Table 4. Quality of life weights

Variable	Baseline values	Range
Quality of life weights for CIN		
CIN1	0.97	0.97–1.00
CIN2, 3	0.93	0.93–1.00
Quality of life weights for invasive cancer		
Stage I	0.65	0.49–0.81
Stage II	0.56	0.42–0.70
Stage III	0.56	0.42–0.70
Stage IV	0.48	0.36–0.60
Quality of life weights after treatment for invasive cancer		
Stage I	0.97	0.73–0.99
Stage II	0.9	0.68–0.98
Stage III	0.9	0.68–0.98
Stage IV	0.62	0.47–0.78

Results

Reduction in lifetime risk of cancer

Figure 2 shows the lifetime risk of cervical cancer by strategy estimated from a two-dimensional probabilistic sensitivity analysis. The range represents the minimum and maximum numbers of cervical cancer incidence per 100 000 population and its interquartile range (IQR). The bars represent the median value. Increasing the coverage of screening from the current level of 20–50 and 80% will substantially reduce the number of incident cervical cancer cases by 45.5% (IQR 42.0–48.7) and 63.1% (IQR 60.5–65.7), respectively. Combined strategies of 20, 50 and 80% screening coverage rate yields, respectively, a 66.1% (IQR 68.3–64.2), 80.9% (IQR 78.6–83.3) and 86.8% (IQR 85.4–87.9) reduction in cervical cancer incidence.

Total costs and QALYs of vaccination and screening programmes

Total QALYs gained per 100 000 population for each strategy showed slight increase as the screening coverage increases and the universal vaccination is added (Figure 3). Figure 4 shows cost per person for each strategy. The squares represent average values and the range represents average value ± 2 SD. Costs of strategies including vaccination are approximately four times higher than that of strategies without vaccination. Increasing the screening coverage rate was cheaper than introducing vaccination for all 11-year-old girls.

Incremental cost-effectiveness ratio

Table 5 shows the ICER of each strategy compared with its next best alternative strategy. Using the default model values, 50% screening coverage with a vaccination strategy was the most cost-effective when using a willingness to pay for a QALY threshold of 4,500,000 yen (≅ US$500,000) (Figure 5).

Sensitivity analysis on vaccine efficacy

We performed a sensitivity analysis on vaccine efficacy. The vaccine efficacy is determined by the combination of risk ratios to acquire HPV16/18 and other HR in our model. Table 6 shows cost and QALYs derived from the reference vaccine efficacy, minimum and maximum vaccine efficacy per 1000 people. Differences in vaccine efficacy would result in the differences in programme costs ranging from approximately 4,000,000–8,000,000 yen (≅US$480,000–960,000).

Table 7 shows the ICERs derived from the sensitivity analysis. The current strategy is dominated by strategies with a higher screening rate. A screening rate of 20% with a vaccination strategy is always ruled out because of extended dominance. The ICER for a screening rate of 50 and 80% with vaccination strategies was sensitive to the

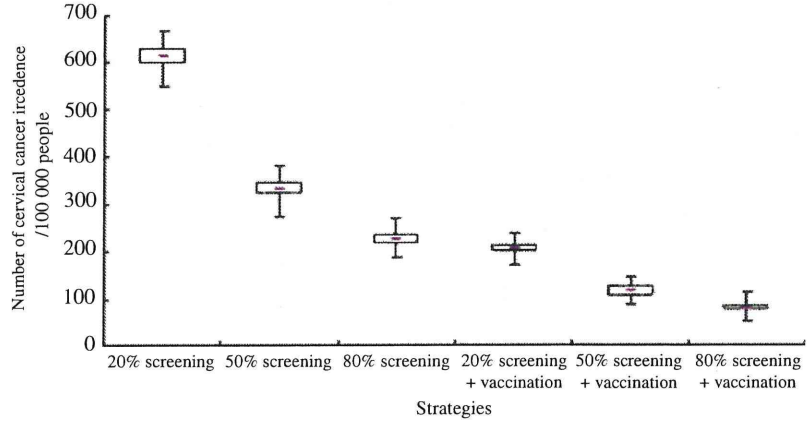

Figure 2. Lifetime risk of cancer for each strategy.

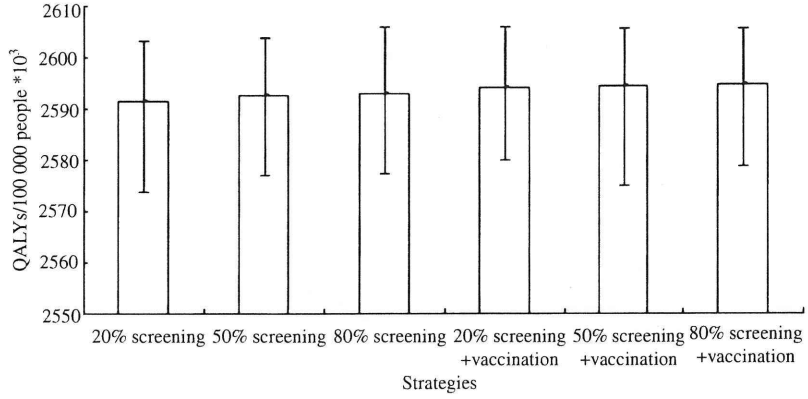

Figure 3. Total QALYs per 100,000 people for each strategy.

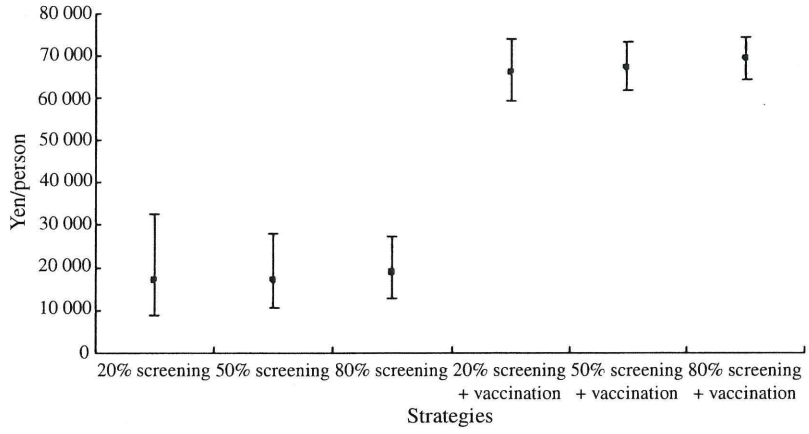

Figure 4. Cost per person for each strategy.

Table 5. Cost effectiveness of alternative screening and vaccination strategies

Strategy	Incremental cost effectiveness Ratio* (Yen/QALY)	
20% Screening	–	Dominated
50% Screening	658	
80% Screening	571 015	
20% Screening + vaccination	–	Extended Dominance
50% Screening + vaccination	2 920 636	
80% Screening + vaccination	8 568 182	not cost effective

*Ratio of additional costs and benefits of a particular strategy compared with the previous strategy.

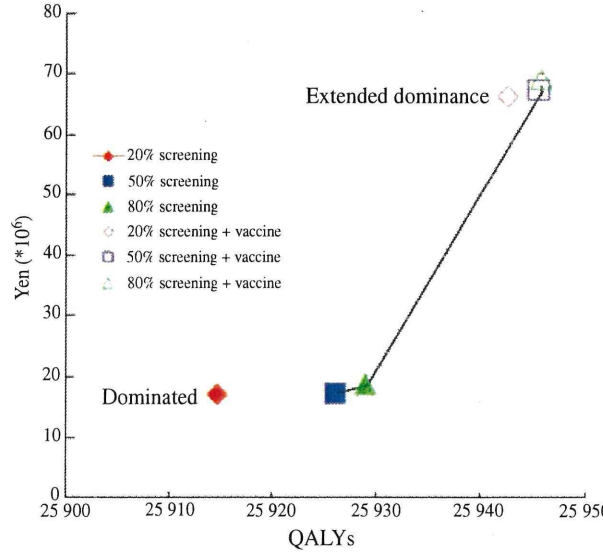

Figure 5. Cost and QALYs per 1000 people.

differences in incremental costs and effectiveness given by the result of a two-dimensional probabilistic sensitivity analysis of the model with each vaccine efficacy. With the

Table 6. Costs and QALYs per 1000 people of varied vaccine effect

Strategy	Minimum vaccine effect*		Baseline vaccine effect*		Maximum vaccine effect*	
	Cost (¥)	QALYs	Cost (¥)	QALYs	Cost (¥)	QALYs
Screening 20% + vaccination	69,561,000	25 933.88	66,114,000	25 942.64	62,628,000	25 950.77
Screening 50% + vaccination	70,300,000	25 937.33	67,334,000	25 945.54	64,300,000	25 953.22
Screening 80% + vaccination	72,129,000	25 940.81	69,219,000	25 945.76	66,277,000	25 953.07

*Minimum vaccine effect means relative risks of 0.48 for persistent HPV16 and 18 infection and 0.7 for persistent HPV high-risk type excluding 16, 18 infection. Baseline vaccine effect means relative risks of 0.12 for persistent HPV16 and 18 infection and 0.5 for persistent HPV high-risk type excluding 16, 18 infection. Maximum vaccine effect means relative risks of 0.03 for persistent HPV16 and 18 infection and 0.3 for persistent HPV high-risk type excluding 16, 18 infection.

