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Original Article

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The first-round results of a population-based cohort study of HPV testing in Japanese cervical cancer screening: baseline characteristics, screening results, and referral rate

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ABSTRACT

Objective: In 2013, a cohort study aimed to clarify the positive and negative effects of introducing the human papillomavirus (HPV) testing for population-based cervical cancer screening has been launched in Japan. This study included four screenings during the subsequent 7-year follow-up period. We aim to describe the results of the first round of this study on cervical cancer screening here.
Methods: This study began in September 2013 with recruitment completed in March 2016. Women aged 30–49 years were divided into 2 groups: those who received uterine cervical cytology alone in the first year (control group), or those who received a combination of cytology and HPV testing (intervention group), based on their age. After first screening, women with positive result of cytology or positive HPV test required referral. We summarized the results of the first round of cervical cancer screening.
Results: Of the 25,074 women who were eligible for the study, 13,845 women (55.2%) were screened with cytology alone; 11,229 women (44.8%) received a combination of cytology and HPV testing. After screening, 407 women (2.9%) in the control group and 1,003 women (8.9%) in the intervention group required referral, respectively. Adding HPV testing increased referral rate significantly ($p<0.001$).
Conclusion: After first screening, introduction of HPV testing appears to contribute to significantly higher referral rates, suggesting that the number of colposcopies as a detailed examination may increase. These preliminary findings suggest that if HPV testing is

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Conflict of Interest
Dr. Aoki declares receipt of honoraria from Roche Diagnostics K. K. and Hologic Japan, Inc, and expert testimony fees from Sysmex Corporation and Sekisui Medical Co., Ltd. (Inst). Dr. Miyagi declares receipt of honoraria from Roche Diagnostics K. K. Other authors have nothing to declare.

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introduced into screening, medical institutions need to be prepared for an increasing number of follow-up examinations.

Keywords: Cervical Cancer; Human Papillomavirus; Cytology; Cancer Screening

INTRODUCTION

The pivotal role of high-risk human papillomavirus (HPV) infection in the development of cervical cancer is well-established [1,2]. Studies have reported that HPV testing is more sensitive than cytology in cervical cancer screening and have highlighted better detection rates for cervical intraepithelial neoplasia (CIN). Therefore, HPV testing may potentially decrease cervical cancer mortality [3,4]. Several trials have investigated the effectiveness and efficacy of introducing HPV testing into cervical cancer screening programs. Randomized studies comparing HPV testing-based screening with cytology have been conducted in Italy (New Technologies for Cervical Cancer; NTCC) [5,6], England (A Randomised Trial In Screening To Improve Cytology; ARTISTIC) [7,8], the Netherlands (Population Based Screening Study Amsterdam; POBASCAM) [3,9], and Sweden (Swedescreen) [10]. While having adequate sample sizes to compare the effectiveness and efficacy of cytology with that of HPV testing, these studies involved substantially different screening protocols, age groups, and strategies for follow up of screen-positives, limiting generalizability to other countries for reducing their cervical cancer mortality rates [11].

Cervical cytology remains a reliable modality for cervical cancer screening with robust evidence that it can decrease cervical cancer mortality [12-14]. Nonetheless, there are currently many screening options available, including cytology alone, primary HPV testing, combination cytology-HPV testing, and HPV testing and triage with cytology. Adding to this variability cervical cancer screening programs differ by country, especially in terms of policies and strategies regarding the introduction of HPV testing. In Japan, population-based cervical cancer screening using cytology for women aged over 20 years is executed based on guidelines from the Ministry of Health, Labour and Welfare, which defines cytology as a modality of cervical cancer screening [15]. Introduction of HPV testing for cervical cancer screening has the potential for early detection and reduction of cervical cancer, however, a large-scale study that directly compares HPV testing-based screening with cytology-based screening has not been conducted in Japan. Japan still has a need for evaluation of the effectiveness of HPV testing, including benefit of early detection of cervical cancer and harm of false-positive, to establish specific screening strategies suitable for recommendation for a population-based cervical cancer screening program. Our work began as a cohort study from a population-based screening program conducted by selected local governments to evaluate the effectiveness of HPV testing verification services. This was done in the framework of cancer screening promotion services by the Ministry of Health, Labour and Welfare in 2013. Firstly, local governments that had been conducting cancer screening programs under good management were selected to the study. These selected local governments had sufficient infrastructure with call-recall systems for sending invitation letters to eligible screenees, monitoring systems for screening results, and centralizing systems for processing screening data [16]. After the selection of 39 local governments, participant recruitment began in September 2013 and was completed in March 2016. Thus, our study was conducted within the current established population-based screening program in Japan. To estimate the effectiveness of cancer screening under longitudinal conditions, our study consisted of 4

