

Pre-vaccination epidemiology of human papillomavirus infections in Japanese women with abnormal cytology

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Short title: Epidemiology of HPV in Japanese women

Abstract

Aim: To investigate the pre-vaccination epidemiology of genital human papillomavirus (HPV) infections and genotypes in women with abnormal cytology in Nagasaki, Japan.

Methods: We performed Pap smear tests, biopsies and HPV genotype testing in Nagasaki Prefecture from August 2007 through November 2009.

Results: During the study period, serial samples of uterine cervical specimens were obtained from 539 subjects with abnormal cytology and/or squamous intraepithelial lesions (SIL) confirmed previously, or with clinically suspected invasive cervical cancer. In 119 HPV-positive subjects with low-grade SIL, the three most prevalent high-risk HPV genotypes were HPV52 (21.8%; 26/119), HPV16 (20.2%; 24/119) and HPV56 (17.6%; 21/119). In 199 women, 127 HPV-positive subjects with high-grade SIL and 67 HPV-positive subjects with squamous cell carcinoma (SCC), the three most prevalent high-risk HPV genotypes were HPV16 (44.3%; 86/194), HPV52 (20.6%; 40/194) and HPV58 (16.0%; 31/194).

Conclusions: Compared with the distribution of high-risk HPV genotypes in other countries, HPV52 was a more common genotype in Nagasaki. With disease progression to SCC, the distribution of high-risk HPV56 belonging to the A6 HPV family decreased, while HPV16 and HPV52 belonging to the A9 HPV family persisted. Our data provide an important resource to address the case for vaccination against HPV genotypes other than HPV16 and HPV18 in Japan.

Key words: epidemiology, genotype, HPV, infection, uterine cervical neoplasia

Introduction

Persistent infections with human papillomavirus (HPV) are recognized as a major cause of cervical cancer. Genital infections with HPV are very common, and these infections are transmitted by sexual contact.¹ However, HPV infections in most cases disappear naturally in a relatively short period, and induce little risk of developing disease.²⁻⁴ We do not fully know the pathological mechanism that results in HPV infection developing into invasive cervical cancer (CC). During persistent infection, different viral characteristics along with HPV genotype may be important, such as the distribution of each type in the population and the ability to evade the host's immune system. Another important factor in persistent infection could be related to the host, such as the host immune reaction against a specific HPV genotype, and sexual behavior.

The distribution of infectious high-risk HPV genotypes and the prevalence of CC in women varies worldwide. Clarification of the relation between clinical characteristics of CC and specific HPV genotypes in a local region may lead not only to implementation of a preventive strategy in that region, but also to an elucidation of the natural history of HPV infections compared with other regions in the world. In Japan, data on the distribution of HPV genotypes remains inadequate. To evaluate the possible effect of an HPV vaccine, we require knowledge of the pre-vaccination epidemiology of genital HPV infections. Thus, to determine the distribution and natural history of HPV infections in Nagasaki, Japan, we performed HPV genotype testing, cervical cytology and colposcopic biopsies.

Methods

Study population

The study included 625 subjects with abnormal cytology and/or histologically confirmed squamous intraepithelial lesions (SIL), or with clinically suspected invasive CC who required examination by colposcopy and directed biopsy. Cytology and HPV DNA test samples were collected in five hospitals in Nagasaki Prefecture from August 2007 through November 2009. Exclusion criteria were patients who had received therapeutic excisions previously or who had non-squamous neoplasms confirmed histologically. Thus, 86 subjects were excluded from the study.

The study protocol was approved by the Ethical Review Board of Nagasaki University and the other hospitals involved. All women were informed of the purpose of the study and gave their consent.

Sample collection and pathologic diagnoses

Specimens were collected using a Cervex Brush (Rovers Medical Devices, the Netherlands) and suspended in 10 mL of SurePath preservative fluid (Becton, Dickinson & Co., USA). We used the samples from the same vial for cytology with the Bethesda III system (2001) and for HPV genotype testing. Cervical specimens for cytology and HPV genotyping were obtained at each visit from participants who received regular follow-up examinations. The cytologic diagnoses of the specimens were performed by experienced cytoscreeners in a commercial laboratory (SRL, Inc., Japan), and they were blinded from the HPV genotyping test. The histopathological review was performed by experienced pathologists of the Division of Pathology at Nagasaki University Hospital.