Table 7. Sensitivity analysis on vaccine effect (ICER)*

Strategy	Minimum vaccine effect**	Baseline vaccine effect**	Maximum vaccine effect**
Screening 20%	Dominated	Dominated	Dominated
Screening 50%	658	658	658
Screening 80%	Extended dominance	571 015	571 015
Screening 20% + vaccination	Extended dominance	Extended dominance	Extended dominance
Screening 50% + vaccination	Extended dominance	2 920 636	1 874 867
Screening 80% + vaccination	3 745 442	8 568 182	Dominated

*Incremental cost effectiveness ratio (Yen/QALY).
**Minimum vaccine effect means relative risks of 0.48 for persistent HPV16 and 18 infection and 0.7 for persistent HPV high-risk type excluding 16, 18 infection. Baseline vaccine effect means relative risks of 0.12 for persistent HPV16 and 18 infection and 0.5 for persistent HPV high-risk type excluding HPV16, 18 infection. Maximum vaccine effect means relative risks of 0.03 for persistent HPV16 and 18 infection and 0.3 for persistent HPV high-risk type excluding 16, 18 infection.

minimum efficacy, a combined strategy of 80% screening and universal vaccination is most cost-effective. On the other hand, with the maximum and baseline vaccine efficacy, a combined strategy of 50% screening and universal vaccination remains most cost-effective.

Discussion

The introduction of HPV vaccine to the Japanese population has been controversial because the coverage of Pap smear screening is low and the prevalence of HPV types is different from that observed in Western countries.

To date there has been only one study that has assessed the impact of introducing HPV vaccine in Japan.[16] However, this study suffered from several major limitations. It did not distinguish health status related to HPV type 16 and 18 from other high-risk types. We modelled the natural history of each HPV type status; HPV16/18, other HR, and LR. We used different vaccine efficacies depending on the HPV types with a range that was derived from a meta-analysis of the available evidence. The previous study also did not include strategies of varied screening rates without vaccination. The authors analysed the effect of screening atthe currently observed levels ranging from 13.6 to 24.7%, and so the impact of increasing Pap smear coverage was not considered. Instead, the present study compared the strategies of varied screening rates ranging from 20 to 80%.

Our analysis suggests that increasing cervical cancer screening coverage to 50% would halve the incidence of cervical cancer and save programme costs and that the introduction of HPV vaccination would reduce the incidence by two-thirds but result in a four-fold increase in programme costs. Using the model's default values, a combined strategy to expand the coverage of cancer screening up to 50% and the introduction of universal vaccination would be most cost-effective. The results are robust with sensitivity analysis in which the optimum coverage level most likely lies somewhere between 50 and 80%. Our result confirms the need for expanding coverage for Pap smears in Japan as previously suggested,[39] to maximise the impact of the cervical cancer strategy regardless of whether a national vaccine programme is also implemented.

The detection rate of HPV16 and 18 among women with cervical cancer in Japan is reported to be lower than that in other countries.[14] We used the latest age-dependent prevalence data, which consistently show that the younger population has a higher detection rate of HPV16 and 18 than the older population.[20] The prevention of cervical cancer in a young person shows larger QALYs gained than that of an older person because of the longer remaining life expectancy. Hence the effect of vaccine on cancer incidence or QALYs is not as low as might otherwise be expected.

The present study has several limitations. First, we assumed life-long lasting immunity acquired by the vaccine. The vaccine has only been recently introduced, and the latest evidence shows 7.3 years of efficacy and immunogenicity of the vaccine, which was derived from the population of the initial placebo-controlled study.[40] If additional vaccination is required to maintain immunity in the future, then programme costs are slightly underestimated. Second, there is no population-based survival data of women with cervical cancer by stages of FIGO. These data are essential when building a model. However, we managed to adopt and validate data from an existing Japanese regional cancer registry. Third, we did not incorporate the preferences of girls and their parents and the subsequent uptake of vaccine as a result of their preferences. Both effects and costs may be overestimated in that sense. Finally, we did not include the cost for campaigns to increase the coverage of screening and/or vaccination in this analysis, which may underestimate the programme costs but such a bias is minimal given the fact that the majority of costs is incurred by screening, vaccination and treatment interventions.

Vaccination for HPV is attracting considerable policy attention now as a strategy for cervical cancer prevention in Japan. Our analysis showed that increasing the rate of the current screening strategy would halve cancer incidence with a similar cost to the current screening strategy, though vaccination strategies may also be cost effective. We suggest further efforts to expand the current screening programme regardless of what support is provided for vaccination.

Some of the reasons why Pap smear coverage is so low in Japan relate to a lack of knowledge and from the fact that the financial support of the screening programme from the Ministry of Health, Labour and Welfare was discontinued because it was included in the general ones in 1998. Most cities, towns and villages decided to reduce the cost for the screening programme.[41–43] Free tickets for the Pap smear were provided under supplemental budgets for 2009. Distributing free tickets to a target population of certain ages showed a significant increase in the coverage rate by 2.8 times.[44] We need to continue endeavours to increase coverage by effective interventions such as providing free tickets and undertaking awareness campaigns. The involvement of gynaecologists in school education will also support the enhancement of knowledge about cervical cancer prevention and help to increase the coverage rate of screening as has been seen in other countries.[45,46]

Our analysis showed that introducing the HPV vaccination for all 11-year-old girls would reduce cervical cancer incidence to 33.9% with a net cost of only 49,000 yen per person (taking into account the social burden of cancer). Vaccinating all 11-year-old girls would cost 33.7 billion yen. Our analysis showed the cost-effectiveness of vaccination and that it would save future costs. It is important to give priority to policy which is evidence based medically and economically. If the prevalence of HPV infection is reduced as a result of universal vaccination, as our model predicts, then it may be possible to extend the interval between routine screens or to increase the age at which screening is first offered, as suggested in other cost-effectiveness studies.[34,47] The use of the HPV-DNA test in the screening programme is one choice that should be evaluated in the future.

In conclusion, the introduction of HPV vaccine in Japan is cost-effective as in other countries. It is more cost-effective to increase the coverage of the Pap smear along with the universal administration of HPV vaccine. Only by doing so, can the scarce healthcare resources be efficiently and effectively used to reduce the burden from cervical cancer in Japan.

Disclosure of interests

None of the authors have any conflicts of interest to declare.

Contribution to authorship

NY contributed to the study design of the current paper, model construction, data acquisition, data analysis and interpretation, drafting and revising the manuscript. RM contributed to the study design of the current paper, model construction, results interpretation and revising the manuscript. PJ contributed to the model construction, results interpretation and the critical review of the manuscript. YO contributed to the study design of the current paper. KK contributed to the model construction, data acquisition and interpretation of the results. KS and YT contributed to the study design of the current paper and interpretation of the results. All authors approved the final version of the manuscript.

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CASE REPORT

Interstitial pneumonitis induced by pegylated liposomal doxorubicin in a patient with recurrent ovarian cancer

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Abstract Interstitial pneumonitis after treatment with pegylated liposomal doxorubicin (PLD) has been rarely reported. We describe herein a case of interstitial pneumonitis in a 49-year-old woman with relapsed ovarian carcinoma treated with PLD. Twenty-five days after the second administration of PLD, she presented with fever and dry cough, and chest CT scans revealed bilateral interstitial infiltrates and ground-glass opacities. She was diagnosed to have interstitial pneumonitis induced by PLD. Steroid therapy improved her symptoms.