rounds of screening during a 7-year follow-up period. Here, we aimed to describe here the first-round cervical cancer screening results of basal characteristics, results of screening, and referral rate.

MATERIALS AND METHODS

In this study, 39 local governments throughout Japan participated in this HPV testing trial for population-based cervical cancer screening. Details of the study design, including the selection of the 39 local governments, allocation details, and exclusion criteria, have been described previously [16]. Briefly, women between 30 and 49 years old who received cervical cancer screening conducted by their local government were eligible to participate in the study. All women provided informed consent and underwent either cytology testing alone (control group) or combined cytology and HPV testing (intervention group) as screening in the first year. Based on starting year of Japan's HPV vaccination program, women who participated were not in the eligible age ranges for participating in the HPV vaccination programs.

Allocation was based on age, in general, participants aged 30, 35, 40, and 45 years were allocated to the intervention group and the remaining participants aged 31–49 were allocated to the control group. We assigned the age group corresponding to the age of the intervention group to the control group.

None of the participating women had a history of receiving an HPV vaccination through the Japan's national immunization program. Selection of cytology technique (either conventional cytology [CC] or liquid-based cytology [LBC]) and HPV testing product was left to the discretion of each local government responsible for conducting a cervical cancer screening program, though the study recommended each local government select a single cytology technique and a single HPV testing product for use for all participants in their screening program. Cytology results were reported in accordance with the Bethesda system 2001. After first screening, women with atypical squamous cells of undetermined significance (ASC-US) and worse than ASC-US (>ASC-US), or positive HPV (HPV+) test were considered as a screened positive and required referral. This study centralized all data to monitor situations to ensure all participants received equal quality of health services from their local governments as the first trial for cancer screening program in Japan.

In the control group, women with ASC-US were referred for triage with HPV testing, and women with >ASC-US were referred for immediate colposcopy and biopsy. In the intervention group, women with ASC-US/HPV negative (HPV–) and negative for intraepithelial lesion or malignancy (NILM)/HPV+ were referred for cytology after 12 months. Women with ASC-US/HPV+ and >ASC-US/regardless of HPV status were referred for immediate colposcopy and biopsy. Women with NILM in the control group and NILM/HPV- in the intervention group were invited to further screenings at 2-year intervals using cytology alone (**Fig. 1**). In our study, the sample size was determined to detect a 25% difference CIN3 or worse (CIN3+) between the intervention and control groups at a conventional significance level of p=0.05 with a power of 80% during the study period. The calculated cumulative incidence of CIN3+ in the target age group was 2.3% in the control group and 1.7% in the intervention group. Thus, we considered 10,000 women as a sufficient sample size for each group [16].

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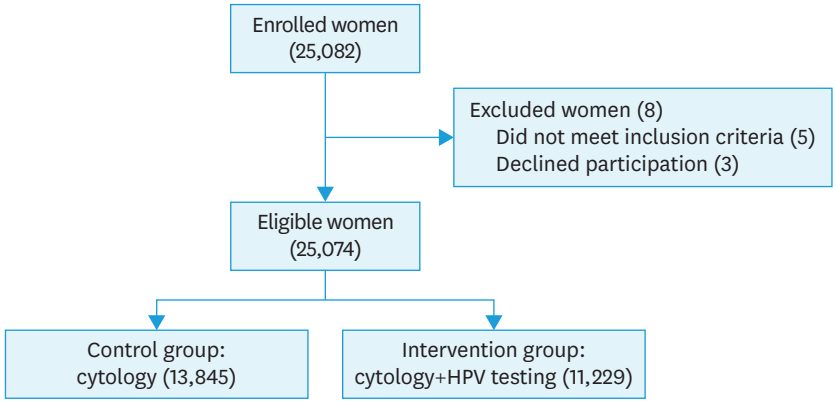

Fig. 1. Diagram of women through the baseline phase. Total 25,082 women enrolled the study and of 25,074 women who were eligible for the study, 13,845 women (55.2%) were screened with cytology alone (control group); 11,229 women (44.8%) received a combination of cytology and HPV testing (intervention group). HPV, human papillomavirus.

