$), this difference could be due to variations in PCR assays. Still a similarly elevated prevalence was sustained across all ages.

Our observed age curve was similar to shallow U-shaped curves reported in other cohort studies in rural Gambia and other areas of Africa and Latin America.[9,18,25,26] Our study found an elevated occurrence of HPV35 (2.4%) and observed that HPV35 was present in 18.8% of high-grade cytology abnormalities, second highest to HPV16 (21.9%). Such over-representation of HPV35 has been observed in other studies across Africa.[16,17,27–29]

We did not obtain human immunodeficiency virus (HIV) seroprevalence data from women in our study. However, we believe that HIV is unlikely to explain the elevated HPV prevalence in older women.

Our field study confronted several limitations worth mentioning. Participation was lowest among women age ≤20, preventing definitive interpretations of the prevalence of HPV in young women age 15–20. The risk of HPV infection in this age group was lower than anticipated for a sexually

Epidemiology

Table 2. Absolute risk (positive predictive value) of cytologic abnormality among women testing PCR positive for one or more carcinogenic HPV genotypes[1] by age group

| Age (years) | HPV+ | Cytology[2] | | | | | | | |
| | | Normal | | ASC-US[3] | | LSIL[4] | | HSIL+[5] | |
		n	%	n	%	n	%	n	%
15–29	49	26	54.2	7	14.6	13	27.1	2	4.2
30–39	28	18	69.2	3	11.5	2	7.7	3	11.5
40–49	32	15	51.7	4	13.8	4	13.8	6	20.7
50–59	27	17	73.9	1	4.4	1	4.4	4	17.4
60–69	41	30	76.9	2	5.1	3	7.7	4	10.3
70+	11	7	70.0	1	10.0	0	0.0	2	20.0
Total	188	113	64.6	18	10.3	23	13.1	21	12.0

[1]HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. [2]Cytology results are not available for 13 HPV+ women. [3]Atypical squamous cells of undetermined significance (ASC-US) include three diagnoses of ASC, rule out HSIL (ASC-H). [4]Low-grade squamous intraepithelial lesion (LSIL). [5]High-grade intraepithelial lesion (HSIL) includes three diagnoses of carcinoma.

transmitted infection, and it is possible that our study failed to identify an early crest of HPV infection following the beginning of sexual activity in the teenage years. In addition, our understanding of HPV prevalence and cytologic abnormalities is most challenging for women over 70 years old. In our study, HPV prevalence declined in this age group, and we hypothesize that this marked difference was caused by poor sampling from atrophic or distorted cervices. Conversely, the prevalence of ASC-US abnormalities was unexpectedly high in this age group and again likely represents increased benign abnormalities including those associated with atrophy.

Despite using standard liquid-based collection and processing of cytology samples, expert cytopathologists in the United States found some of the specimens difficult to interpret because of poorly preserved and shredded cell material as well as grossly bloody specimens. Yet, we note that the proportion of women with any cytologic abnormality (9.4%) in Irun was similar to the few available population-based studies from West Africa (6.7–9.5%).[16–18]

In summary, we conclude that in some West African settings like Irun, HPV screening might have acceptable performance characteristics if testing is tailored to the underlying, age-specific prevalence and PPV of HPV infection for identifying cervical precancer. Women age 30–49 are optimal candidates, because they are past the initial peak of transient HPV infection and have reached the age when there is increasing probability of detecting treatable, high-grade disease. The remaining dilemma is how to prevent cervical cancer in older women, for whom treatment with cryotherapy might fail.

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Epidemiology