Keywords Interstitial pneumonitis · Pegylated liposomal doxorubicin · Drug induced · Japanese · Ovarian cancer

Introduction

Pegylated liposomal doxorubicin (PLD) is an active drug in recurrent ovarian cancer as demonstrated in trials in the second-line chemotherapy [1–3]. It has been designed to enhance the efficacy and to reduce the toxicities of doxorubicin such as cardiotoxicity, hematologic toxicity, and alopecia by using a unique delivery system: a polyethylene glycol coat [4, 5]. Whereas hand-foot syndrome and planter palmar erythema are widely recognized as adverse effects of PLD, few cases of interstitial pneumonitis after treatment with PLD have been reported. Here, we describe a case of interstitial pneumonitis induced by PLD.

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Case report

A 48-year-old woman (gravida 4, para 3) with recurrent ovarian cancer was started on third-line chemotherapy with PLD (50 mg/m^2/4 weeks). She was initially diagnosed in February 2009 and underwent complete debulking surgery for a stage IIIC serous ovarian adenocarcinoma. Postoperatively, she received adjuvant chemotherapy with six cycles of paclitaxel (175 mg/m^2) and carboplatin (AUC 6). Four months later, because of peritoneum dissemination and elevation of CA125, she was treated with weekly CPT-11 (95 mg/m^2/week) with progressive disease after four cycles. In April 2010, PLD was given under her excellent performance status.

Twenty-three days after the first administration of PLD, she developed a fever from which she recovered without any treatment. However, 25 days after the second administration of PLD, she presented to our hospital with fever, chill, dry cough, and dyspnea (grade 3 according to Common terminology criteria for adverse events, version 4.0). Physical examination was remarkable for bilateral fine crackles at the lung bases. A chest X-ray and chest CT scans revealed bilateral interstitial infiltrates and ground-glass opacities, though chest CT scans performed before PLD therapy showed clear lung field (Fig. 1a, b). Oxygen saturation by pulse oximetry was 89% on room air and arterial blood gas analysis showed hypoxia (FiO_2 0.32, PaO_2 90.5 mmHg, alveolar-arterial oxygen gradient 94.9 mmHg). Laboratory analysis revealed white blood cells of 2,500/μl with 78% neutrophils, lactate dehydrogenase of 347 IU/l, C-reactive protein of 14.32 mg/dl, and Krebs von den Lungen-6 (KL-6) of 227 U/ml.

Her clinical course and laboratory data indicated that she has interstitial pneumonitis probably induced by PLD. She had not received granulocyte colony-stimulating

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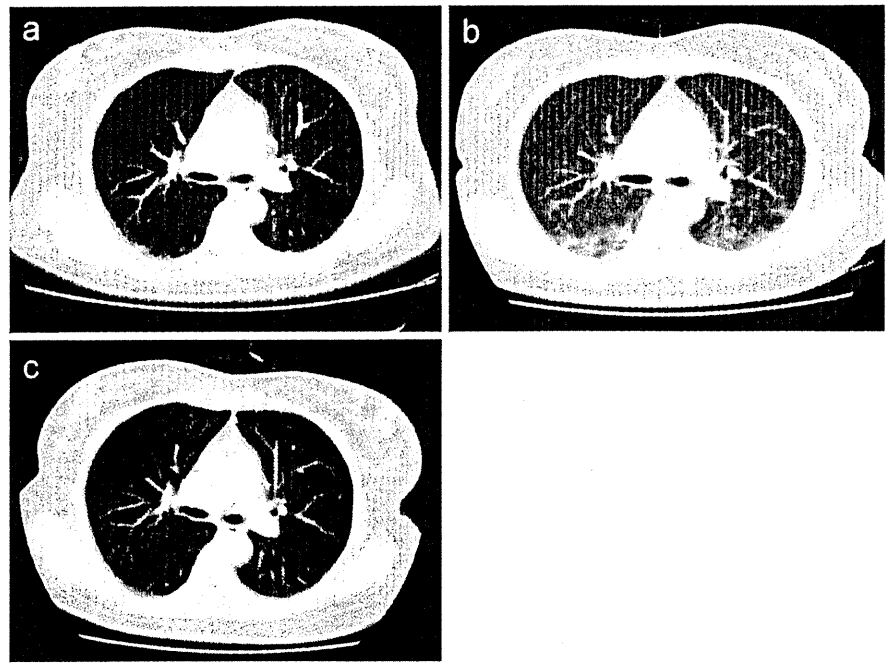

Fig. 1 **a** Chest computed tomography (CT) scan before PLD therapy showed clear lung field. **b** Twenty-six days after second administration of PLD, CT revealed bilateral interstitial infiltrates and ground-glass opacities. **c** Two months after steroid therapy, CT showed significant improvement

factor, which could induce interstitial pneumonitis. In addition to PLD, she received ascorbic acid, pyridoxal phosphate hydrate, rebamipide, and brotizolam. As they were all unlikely to induce interstitial pneumonitis, administration of these drugs except PLD was continued. The patient was treated with intravenous methylprednisolone 500 mg/day for 3 days. Azithromycin 1,000 mg per os and intravenous cefepime 4 g/day were administrated until all examinations of infection proved to be negative, including blood culture, β-D-glucan, influenza antigen detection, urinary pneumococcal antigen test, Chlamydia IgA/IgG, candida antibody assays, and galactomannan antigen of aspergillosis.

After the steroid pulse therapy, symptoms resolved promptly and lung function tests improved remarkably. Two months after the diagnosis of interstitial pneumonitis, a chest CT scan showed significant improvement (Fig. 1c). PLD was discontinued and her chemotherapy regimen was changed to docetaxel (70 mg/m^2). She has not shown any respiratory symptoms after cessation of PLD. Currently, she is alive with disease 24 months after the surgery and undergoing fifth-line chemotherapy.

Discussion

Pegylated liposomal doxorubicin is a reformulated version of doxorubicin, which takes the active agent doxorubicin and places it into a phospholipid bilayer called a liposome and another outer layer of methoxypolyethylene glycol. This coating allows PLD to evade detection and destruction by the immune system and to remain longer in the blood circulation.

PLD has a different toxicity profile compared with free doxorubicin. Though cumulative cardiac toxicities are unique to free doxorubicin, cardiac toxicities associated with PLD are rarely reported. Toxicities relatively unique to PLD are hand-foot syndrome or plantar palmar erythema (PPE), which are rarely reported with free doxorubicin.

It is reported that lung toxicity induced by doxorubicin is rare. Several cases of interstitial pneumonitis associated with doxorubicin or PLD have been described [6, 7]. It was unclear whether the lung toxicities were directly attributable to doxorubicin in published case reports, because all patients were concurrently receiving other agents, mostly antineoplastic drugs, which were also implicated in causing lung toxicity.

In our case, though the symptoms were initially severe, discontinuation of PLD and steroid therapy immediately resolved them. Serum KL-6 levels have been reported to correlate with grade of interstitial lung disease [8]. Normal serum KL-6 level in this case might associate with her excellent clinical course.

Two possible mechanisms of drug-induced interstitial pneumonitis have been described, one of which is the direct toxicity of the drug to the pulmonary organ and the other is immunological mechanism, although the etiology of PLD-induced interstitial pneumonitis is unclear.

Drug-induced pulmonary toxicity in Japan got a great deal of attention because of pulmonary toxicity induced by molecular-targeted chemotherapeutic drugs, gefitinib and an antirheumatic drug, leflunomide. It is reported that the

rates of interstitial lung disease associated with gefitinib and leflunomide are 2 and 1.1% in Japan and 0.3 and 0.02% in the United States, respectively. These data indicate that chemotherapeutic-drug-induced pulmonary toxicity is more frequent in Japan than in other nations [9, 10]. Fatal pneumonitis induced by gefitinib or leflunomide is less frequent in other Asian countries than in Japan. It may be that such drugs including PLD cause fatal pneumonitis predominantly in Japanese. The differences of genetic background or lifestyle between Japanese and non-Japanese might be involved in this event.