We defined characteristics of women screened in the first round of testing including age, geographical area, collection methods of cytology samples (cytology technique and sampling devices), and HPV testing products. Cytology results, including unsatisfactory results, were compared between the control and intervention groups. For the intervention group, screening results of both cytology and HPV testing were reported, and cytology results were compared by HPV status. We evaluated any differences in significance in various characteristics or screen results using χ^2 tests and considered $p<0.05$ to be statistically significant. Statistical analyses were conducted using SPSS for Windows (version 26.0; IBM Corp., Armonk, NY, USA).

This study was approved by the Institutional Review Board at Keio University School of Medicine (approval number: 20130139, 20140037) and registered at the University Hospital Medical Information Network Clinical Trial Registration (UMIN-CTR), Japan (number: UMIN000014720). The study was conducted in accordance with the Declaration of Helsinki and the Ethical Guideline on Clinical Studies of the Ministry of Health, Labour and Welfare of Japan. This study was funded by the Health, Labour and Welfare Sciences Research Grants (H25-Ganrinsyo-Shitei-001), a fund of the Commission for Sciences Research from the Ministry of Health, Labour and Welfare (H26-Kakushintekigan-Ippan-016), and the Practical Research for Innovative Cancer Control from Japan Agency for Medical Research and Development (AMED, 15ck0106020h0002, 16ck0106020h0003, 17ck0106275h0001, 18ck0106275h0002, 19ck0106275h0003, 20ck0106559h0001).

RESULTS

During the recruitment period, 25,082 women agreed to participate in this study. Of these, 5 women did not meet the inclusion criteria and three women withdrew from participating. A total of 25,074 eligible women were allocated to either the control or intervention group based upon their ages. In the control group, 13,845 women (55.2% of total) were screened with cytology alone. In the intervention group, 11,229 women (44.8% of total) were screened with cytology and HPV testing (**Fig. 2**). As shown in **Table 1**, age distributions differed between the control and intervention groups, with an average age of 37.2 (95% confidence

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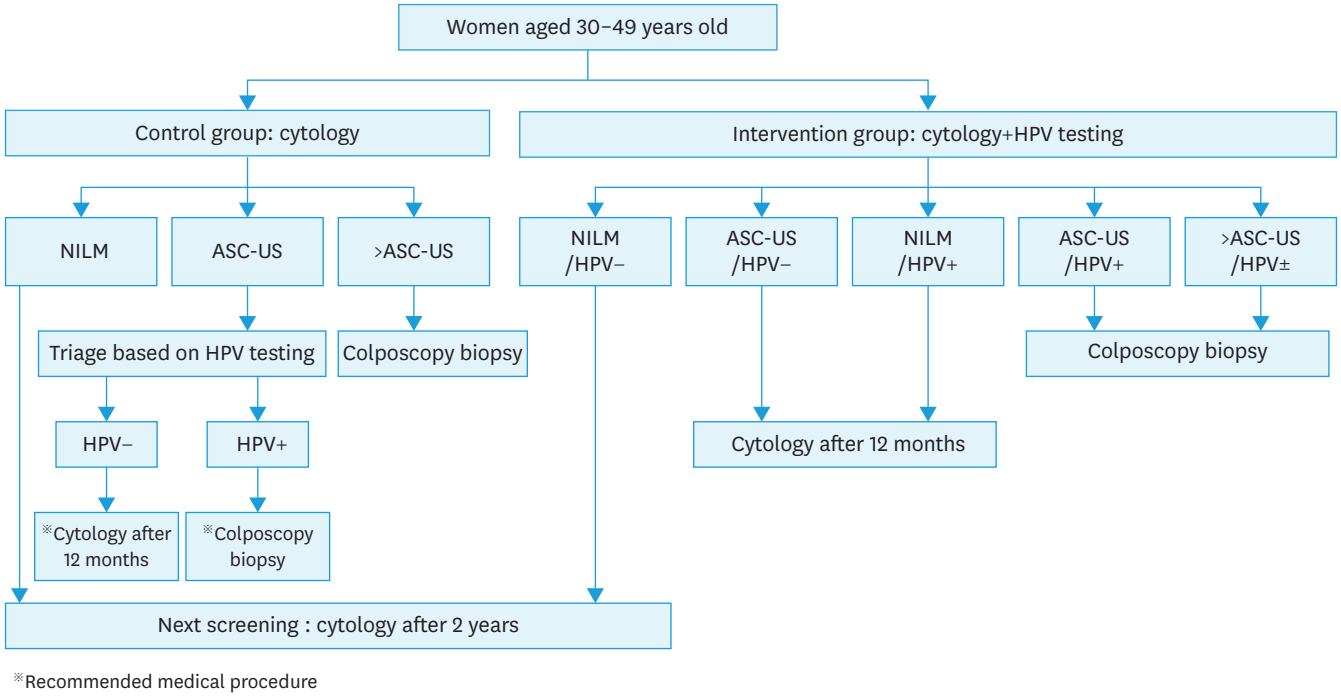