Drug-induced interstitial pneumonitis should be taken into consideration in the differential diagnosis of otherwise unexplained ground-glass lung lesions. Pulmonary toxicity induced by PLD is rare, but awareness of this toxicity is important, since it could be lethal. Additional investigation is required to elucidate how PLD induces interstitial pneumonitis or whether PLD-induced interstitial pneumonitis is more frequent in Japanese.

Conflict of interest No author has any conflict of interest.

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ORIGINAL ARTICLE

Subsequent risks for cervical precancer and cancer in women with low-grade squamous intraepithelial lesions unconfirmed by colposcopy-directed biopsy: results from a multicenter, prospective, cohort study

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Abstract
Objective To investigate the natural course of low-grade squamous intraepithelial lesions (LSILs) that cannot be histologically confirmed by colposcopy-directed biopsy.
Methods In a multicenter, prospective, cohort study of Japanese women with LSILs, we analyzed the follow-up data from 64 women who had a negative biopsy result at the initial colposcopy (biopsy-negative LSIL) in comparison with those from 479 women who had a histologic diagnosis of cervical intraepithelial neoplasia grade 1 (LSIL/CIN1). Patients were monitored by cytology and colposcopy every 4 months for a mean follow-up period of 39.0 months, with cytologic regression defined as two consecutive negative smears and normal colposcopy.
Results In women with biopsy-negative LSILs, there were no cases of CIN3 or worse (CIN3+) diagnosed within 2 years; the difference in the 2-year risk of CIN3+ between the two groups was marginally significant (0 vs. 5.5%; $P = 0.07$). The cumulative probability of cytologic regression within 12 months was much higher in the biopsy-

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negative LSIL group (71.2 vs. 48.6%; $P = 0.0001$). The percentage of women positive for high-risk human papillomaviruses (hrHPVs) was significantly lower in the biopsy-negative LSIL group than in the LSIL/CIN1 group (62.1 vs. 78.4%; $P = 0.01$); however, the 12-month regression rate of biopsy-negative LSIL was similar between hrHPV-positive and -negative women (67.3 vs. 74.4%, $P = 0.73$).

Conclusion In women with biopsy-negative LSILs, the risk of CIN3+ diagnosed within 2 years was low; furthermore, approximately 70% underwent cytologic regression within 12 months, regardless of HPV testing results. Biopsy-negative LSILs may represent regressing lesions rather than lesions missed by colposcopy.

Keywords Low-grade squamous intraepithelial lesion · Colposcopy · Human papillomavirus · Cervical intraepithelial neoplasia

Introduction

In the Bethesda System for cytologic reporting, a low-grade squamous intraepithelial lesion (LSIL) represents mild cervical abnormalities, including cellular changes associated with human papillomavirus (HPV) infection and cervical intraepithelial lesion grade 1 (CIN1) [1]. However, approximately 15–20% of women with a cytologic interpretation of LSIL have a grade 2 (CIN2) or grade 3 (CIN3) cervical intraepithelial lesion, which are immediately treated with cervical ablation, loop electrosurgical excision procedure (LEEP) or cone biopsy [2, 3]. Therefore, women with LSILs usually undergo a colposcopy-directed biopsy for histologic evaluation of cervical abnormalities. Of the women with LSIL cytology, 40–60% are found to have a histologic diagnosis of CIN1 at the initial colposcopy, while 15–30% have a negative biopsy result [2, 3]. According to the 2006 American Society for Colposcopy and Cervical Pathology consensus management guidelines [4], the follow-up strategy for women with a negative biopsy result is identical to that of women with CIN1; that is, both groups are followed with either repeated cytology at 6 and 12 months or HPV testing at 12 months. However, the natural course of LSILs that cannot be histologically diagnosed by colposcopy-directed biopsy has not been well documented.

The Japan HPV and Cervical Cancer (JHACC) cohort study was designed to identify determinants of regression and progression of low-grade cervical abnormalities [5, 6]. In the primary analysis, we used only the follow-up data from 570 women with cytologic LSIL and histologically confirmed CIN1 or CIN2 lesions, and demonstrated HPV type-specific risks of LSIL persistence and progression [5]. In the present study, we analyzed the follow-up data of 64 women with biopsy-negative LSIL who were excluded from the main analysis cohort.

Methods

Study design

This study represents a secondary analysis of data from the prospective non-intervention cohort study conducted by the JHACC study group for identifying determinants of LSIL/CIN regression and progression. Details of the design, methods, and primary results have been provided in more detail elsewhere [5, 6]. Briefly, 905 women with mildly abnormal cytology were recruited from nine hospitals that performed conventional Pap smears, colposcopy and cervical biopsies. The inclusion criteria of this secondary analysis were: evident LSIL cytology; histologic diagnosis of CIN1 or less at initial colposcopy and biopsy; age 18–54 years; first detection of cervical abnormality; and a sufficient number (two or more) of follow-up visits. Women entered the study voluntarily after giving their signed informed consent. Cervical smears were classified according to the Bethesda System [1]. At the time of study entry, two (or more) small cervical specimens were taken by colposcopy-directed punch biopsy and stained with hematoxylin and eosin (H&E). A histologic diagnosis was determined based on the World Health Organization (WHO) classification system. Two cytopathologists (Y.H. and Masafumi Tsuzuku) and two pathologists (R.F. and T.K.) reviewed all cytologic and histologic specimens collected at the time of entry. Patients were tested for cervical HPV DNA, serum IgG antibodies to cytomegalovirus (CMV), *Chlamydia trachomatis*, and herpes simplex virus type 2 (HSV2) at the time of entry. The researchers who performed the assays were blinded to the clinical data collected from the study subjects. Information regarding smoking and sexual behavior was obtained from a self-administered questionnaire. Patients were routinely followed at 3- to 4-month intervals and received cytologic and colposcopic examinations at each visit. To avoid interference from the biopsy procedure on the natural course of the disease, a cervical biopsy was performed during the follow-up period only when Pap smears and colposcopic findings were suggestive of the presence of CIN3 or worse (CIN3+). A cytology result of HSIL triggered colposcopy-guided biopsy during follow-up examinations. The two cytopathologists and the two pathologists reviewed all cytologic and histologic specimens collected for the diagnosis of CIN3+. We chose an end-point of CIN3 or cancer rather than CIN2 or higher because CIN2 likely represents a heterogenous collection of cervical abnormalities [7, 8], only some of which progress to CIN3 [5, 9]. In this analysis, we defined regression as normal colposcopy results and at least two consecutive negative Pap smears. Persistent lesions were defined as lesions that did not regress or were diagnosed with CIN3+ during the follow-up period.

Overall, the study subjects consisted of 554 women who had a negative biopsy result (biopsy-negative LSIL; $n = 64$) or a histologic diagnosis of CIN1 (LSIL/CIN1; $n = 491$) at the initial colposcopy for LSIL cytology. Unfortunately, data from cervical samples, blood samples, or questionnaires were not available in all 554 study subjects. Cervical HPV data were not available in 21 women because of insufficient samples, while data on serum antibodies to sexually transmitted agents were lacking in 23 women. In addition, 54 women gave no responses to a self-administrated questionnaire. The study protocol was approved by the ethical and research review boards of the participating institutions.

HPV genotyping

We detected HPV DNA in cervical samples by polymerase chain reaction (PCR)-based methodology, as previously described [10]. In brief, exfoliated cells from the ectocervix and endocervix were collected in a tube containing 1 ml of phosphate-buffered saline (PBS) and stored at −30°C until DNA extraction. Total cellular DNA was extracted from cervical samples by a standard sodium dodecyl sulfate (SDS)–proteinase K procedure. HPV DNA was PCR amplified by using consensus primers (L1C1/L1C2 + L1C2M) for the HPV L1 region. A reaction mixture without template DNA was included in every set of PCR runs as a negative control. Primers for a fragment of the β-actin gene were also used as a control to rule out false-negative results for samples in which HPV DNA was not detected. HPV types were identified by an analysis of restriction fragment length polymorphism (RFLP), which has been shown to identify at least 26 types of genital HPVs [11].

IgG antibody against sexually transmitted agents

The level of IgG antibodies to *Chlamydia trachomatis* and HSV2 was determined by using commercially available enzyme-linked immunosorbent assay (ELISA) kits: *Chlamydia trachomatis* (HITAZYME; Hitachi Chemical, Tokyo, Japan) and HSV2 (HerpeSelect 2 ELISA IgG; Focus Diagnostics, Cypress, CA, USA). The serologic assay for *Chlamydia trachomatis* utilizes purified EB outer-membrane proteins of the *Chlamydia trachomatis* L2 strain as antigens and does not detect antibody to *Chlamydia pneumoniae* [12]. These serologic assays were performed at a clinical testing laboratory (SRL, Tokyo, Japan).

Statistical analysis

All time-to-event analyses were based on the actual date of the visits. For regression or progression, time to event was measured from the date of the index visit (i.e., the first instance of an abnormal cytology result) to the date of the visit at which cytologic transition to normal occurred or CIN3+ was first detected. Women whose lesions persisted or who dropped out of the study were censored at their last recorded return visit dates. Subjects who had only one negative colposcopy/cytology result before loss to follow-up were censored at the last date of positive Pap tests. Subjects who were biopsied were censored at the time of their biopsy, regardless of the biopsy results, to reduce the potential for interference by the biopsy procedure on estimates of time of regression. Cumulative probability of LSIL regression or progression was estimated by using the Kaplan–Meier method and compared with a log-rank test. All analyses were carried out using the JMP 7.0J (SAS Institute, Cary, NC, USA) statistics packages. Two-sided P values were calculated throughout and considered to be significant at less than 0.05.

Results

We analyzed the follow-up data from a total of 554 women with LSIL cytology who had a negative biopsy result (biopsy-negative LSIL; $n = 64$) or a histologic diagnosis of CIN1 (LSIL/CIN1; $n = 491$) at the initial colposcopy. Distributions of baseline characteristics between these two groups are presented in Table 1. The women with biopsy-negative LSILs were older than the women with LSIL/CIN1 (mean age ± SD 38.8 ± 9.2 vs. 36.2 ± 7.7 years); however, the difference in the age distribution between the two groups was only marginally significant ($P = 0.07$). Cervical HPV infections were found in 75.0% of women with biopsy-negative LSILs and in 84.6% of women with LSIL/CIN1 results and the difference was statistically significant ($P = 0.02$). The percentage of women positive for high-risk human papillomaviruses (hrHPVs) was also significantly lower in the biopsy-negative LSIL group than in the LSIL/CIN1 group (62.1 vs. 78.4%; $P = 0.01$). The percentage of women who had smoked was lower in the biopsy-negative LSIL group (32.6 vs. 48.7%), but the difference was only marginally significant ($P = 0.07$). The number of lifetime sexual partners was significantly greater among women with LSIL/CIN1 than among women with biopsy-negative LSILs ($P = 0.001$). The age at first sexual intercourse was also lower among women with LSIL/CIN1 compared to women with biopsy-negative LSILs, although the difference was only marginally significant ($P = 0.06$). Women with LSIL/CIN1 were likely to have a higher IgG antibody titer against *Chlamydia trachomatis* than women with biopsy-negative LSILs; however, the difference was not significant ($P = 0.25$). The IgG reactivity to HSV2 was similar between the two groups ($P = 0.82$). At least two

Table 1 Characteristics of the study subjects

	Cytology and histology		P values[†]
	Biopsy-negative LSIL ($n = 64$)[a]	LSIL/CIN1 ($n = 479$)	
Age (years)			
Mean (SD)	38.8 (9.2)	36.2 (7.7)	
18–29	11 (17.2%)	95 (19.8%)	0.07
30–39	21 (32.8%)	215 (44.9%)	
40+	32 (50.0%)	169 (35.3%)	
HPV typing			
Positive for high-risk types[b]	36 (62.1%)	359 (78.0)	0.01
Negative for high-risk types	22 (37.9%)	101 (22.0%)	
Positive for any HPV	48 (77.4%)	405 (88.0%)	0.02
Negative for any HPV	14 (22.6%)	55 (12.0%)	
Smoking			
Never smokers	37 (63.8%)	222 (51.3%)	0.07
Smokers	21 (36.2%)	211 (48.7%)	
Current smokers	16 (27.6%)	143 (33.0%)	
Former smokers	5 (8.6%)	68 (15.7%)	
Number of lifetime sexual partners			
1	23 (39.6%)	79 (18.1%)	0.001
2–3	13 (22.4%)	129 (29.5%)	
4+	22 (37.9%)	229 (52.4%)	
Age at first sexual intercourse (years)			
≤20	12 (20.3%)	147 (34.2%)	0.06
21–23	26 (44.1%)	179 (41.6%)	
≥24	21 (35.6%)	104 (24.2%)	
IgG antibodies to *Chlamydia trachomatis*			
Low	27 (45.0%)	166 (36.1%)	0.25
Mid	20 (33.3%)	150 (32.6%)	
High	13 (21.7%)	144 (31.3%)	
IgG antibodies to HSV2			
Low	23 (38.3%)	158 (34.3%)	0.82
Mid	19 (31.6%)	150 (32.6%)	
High	18 (30.0%)	152 (33.0%)	

[†] These P value were calculated by the χ^2 test

[a] Biopsy-negative LSIL denotes women with LSILs that had a negative biopsy result at the initial colposcopy

[b] HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 were classified into high-risk HPV types

biopsies were taken at the initial colposcopy and there was no difference in the number of biopsies between the two groups.

Patients were monitored by cytologic and colposcopic testing at intervals of 3–4 months. Among women with biopsy-negative LSILs, no case was diagnosed with CIN3+ within 2 years; the difference in the cumulative risk of CIN3+ diagnosed within the next 2 years between the two groups was marginally significant (0 vs. 5.5%; $P = 0.07$ by log-rank test; Fig. 1a). In women with biopsy-negative LSILs, the majority of cytologic regression occurred within 12 months. The cumulative probability of cytologic regressions within 12 months was much higher in women with biopsy-negative LSILs than in women with LSIL/CIN1 (71.2 vs. 48.6%; $P = 0.0001$; Fig. 1b). The 2-year rate of cytologic regression was also significantly different between the two groups (75.1 vs. 64.0%; $P = 0.003$). Cytologic regression occurred more quickly in women with biopsy-negative LSILs than in women with LSIL/CIN1 (median time to regression: 6.3 vs. 12.4 months). In the women with biopsy-negative LSILs, the 12-month cumulative probability of cytologic regression was similar between hrHPV-positive and -negative women (67.3 vs. 74.4%; $P = 0.74$); median time to regression was also similar between hrHPV-positive and -negative women (5.4 vs. 7.7 months; $P = 0.45$; Fig. 2a). In women with LSIL/CIN1, however, detection of hrHPVs significantly influenced the 12-month rate of cytologic regression (hrHPV-positive [45.2%] vs. hrHPV-negative [62.6%]; $P = 0.006$; Fig. 2b).

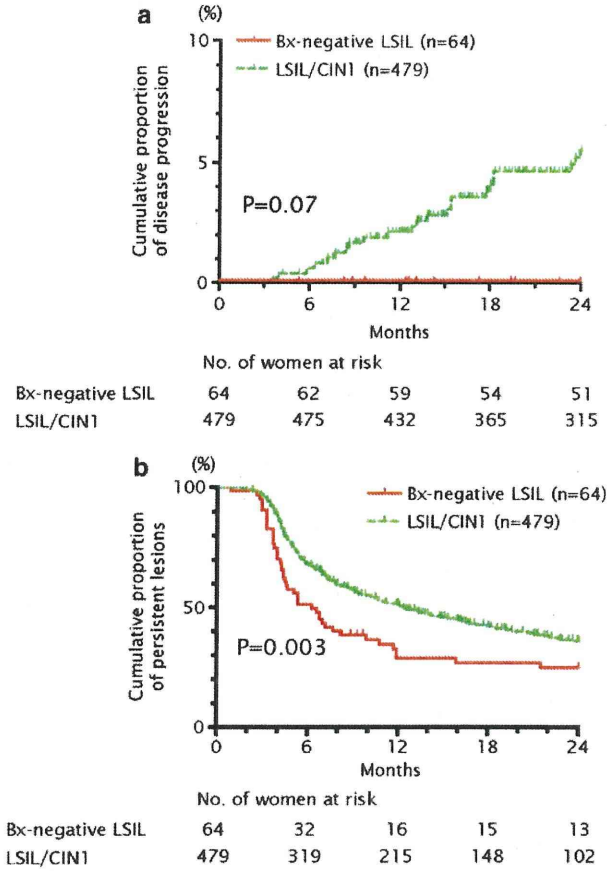

Fig. 1 Cumulative probabilities of CIN3+ diagnosis and cytologic regression within 2 years. A Kaplan–Meier plot was used to estimate the cumulative probabilities of CIN3+ diagnosis (**a**) and cytologic regression (**b**) within 2 years among women with biopsy-negative LSILs (*solid line*) or LSIL/CIN1 (*dashed line*). *P* values were calculated by the log-rank test