Fig. 2. Clinical management and timeline according to study group. Women with NILM in the control group and NILM/HPV- in the intervention group were invited to further screenings at two year intervals using cytology alone. Besides NILM and NILM/HPV-, clinical management is set up depend on cytological abnormality and HPV infection status.
ASC-US, atypical squamous cells of undetermined significance; >ASC-US, abnormal cytology worse than atypical squamous cells of undetermined significance; HPV–, a negative human papillomavirus test result; HPV+, a positive human papillomavirus test result; NILM, negative for intraepithelial lesion or malignancy.

interval [CI]=32.0–42.3) years in the control group versus 35.9 (95% CI=31.2–40.6) years in the intervention group. The geographical distribution of the screenees was spread throughout Japan. The highest participation was found in Kanto area (including the Tokyo metropolitan area), which reached over 60% in both groups. For cytology techniques, LBC was used less than 20% compared with CC in both groups (LBC 19.5%, CC 80.5% in the control group; LBC 14.7%, CC 85.3% in the intervention group). For cytology sampling devices, more than 90% of samples were collected by brush or scraper (cytobrush, plastic spatula, or cytopic) in both groups, however 4.5% and 8.1% were collected by cotton swabs in the control and intervention groups, respectively. When HPV testing was performed, samples tested with the COBAS 4800 HPV test®, HC II HPV DNA test®, and Cervista high risk HPV test® were 63.4%, 24.3%, and 11.3% respectively. In our study, a total of seven samples (0.03%) were considered to have unsatisfactory results because of insufficient sample preparation. The details of the unsatisfactory samples are shown in the **Supplementary Table 1**.

As shown in **Table 2**, after the first-round of screening, a total of 407 women (2.9%) screened positive in the control group and 1,003 women (8.9%) screened positive in the intervention group. The positive screening rate was more than double in the intervention group across all age categories, and ranged from 6.6% to 12.1% in the intervention group versus 1.8% to 3.5% in the control group.

The summarized cytology results by age category are shown in **Table 3**. The overall cytology positive rate of 3.7% in the intervention group was significantly higher than the 2.9% positive rate in the control group ($p<0.001$). This trend was found in the 30–34-year age group and in

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Table 1. Baseline characteristics of study population

Characteristics	Control (n=13,845)	Intervention (n=11,229)	p-value*
Age (yr)			<0.001
30–34	3,014 (21.8)	3,272 (29.1)	
35–39	4,306 (31.1)	3,552 (31.6)	
40–44	4,075 (29.4)	3,525 (31.4)	
45–49	2,450 (17.7)	880 (7.8)	
Area (number of local governments)			<0.001
Hokkaido·Tohoku (n=6)	92 (0.7)	244 (2.2)	
Kanto (n=8)	10,579 (76.4)	7,240 (64.5)	
Toukai (n=4)	160 (1.2)	524 (4.7)	
Kinki (n=6)	460 (3.3)	1,037 (9.2)	
Chugoku·Shikoku (n=9)	2,185 (15.8)	836 (7.4)	
Kyushu (n=6)	369 (2.7)	1,348 (12.0)	
Cytology technique			<0.001
Liquid based cytology	2,694 (19.5)	1,654 (14.7)	
Conventional cytology	11,151 (80.5)	9,575 (85.3)	
Sampling device			<0.001
Cytobrush	12,083 (87.3)	9,065 (80.7)	
Plastic spatula	111 (0.8)	551 (4.9)	
Cytopick	1,026 (7.4)	690 (6.1)	
Cotton swabs	618 (4.5)	912 (8.1)	
Others	4 (0.0)	10 (0.1)	
Unknown	3 (0.0)	1 (0.0)	
HPV testing (kit)			n/a
COBAS 4800 HPV	n/a	7,114 (63.4)	
HPV DNA QIAGEN HC II	n/a	2,729 (24.3)	
Cervista HPV HR	n/a	1,268 (11.3)	
Amplicor HPV	n/a	67 (0.6)	
Others	n/a	51 (0.5)	

Values are presented as number of participants (%).
HPV, human papillomavirus; n/a, not applicable.
*The p-value from Pearson's χ^2 test.

Table 2. Results of screening test by age group

Screen result	Control	Intervention	p-value*
Total			<0.001
Negative†	13,432 (97.0)	10,226 (91.1)	
Positive‡	407 (2.9)	1,003 (8.9)	
Undecidable§	6 (0.0)	0 (0.0)	
30–34 years			<0.001
Negative†	2,911 (96.6)	2,875 (87.9)	
Positive‡	101 (3.4)	397 (12.1)	
Undecidable§	2 (0.1)	0 (0.0)	
35–39 years			<0.001
Negative†	4,156 (96.5)	3,253 (91.6)	
Positive‡	149 (3.5)	299 (8.4)	
Undecidable§	1 (0.0)	0 (0.0)	
40–44 years			<0.001
Negative†	3,961 (97.2)	3,291 (93.4)	
Positive‡	112 (2.7)	234 (6.6)	
Undecidable§	2 (0.0)	0 (0.0)	
45–49 years			<0.001
Negative†	2,404 (98.1)	807 (91.7)	
Positive‡	45 (1.8)	73 (8.3)	
Undecidable§	1 (0.0)	0 (0.0)	

Values are presented as number of participants (%).
ASC-US, atypical squamous cells of undetermined significance; HPV, human papillomavirus; NILM, negative for intraepithelial lesion or malignancy.
*The p-value from Pearson's χ^2 test; †NILM result in control group and NILM with HPV negative results in intervention group; ‡Cytology abnormality include ASC-US in control group and either cytology abnormality include ASC-US or HPV positive result in intervention group; §There is no cytological screening results in control group. There is no screening results neither cytology nor HPV testing in intervention group.

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Table 3. Results of cytology and HPV testing by age group

Age group	Screen result	Control (n=13,845)	Intervention (n=11,229)	p-value*
Total	NILM	13,432 (97.0)	10,809 (96.3)	<0.001
	ASC-US	166 (1.2)	203 (1.8)	
	>ASC-US	241 (1.7)	216 (1.9)	
	Unsatisfactory	6 (0.0)	1 (0.0)	
	Cytology positive†	407 (2.9)	419 (3.7)	<0.001
	HPV positive‡	n/a	855 (7.6)	n/a
30–34 years	NILM	2,911 (96.6)	3,117 (95.3)	0.001
	ASC-US	33 (1.1)	79 (2.4)	
	>ASC-US	68 (2.3)	75 (2.3)	
	Unsatisfactory	2 (0.1)	1 (0.0)	
	Cytology positive†	101 (3.4)	154 (4.7)	0.008
	HPV positive‡	n/a	347 (10.6)	n/a
35–39 years	NILM	4,156 (96.5)	3,437 (96.8)	0.395
	ASC-US	60 (1.4)	56 (1.6)	
	>ASC-US	89 (2.1)	59 (1.7)	
	Unsatisfactory	1 (0.0)	0 (0.0)	
	Cytology positive†	149 (3.5)	115 (3.2)	0.548
	HPV positive‡	n/a	260 (7.3)	n/a
40–44 years	NILM	3,961 (97.2)	3,414 (96.9)	0.419
	ASC-US	53 (1.3)	51 (1.4)	
	>ASC-US	59 (1.4)	60 (1.7)	
	Unsatisfactory	2 (0.0)	0 (0.0)	
	Cytology positive†	112 (2.7)	111 (3.1)	0.367
	HPV positive‡	n/a	193 (5.5)	n/a
45–49 years	NILM	2,404 (98.1)	841 (95.6)	<0.001
	ASC-US	20 (0.8)	17 (1.9)	
	>ASC-US	25 (1.0)	22 (2.5)	
	Unsatisfactory	1 (0.0)	0 (0.0)	
	Cytology positive†	45 (1.8)	39 (4.4)	<0.001
	HPV positive‡	n/a	55 (6.3)	n/a