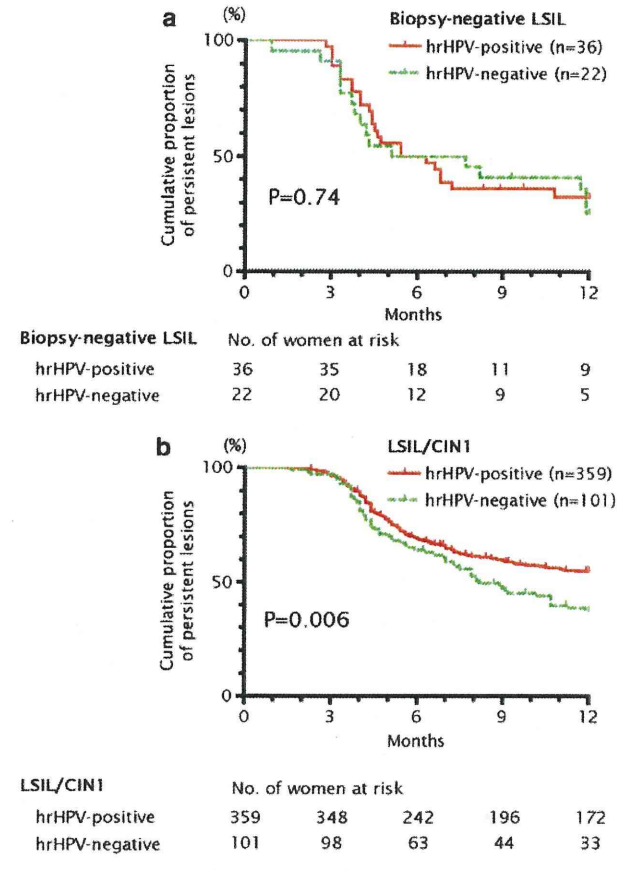

Fig. 2 Cumulative probabilities of cytologic regression within 12 months according to detection of hrHPVs. A Kaplan–Meier plot was used to estimate the cumulative probabilities of cytologic regression within 12 months among women with biopsy-negative LSILs (**a**) or LSIL/CIN1 (**b**) according to hrHPV detection. *P* values were calculated by the log-rank test

Discussion

Colposcopy-directed biopsies are recommended for women with LSIL cytology, primarily to exclude a high-grade lesion. Although approximately 15–30% of those women have a negative biopsy result [2, 3], they are routinely subjected to follow-up because of uncertainty about the risk of precancerous lesions missed by a colposcopic biopsy. In the present study, women with a biopsy-negative LSIL (i.e., "unconfirmed" LSIL) were at substantially low risk of CIN3 or cancer diagnosed within the following 2 years. The women with biopsy-negative LSILs were also significantly more likely to have cytologic regression than women with LSILs underlying CIN1. Some cases of biopsy-negative LSIL may be based on false-positive cytology because the percentage of women negative for any HPV was significantly higher in the biopsy-negative LSIL group than in the LSIL/CIN1 group. Additionally or alternatively, biopsy-negative LSILs may represent currently regressing lesions. This may be supported partially by the higher percentages of women in the biopsy-negative LSIL group who did not have cervical cancer risk factors, such as detection of hrHPVs, smoking, higher sexual activity and infections with *Chlamydia trachomatis* [13–16]. Several studies have reported that LSIL is more likely to regress to normal cytology among hrHPV-negative women or women who never smoked [5, 6, 17]. Interestingly, the 12-month regression rate of biopsy-negative LSIL was high, even among hrHPV-positive women. Low-grade lesions currently regressing to normal cytology may be difficult to confirm by colposcopy-guided biopsies because of the small lesion size, lower-grade colposcopic impression and/or weak pathologic findings.

Data on the natural course of biopsy-negative LSILs are limited. Pretorius et al. [18] reported that the subsequent risk of CIN3+ among women with histologically unconfirmed atypical squamous cells of undetermined significance (ASC-US) or LSIL cytology was low (1.8%). This

result was consistent with our observation; however, it was based on retrospective analyses of previous data including ASC-US cytology. In the ALTS (ASCUS-LSIL Triage Study) report [2], the risk of CIN3+ diagnosed within 2 years after unconfirmed LSIL was higher compared with the present study (6.1 vs. 0%). The difference between our results and the ALTS data may be explained by the difference in study design between the two studies. In the ALTS study, all women had an exit colposcopy and biopsy at 2 years after the semiannual follow-up by repeated cytology. Although our study subjects received both cytologic and colposcopic examinations at each visit at 3- to 4-month intervals, we did not routinely perform a colposcopic biopsy 2 years later. This may have resulted in an underestimation of the 2-year risk for CIN3+ in our study. Additionally, the sensitivity of the enrollment colposcopy may have affected the results from these two prospective studies. Recent studies have showed that initial colposcopy-directed biopsy are not as sensitive as we had previously assumed [19]. Thus, at least two directed biopsies, random biopsy or endocervical curettage are recommend to increase the sensitivity of the initial colposcopy [20–22]. In the ALTS study, 77.6% of women had null or only one biopsy at enrollment colposcopy [20]. By contrast, two (or more) biopsies were taken at entry in our study subjects. The number of biopsies may have increased the risk of misclassification errors of cervical lesions at enrollment. Although central pathologic review systems were employed in both studies, the limitation of histopathologic diagnosis (i.e., poor reproducibility in CIN grading) may also have affected disease classification at enrollment and during follow-up [7, 8, 23].

The current US guidelines advise that women with LSIL cytology and a histologic diagnosis of CIN1 or less should be followed with repeated cytology at 6 and 12 months or, alternatively, hrHPV testing at 12 months [4]. Our data also confirmed that these management strategies are sufficiently safe. A previous study reported that there was no significant difference in the subsequent risk of CIN2/3 between women with no disease documented by initial colposcopy-directed biopsy and women with histologically confirmed CIN1 [24]. However, the study was based on retrospective analyses, which was limited by the small sample size (negative biopsy $n = 43$; CIN1 $n = 30$) and included women with various cytologic abnormality profiles. In the present study, the risk of CIN3+ diagnosed within the following 2 years and the likelihood of LSIL regression were obviously different between women with biopsy-negative LSILs and women with LSIL/CIN1. The 2-year follow-up in ALTS of women with CIN1 or less has indicated that the subsequent risk of CIN2 or higher varies little with respect to the findings at the initial colposcopy [2]. However, when the analysis was confined to the risk of CIN3 or higher among women with LSILs, there was a marginal tendency for a higher risk of subsequent CIN3 that was associated with CIN1 compared with <CIN1 (10.5 vs. 6.1%). Based on these observations, the follow-up strategy for women with biopsy-negative LSILs may be better differentiated from that for women with LSIL/CIN1 results in terms of quality-of-life and cost. Our data suggest that follow-up by repeated cytology at 12 months may be appropriate for women with biopsy-negative LSIL when two or more colposcopy-directed biopsies are taken at the initial colposcopy.

In conclusion, the risk of CIN3+ diagnosed within 2 years was low in women with biopsy-negative LSILs; furthermore, approximately 70% showed cytologic regression within 12 months, regardless of HPV testing results. Our data suggest that biopsy-negative LSILs may represent false-positive cytology or currently regressing lesions rather than lesions missed by colposcopy. However, the sample size of the present study was small; thus, to confirm our results, further prospective studies with larger sample sizes will be needed.

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Conflict of interest We declare that we have no conflict of interest relevant to this article. The supporting organization played no role in the design and implementation of the study; the collection, management, analysis, and interpretation of the data; and the preparation, review, or approval of the manuscript.