Values are presented as number of participants (%).
ASC-US, atypical squamous cells of undetermined significance; >ASC-US, abnormal cytology worse than, atypical squamous cells of undetermined significance; HPV, human papillomavirus; n/a, not applicable; NILM, negative for intraepithelial lesion or malignancy; Unsatisfactory, unsatisfactory sample preparation.
*The p-value from Pearson's χ^2 test; †This indicates that the sum of ASC-US and >ASC-US; ‡This indicates that a positive HPV test result.

the 45–49-year age group (p=0.001 and p<0.001, respectively). The high rates were especially marked for the ASC-US results, which was doubled in those age groups in the intervention group compared to control group. The summarized results of HPV testing are shown in **Table 3**. Regarding positive and negative results of HPV testing, a total of 855 women (7.6%) were HPV positive in the intervention group. The rates of HPV positives at 30–49 years of age by 5-year age increments were 10.6%, 7.3%, 5.5%, and 6.3%, and the HPV positives rates for women in their 30s tended to be higher than those of women in their 40s.

Table 4 shows the summarized results of the cytology and HPV infection statuses by age groups in the intervention group. There was no statistical difference in the HPV positive rate by different inspection products of HPV testing (data not shown). In all age groups, the proportion of cytology results was significantly different (p<0.001) among HPV infection status. For the HPV infection status with a cytology result of NILM, 10,226 women were HPV negative and 580 women were HPV positive. Thus, a negative result of HPV testing is highly associated with a normal cytology result, and younger women tended to acquire HPV infections. In contrast, the cytology positive rate in the HPV positive group was 29.2% to 38.2%, and was higher when compared with the 1.2% to 2.2% in the HPV negative group. Overall 418 women (203 women were ASC-US and 215 women were >ASC-US) were cytology

Table 4. Results of cytology according to HPV status by age group for intervention group

Age group	Cytology result	HPV−	HPV+	p-value*
Total	NILM	10,226 (98.6)	580 (67.8)	<0.001
	ASC-US	94 (0.9)	109 (12.7)	
	>ASC-US	49 (0.5)	166 (19.4)	
	Unsatisfactory	1 (0.0)	0 (0.0)	
	Cytology positive†	143 (1.4)	275 (32.2)	
30–34 years	NILM	2,875 (98.3)	241 (69.5)	<0.001
	ASC-US	30 (1.0)	49 (14.1)	
	>ASC-US	18 (0.6)	57 (16.4)	
	Unsatisfactory	1 (0.0)	0 (0.0)	
	Cytology positive†	48 (1.6)	106 (30.5)	
35–39 years	NILM	3,253 (98.8)	184 (70.8)	<0.001
	ASC-US	25 (0.8)	31 (11.9)	
	>ASC-US	13 (0.4)	45 (17.3)	
	Unsatisfactory	0 (0.0)	0 (0.0)	
	Cytology positive†	38 (1.2)	76 (29.2)	
40–44 years	NILM	3,291 (98.8)	121 (62.7)	<0.001
	ASC-US	28 (0.8)	23 (11.9)	
	>ASC-US	11 (0.3)	49 (25.4)	
	Unsatisfactory	0 (0.0)	0 (0.0)	
	Cytology positive†	39 (1.2)	72 (37.3)	
45–49 years	NILM	807 (97.8)	34 (61.8)	<0.001
	ASC-US	11 (1.3)	6 (10.9)	
	>ASC-US	7 (0.8)	15 (27.3)	
	Unsatisfactory	0 (0.0)	0 (0.0)	
	Cytology positive†	18 (2.2)	21 (38.2)	

Values are presented as number of participants (%).
ASC-US, atypical squamous cells of undetermined significance; >ASC-US, abnormal cytology worse than atypical squamous cells of undetermined significance; NILM, negative for intraepithelial lesion or malignancy; Unsatisfactory, unsatisfactory sample preparation; HPV−, a negative human papillomavirus test result; HPV+, a positive human papillomavirus test result.
*The p-value from Pearson's χ^2 test; †This indicates that the sum of ASC-US and >ASC-US.

positive in intervention group. For HPV infection status among women with NILM, 580 women (5.4%) were HPV positive and 10,226 women (94.6%) were HPV negative. Among women with ASC-US, 109 women (53.7%) were HPV positive and 94 women (46.3%) were HPV negative. Among women with >ASC-US, 166 women (77.2%) were HPV positive and 49 women (22.8%) were HPV negative. HPV positivity tended to increase as the findings of cytology become severer.

DISCUSSION

The implementation of HPV testing for cervical cancer screening program has been examined mainly in European countries, and several studies have reported the effectiveness of HPV testing for screening programs [11]. Further, some countries such as the Netherlands and Australia have switched their population-based screening program from cytology-based screening to primary HPV screening. To introduce HPV testing for a population-based screening program in Japan, it is essential to conduct an implementation study to assess the effectiveness of HPV testing and the feasibility of operating HPV testing-based screening with current Japanese resources. Cervical cancer incidence, mortality, and quality of screening management differ greatly between countries. Thus, this cohort study was designed to evaluate the effectiveness of HPV testing within the current population-based screening environment.

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Previously, we calculated 10,000 women as a sufficient study size for both control and intervention groups to detect primary endpoint of cumulative incidence of CIN3+ [16]. During the enrollment period, we successfully recruited more than 10,000 women for each group. Therefore, our study had a sufficient sample size to be able to distinguish the cumulative incidence of CIN3+ between the groups. When comparing the characteristics of the groups, the average age of participants was 1.3 years younger in the intervention group. A possible reason is that most of the participating local governments provided free HPV testing to 30, 35, and 40-year-old women in the first year of this study. Therefore, relatively younger women were willing to enroll in the intervention group. When assessing cervical cancer screening in Japan, it should be targeted at all ages over 20 years old in line with the current screening. Since this study was launched as a cohort study to observe the HPV testing verification services, the target was designated for the age group of 30–49, with the aim of being compared in the age group with a relatively high prevalence of cervical cancer.

Regarding cytology techniques, more than 80% of cytology samples were processed with CC, while processing with LBC remained below 20% in both groups. For cytology sampling devices, more than 90% of cytology sampling devices were brushes or spatulas in both groups. We expected that all cytology samples were taken by brush or spatula in the intervention group, however 8.1% samples were taken by cotton swabs. These results indicated that some medical facilities still used cotton swabs for HPV testing as part of their usual screening practices. Regarding inspection products of HPV testing, more than half of HPV testing in this study were COBAS 4800 HPV test®. This type of test has the ability to detect and discern the HPV 16 and 18 types from other high-risk HPV types independently. However, this study did not consider individual HPV types.