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Do Neutralizing Antibody Responses Generated by Human Papillomavirus Infections Favor a Better Outcome of Low-Grade Cervical Lesions?

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To determine the role of neutralizing antibody generated by human papillomavirus (HPV) infections, baseline levels of serum neutralizing antibodies directed against HPV 16 and cervical HPV DNA were determined in 242 unvaccinated women with low-grade cervical abnormalities, who were then monitored by cytology and colposcopy every 4 months. In women infected with HPV 16 (n = 42), abnormal cytology persisted longer in those positive for HPV 16-specific neutralizing antibodies at baseline (median time to cytological regression: 23.8 vs. 7.2 months). Progression to cervical precancer (cervical intraepithelial neoplasia grade 3) within 5 years occurred only among women carrying HPV 16-specific neutralizing antibodies ($P = 0.03$, log-rank test). In women infected with types other than HPV 16 (n = 200), detection of HPV 16-specific neutralizing antibodies was not correlated with disease outcome. In conclusion, development of specific neutralizing antibodies following natural HPV 16 infection did not favor a better outcome of low-grade cervical lesions induced by HPV 16 or by other types; rather, detection of neutralizing antibodies generated by current infection may reflect viral persistence and thus help identify those who are at high risk of disease progression. ***J. Med. Virol. 84:1128–1134, 2012.***

KEY WORDS: human papillomavirus; neutralizing antibody; low-grade squamous intraepithelial lesion; progression; cervical intraepithelial neoplasia

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INTRODUCTION

Human papillomavirus type 16 (HPV 16) is the most common genotype detected in cervical cancer worldwide [Muñoz et al., 2003]. HPV 16 virus-like particles (VLPs) are obtained through the self-assembly of the major capsid protein L1 expressed in insect or yeast cells. HPV 16 VLP-based ELISA has been used to detect HPV 16 capsid antibody responses following natural infections [Frazer, 2009]. More than half of women infected by HPV 16 produce serum IgG antibodies [Carter et al., 2000]. HPV 16 VLP IgG antibodies are more commonly detected in women who have been persistently positive for HPV 16 DNA at different time points [de Gruijl et al., 1997; Sasagawa et al., 1998]. In women not infected currently with HPV 16, the presence of ELISA antibody responses to HPV 16 VLPs correlated with a significantly reduced risk of subsequent new HPV 16 infection, although the risk was reduced by only 50% compared to that of seronegative women [Ho et al., 2002; Safaeian et al., 2010].

In the case of many viruses, the presence of serum neutralizing antibodies is a correlate of immune protection. Recently, production of HPV 16 pseudovirions encapsidating a secreted alkaline phosphate (SEAP) reporter gene has allowed efficient measurement of specific HPV 16 neutralizing antibodies in women infected with HPV 16 or vaccinated with HPV 16 VLPs. Although serum antibody titer measured by VLP-based ELISA correlated with the neutralizing antibody titer, the neutralization assay was found to be more sensitive and type-specific than the VLP-based ELISA detecting total antibodies to HPV 16 capsids [Pastrana et al., 2004]. Immunization with VLP-based vaccines elicits neutralizing antibodies and protects against new infections in animals and humans [Stanley, 2010]. However, there have been few studies assessing the role of neutralizing antibodies in women infected currently with HPV or in those with cervical diseases. The neutralization activity against HPV may prevent persistence of infection by inhibiting viral spread between cervical epithelial cells or act as markers of host immunity responses to control cervical HPV infection.

Most low-grade cervical lesions are known to regress spontaneously, whereas only a small fraction progress to precancer lesions and cervical cancer [Östör, 1993; Melnikow et al., 1998; Holowaty et al., 1999]. In the current study, baseline serum neutralizing antibodies against HPV 16 were measured to assess possible association with clinical outcomes of low-grade cervical lesions induced by HPV 16 or other HPV types.

MATERIALS AND METHODS

Study Design

Follow-up data from the Japan HPV And Cervical Cancer (JHACC) study (a prospective nonintervention cohort study conducted to assess regression and progression of low-grade cervical abnormalities [Matsumoto et al., 2010, 2011]) were used in this study. Among a total of 570 study subjects, 242 women, whose serum samples were available for the HPV 16 neutralizing assay, were enrolled in the present study. Details of the design and methods have been provided elsewhere [Matsumoto et al., 2011]. Briefly, women with low-grade squamous intraepithelial lesions which were diagnosed as cervical intraepithelial neoplasia grade 2 or less on initial biopsy were recruited from nine hospitals. All volunteer patients provided written informed consent. Cervical smears were classified according to the Bethesda System [Solomon et al., 2001]. On enrollment, two cervical punch biopsy specimens were collected and stained with hematoxylin and eosin (H&E). Histological diagnosis was based on the World Health Organization (WHO) classification system. All cytological and histological specimens were reviewed by two cytopathologists (Y. H. and Masafumi Tsuzuku) and two pathologists (R. F. and T. K.). Levels of serum neutralizing antibodies to HPV 16 and cervical HPV DNA were also determined on enrollment. Researchers conducting the assays were blinded to the corresponding clinical data collected from the patients. The patients meeting enrollment criteria were followed at 3- to 4-month intervals with cytology and colposcopic examination. Cervical HPV DNA was determined at 24 months after enrollment. To avoid interference with the natural course of the disease, a cervical biopsy was performed only when the follow-up findings suggested progression to cervical intraepithelial neoplasia grade 3 or worse. The two cytopathologists and two pathologists reviewed all the cytological and histological specimens collected for diagnosis of disease progression. Progression was defined histologically as the presence of cervical intraepithelial neoplasia grade 3 lesions. In this analysis, regression was defined as normal colposcopy and at least two consecutive negative cervical smears. Lesions which did not regress or progress during the follow-up period were defined as persistent lesions.

The study protocol was approved by the ethics and research review boards of the participating institutions.

HPV Genotyping

HPV DNA in cervical samples was determined by the polymerase chain reaction (PCR), as described previously [Yoshikawa et al., 1991]. Briefly, exfoliated ecto- and endocervical cells were placed in a tube containing 1 ml phosphate buffered saline and stored at −30°C until DNA extraction. Total cellular DNA was extracted by a standard sodium dodecyl sulfate (SDS)–proteinase K procedure. HPV DNA was amplified by PCR using consensus-primers (L1C1/L1C2 + L1C2M) for the HPV L1 region. A reaction mixture without template DNA was included in every

set of PCR runs as a negative control. In addition, primers for a fragment of the β-actin gene were used as an internal control to assess the quality and quantity of template DNA in each PCR specimen. HPV types were identified by restriction fragment length polymorphism (RFLP), which has been shown to distinguish at least 26 types of genital HPVs [Nagano et al., 1996].

To minimize misclassification errors of HPV 16 DNA, HPV 16 infection was confirmed by PCR-amplified DNA sequencing using HPV 16 E6-specific synthetic primers (5-GACATTTTATGCACCAAAAG-3 and 5-GTATCT CCATGCATGATTAC-3, spanning nt 75–575) [Matsumoto et al., 2000].

Preparation of Pseudovirions

Dr. J. T. Schiller kindly donated three plasmids for this study: pYSEAP, expressing secreted alkaline phosphatase (SEAP); p16L1h, expressing HPV 16 L1; and p16L2h, expressing HPV 16 L2. 293FT cells (Invitrogen, Carlsbad, CA) cultured in two 10-cm culture dishes (6×10^6 cells/dish) for 16 hr were transfected with a mixture of a p16L1h plasmid (13.5 μg), a p16L2h plasmid (3 μg), and pYSEAP plasmid (13.5 μg) using Fugene HD (Rosch Diagnostics, Mannheim, Germany). After 60 h, pseudovirions (PVs) were purified from the cells, as described previously [Kondo et al., 2007], and their infectivity was estimated from the SEAP activity in culture media of infected cells using a colorimetric assay.