The overall positive screening rate was 8.9% in the intervention group and 2.9% in the control group. The higher positive screening rate in the intervention group was mainly from adding HPV positive women, especially those with cytology negative/HPV positive results (NILM/HPV+). Although the same criteria for cytology was used in both groups, overall rates of cytological abnormalities (ASC-US and >ASC-US) in the intervention group were higher than those in the control group ($p<0.001$). The percentage of ASC-US and >ASC-US cases was 1.8% and 1.9%, respectively, in the intervention group versus 1.2% and 1.7%, respectively, in the control group. The trend of cytological abnormality rate was found in 1.3 times higher in the intervention group in the Kanto area and the other 5 regions. It was presumed that the difference in cytological abnormality rate between the control group and the intervention group was not due to regional differences. One of the possible reasons for the high detection rate of cytological abnormalities in the intervention group may be the difference in sensitivity between LBC and CC. Previous studies have reported that the usage of LBC tends to result in higher rates of cytological abnormalities [17,18]. However, over 80% of cytology in both groups were performed with CC. In addition, usage of LBC was lower in the intervention group than in the control group (14.7% vs. 19.5%). Thus, the higher rate of cytological abnormalities in the intervention group was not related to the cytology techniques. A further factor that could affect result of cytological abnormality is HPV infection status may bias staff responsible for reporting cytological results, knowing HPV status, staff may be more likely to report a worse cytological result. Actually, several medical facilities have reported that medical staff made a cytological diagnosis with knowing the results of the HPV test in fiscal 2014. After this report, we have asked medical facilities to make a cytological diagnosis without knowing the HPV status. Another explanation for the higher rate of cytological abnormalities in the intervention group may be due to participating

women who had never been screened before. With the additional testing, women who had never been screened or those who had been irregularly screened, may have preferentially participated in this study. Women who had never been screened might have the potential to increase the risk for cervical cancer [19], which may have affected the higher rates of cytological abnormalities in the intervention group. Whereas this issue might be occur in the situation when the HPV test is first introduced to cervical cancer screening in Japan, but it should be recognized as one of the selection biases of this study.

In our study, the HPV positive rates were 10.6%, 7.3%, 5.5%, and 6.3% in respective 5-year age increments from 30 to 49 years. In previous population based screening based studies involving Japanese women, HPV positive rates were reported as 14.9%, 10.3%, 8.8%, and 8.4% [20], and 13.6%, 7.4%, 5.8%, and 5.4% for these age groups [21]. Our study demonstrated slightly lower HPV positive rates compared with other studies targeting participants of cervical cancer screening. All studies corroborate that there are higher HPV positive rates in those in their 30s compared to those in their 40s.

In the intervention group, 5.4% of women were cytology negative and HPV positive results (NILM/HPV+). The previous study showed that 10.4% of women were NILM/HPV+ [21]. Therefore, adding HPV testing contributed to the increased number of positive women due to women with NILM/HPV+. Among the HPV negative women in the intervention group, 0.8% of women were ASC-US and 4.4% were >ASC-US. As a consideration regarding primary screening by either HPV testing or cytology, the seven-year follow-up period will reveal which screening modality could reduce the CIN3+ incidence by analyzing the results of cytology negative/HPV+ cases or cytology positive/HPV− cases. Analysis of these follow-up data will be useful for consideration of the algorithm most suitable for Japan.

In this study, some limitations may affect the implementation of HPV testing for the population-based screening program. Firstly, this study targeted at relatively younger age group than the current Japanese screening program. Careful consideration should be given to applying the findings of this study to screening examinations of all age groups of women. Secondly, our study design did not select a single HPV testing product or select a single cytology technique (LBC or CC) for the trial, the selection of which was left to the discretion of each local government. These issues could limit the assessment of the efficacy of HPV testing. However, our intention was to clarify the positive and negative impacts of introducing HPV testing into the current population-based screening program in future. In that sense, non-standardized cytology techniques and HPV testing products are strengths of our research and reflect the existing screening environment in Japan. In addition, further analysis of the seven-year follow-up period will reveal whether HPV testing reduces the incidence of CIN3+ or increases over-referral of patients to colposcopy. As such, we expect our findings will represent a realistic picture for implementation of HPV testing for the cervical cancer screening program in Japan.

Compared with the control group, the intervention group showed substantially higher referral rates, especially among younger women. The data suggested that introduction of HPV testing contributes to higher referral rates, suggesting that the number of colposcopies as a detailed examination may increase. These preliminary findings suggest that if HPV testing is introduced into screening, medical institutions need to be prepared for an increasing number of follow-up examinations. We consider our work to be an implementation study and an important step in estimating the realistic impact of introducing this new modality into population-based screening programs.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1
Results of cytology by age group

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