Neutralization Assay

The neutralization assay was performed as described previously [Ochi et al., 2008]. Briefly, 50 μl of serum was diluted with 50 μl of neutralization medium (DMEM [without phenol red], 10% FBS, 1% nonessential amino acids, 1% GlutaMax-I) containing an aliquot of the PV stock (400 pg of HPV 16 L1) giving an optical density of approximately 1.0 in the SEAP activity assay conditions and then incubated for 1 h at 4°C. The amount of PV used was in the linear range of the dose–response curve. The mixture was then inoculated onto 293FT cells that had been cultured in 96-well plates (1×10^4 cells/well) for 16 h. Culture media were harvested after 4.5 days at 37°C, and SEAP activity was determined. The neutralization titer is presented as the reciprocal of the maximum dilution of serum that reduced SEAP activity to half the level of that in the control samples.

Statistical Analysis

All time-to-event analyses were based on the actual date of visits. For regression or progression, time to event was measured from the date of the index visit (i.e., the first instance of an abnormal cytology result) to the date of the visit at which cytological transition to normal or to cervical intraepithelial neoplasia grade 3 was first detected. Women whose lesions persisted or who dropped out of the study were censored at their last recorded return visit dates. Patients who had only one negative colposcopy/cytology result before loss to follow-up were censored at the last date of positive Pap tests. Subjects who were biopsied were censored at the time of their biopsy, regardless of the biopsy results, to reduce the potential for interference by the biopsy procedure on estimates of time of regression. Cumulative probability of disease regression or progression was estimated using the Kaplan–Meier method and compared with a log-rank test, and the Cox regression model was used for statistical adjustments. Patient age (18–29, 30–39, or 40–54 years of age) and initial biopsy results (cervical intraepithelial neoplasia grade 1 or 2) were included in the multivariate model for adjustments. The χ^2 or Fisher's exact test was used to determine whither presence of HPV 16-specific neutralizing antibodies is significantly associated with risk of viral persistence. All analyses were carried out using the JMP 7.0J statistics packages (SAS Institute, Cary, NC). Two-sided P-values were calculated throughout and differences were considered significant for $P < 0.05$.

RESULTS

A total of 242 women with low-grade squamous intraepithelial lesions (206 with cervical intraepithelial neoplasia grade 1 and 36 with grade 2) were enrolled in this study. Mean age was 35.7 years (range 19–52 years). The total number of clinical visits was 3850 and the mean follow-up time was 46.2 months (median 43.2; range 6.8–84.9). During the follow-up period, 26 lesions progressed to cervical intraepithelial neoplasia grade 3, and 159 spontaneously regressed to normal cytology. No progression to invasive cancer was observed.

Detection of serum neutralizing antibodies against HPV 16 was strongly associated with the presence of viral DNA in the cervix (Table I), consistent with previous reports [Ochi et al., 2008]. The detection rate of HPV 16-specific neutralizing antibodies was much higher in women infected with HPV 16 than in those infected with other HPV types or those without any HPV DNA (59.5% vs. 12.5%, $P < 0.0001$ by χ^2 test). HPV 16-specific neutralizing antibodies were detected in 10.4% (19/182) of women with HPV strains other than HPV 16 and in 33.3% (6/18) of those without any HPV DNA. In the great majority of those with HPV 16 neutralizing antibodies, titers ranged between 40 and 320. High-titer (≥1,280) neutralizing sera were found in two women infected with HPV 16 and in one without any HPV DNA.

In those infected with HPV 16, a trend was observed for longer persistence of low-grade squamous intraepithelial lesions in HPV 16-specific neutralizing antibody carriers (median time to cytological regression: 23.8 months vs. 7.2 months, Fig. 1A), but the difference did not reach statistical significance ($P = 0.18$). Statistical adjustments for age and initial

TABLE I. The Association Between Cervical HPV Genotypes and Neutralizing Antibodies Against HPV 16

		Neutralizing antibodies against HPV 16							
		Neutralization		Neutralizing titer					
Cervical HPV genotype	N	Negative	Positive (%)	40	80	160	320	640	≥1,280
HPV 16 DNA positive	42	17	25 (59.5%)*	7	6	2	6	2	2
HPV 16 alone	34	13	21 (61.8%)	4	5	2	6	2	2
Multiply infected	8	4	4 (50.0%)	3	1	0	0	0	0
HPV 16 DNA-negative	200	175	25 (12.5%)*	4	8	6	2	3	1
Negative	18	12	6 (33.3%)	0	1	1	0	2	1
HPV 6	2	2	0 (0.0%)	0	0	0	0	0	0
HPV 18	9	8	1 (11.1%)	0	0	0	1	0	0
HPV 31	5	4	1 (20.0%)	0	1	0	0	0	0
HPV 33	2	1	1 (50.0%)	1	0	0	0	0	0
HPV 35	4	4	0 (0.0%)	0	0	0	0	0	0
HPV 39	4	4	0 (0.0%)	0	0	0	0	0	0
HPV 51	29	27	2 (6.9%)	1	1	0	0	0	0
HPV 52	35	31	4 (11.4%)	0	2	1	0	1	0
HPV 53	4	4	0 (0.0%)	0	0	0	0	0	0
HPV 56	23	22	1 (4.3%)	0	1	0	0	0	0
HPV 58	24	22	2 (8.3%)	1	1	0	0	0	0
HPV 59	2	1	1 (50.0%)	0	1	0	0	0	0
HPV 61	1	1	0 (0.0%)	0	0	0	0	0	0
HPV 66	6	5	1 (16.7%)	0	0	1	0	0	0
HPV 68	2	2	0 (0.0%)	0	0	0	0	0	0
Undetermined	14	12	2 (14.3%)	0	0	1	1	0	0
Multiple infection	16	13	3 (18.8%)	1	0	2	0	0	0

*The difference was statistically significant ($P < 0.0001$ by χ^2 test).

biopsy results (cervical intraepithelial neoplasia grade 1 or 2) did not change this finding (data not shown). Interestingly, progression to cervical precancer occurred only in those who had HPV 16-specific neutralizing antibodies at the baseline (Fig. 1B), and the associated risk of progression was statistically significant ($P = 0.03$ by log-rank test). Among women with cervical intraepithelial neoplasia grade 2 lesions, all (3/3, 100%) who had HPV 16-specific neutralizing antibodies at the baseline were diagnosed with grade 3 lesions within 5 years, while no such progression was observed in those who did not have HPV 16 neutralizing antibodies (0/5, 0%) ($P = 0.01$ by log-rank test). Among women with cervical intraepithelial neoplasia grade 1, 13.6% (3/22) positive for HPV 16 neutralizing antibodies were diagnosed with grade 3 disease within 5 years, while no such progression was found in those without HPV 16 neutralizing antibodies (0/12, 0%) ($P = 0.20$ by log-rank test). Adjusted P-values could not be calculated in the Cox proportional hazard model because no event occurred among women without serum HPV 16 neutralizing antibodies.

Neutralizing antibody responses resulting from previous HPV 16 clearance did not favor better outcomes of cytological abnormalities induced by other HPV types. The probability of disease regression within 2 years was not significantly different between women with or without serum HPV 16-specific neutralizing antibodies (44.0% vs. 57.0%, $P = 0.35$ by log-rank test). There was also no significant difference between these two groups in probability of progression to cervical intraepithelial neoplasia grade 3 within 5 years (8.3% vs. 12.0%, $P = 0.44$ by log-rank test). Analyses confined to the various cervical HPV 16-related types studied (HPV 31, HPV 33, HPV 35, HPV 52, and HPV 58) or statistical adjustment for age and initial biopsy results did not change these findings (data not shown).

HPV DNA data at baseline and 24 months were analyzed to determine whether detection of HPV 16-specific neutralizing antibodies was associated with persistent HPV infections. Results at 24 months were available for 149 women. Persistent infection was defined as continued detection at 24 months of HPV genotypes present at baseline. Among women infected with HPV 16 (n = 26), HPV 16 persistence was more common in those positive for serum HPV 16 neutralizing antibodies than in those with none (61.5% vs. 15.4%, Fisher's exact test: $P = 0.04$). Women who had serum HPV 16 neutralizing antibodies were found to be at a much higher risk of persistent infection compared with those who had none (odds ratio 8.06, 95% confidence interval 1.51–51.3). Among women infected with other HPV types (n = 123), HPV 16 neutralizing antibodies were not associated with persistent infections by baseline HPV genotypes (χ^2 test, $P = 0.61$).

DISCUSSION

In women with low-grade cervical lesions induced by HPV 16, progression to cervical precancer (cervical intraepithelial neoplasia grade 3) occurred only