HPV genotyping test

Genotyping of HPV DNA in the SurePath preservative fluid after preparing glass slides was carried out using the Linear Array HPV Genotyping Test kit (Roche Molecular Systems, Inc., USA). The kit uses the PGMY09/PGMY11 primers⁵ to amplify the L1 conserved region. Following

polymerase chain reaction amplification, hybridization of the HPV amplicon was performed using an array of oligonucleotide probes that allowed independent identification of individual HPV genotypes. This kit can detect 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 and CP6108 (89)). For consistency with previous studies, we considered 16 HPV genotypes (16, 18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) as high-risk genotypes, which are related to CC based on previous reports.⁶⁻⁸

RESULTS

Uterine cervical specimens for cytology and HPV DNA testing were collected from 539 women, with a mean age of 42 years in the age range of 19-94, with abnormal Pap smears and/or previously confirmed squamous intraepithelial lesions (SIL) or with clinically suspected invasive CC. In 154 subjects who were negative for intraepithelial lesion or malignancy (NILM), HPV was positive in 67 women (43.5%), with a mean age of 41 years at their initial HPV DNA test. The three most prevalent high-risk HPV genotypes in the NILM group were HPV 52 (23.9%; 16/67), HPV 16 (23.9%; 16/67) and HPV 71 (11.9%; 8/67) (Figure 1A). In 125 subjects with cytologically low-grade SIL (LSIL), HPV was positive in 119 women (95.2%), with a mean age of 40 years at their initial test. The three most prevalent high-risk HPV genotypes were HPV 52 (21.8%; 26/119), HPV 16 (20.2%; 24/119) and HPV 56 (17.6%; 21/119) (Figure 1B). In 128 participants diagnosed with cytologically high-grade SIL (HSIL), HPV infection was present in 127 women (99.2%), with a mean age of 41 years. In 71 women diagnosed cytologically with squamous cell carcinoma (SCC), HPV infection was positive in 67 women (94.4%), with a mean age of 56 years. In these latter two groups, the three most prevalent high-risk HPV genotypes were HPV 16 (44.3%; 86/194), HPV 52 (20.6%; 40/194) and HPV 58 (16.0%; 31/194) (Figure 1C, D). The results of the other 61 subjects who were diagnosed with atypical squamous cells of undetermined significance (ASC-US) and

atypical cells-cannot exclude HSIL (ASC-H) were not considered in this report. However, the HPV genotype distribution of the ASC-US group was similar to that of the LSIL group. The number of participants in the ASC-H group was too small to determine the distribution of HPV genotypes.

The Table shows the multiple HPV infection status by cytological diagnosis. The percentage of single HPV infection was significantly higher in the SCC group (86.6%; 58/67) than in the LSIL group (53.8%; 64/119) ($P < 0.01$, Fisher's exact test).

Figure 2 shows a histogram of patient age distribution and the number of single infections of HPV 16, HPV 18 and HPV 52 in patients diagnosed histologically with cervical intraepithelial lesions grade 3 (CIN3) and invasive CC. The mean age of patients infected with HPV 52 tended to be higher than that of patients with HPV 16 infection but the difference was not statistically significant ($P = 0.07$, Student t-test).

DISCUSSION

The distribution of HPV genotypes in the LSIL group suggests that HPV 52 is the most frequently observed genotype among subjects with persistent HPV infections in Nagasaki. Other investigators also have reported that HPV 52 was dominant among women with normal cytology or cervical neoplastic lesions in Japan.⁸⁻¹⁰ In the general population, the prevalence of HPV genotypes exhibit geographic differences in different countries, though HPV 16 is found to be most prevalent worldwide.^{7,11} In pre-neoplastic and cancer cases, the geographic differences in prevalence of HPV genotype are diminished and HPV 16 tends to be the most dominant all over the world. HPV 18 and HPV 31 infections have also been reported to show higher prevalence in CIN and CC patients, but in the current study, there was a low prevalence of HPV 18 in the HSIL group. In the SCC group, the prevalence of HPV 18 was similar to that of HPV 52 and 58, although there was a lower number of single infection cases. Because four cases of multiple infections included HPV 18 as well as

HPV 16 and 52 infections, the contribution of HPV 18 infection in the SCC group was difficult to evaluate.

In the LSIL group and the HSIL-SCC group, the distribution of HPV genotypes was different; the most marked differences between the HSIL-SCC group and LSIL group were a more than doubling of the HPV 16 genotype and the disappearance of HPV 56 infection in the former. HPV 16, HPV 52 and HPV 58 belong to the same alpha-papillomavirus species No. 9 family (A9 HPV family), which also includes HPV 31, 33, 35 and 67. However, HPV 56 belongs to the A6 HPV family, which also includes HPV 53 and 66.¹² The results indicated that the prevalence of the A6 HPV family was not small, especially in the Nagasaki LSIL group, but this HPV family was less likely to continue into persistent infection, and the observed prevalence of HPV 56 infection was found to be reduced in the HSIL and SCC groups.

Interestingly, the LSIL group had the lowest single infection rate of HPV (53.8%) (Table) and the rate of single infection was higher (67.7%) in the HSIL group. The SCC group showed the highest rate of single infection (86.6%). This finding has also been reported by other investigators and was suggested to support a monoclonal origin for cancer¹⁰.

We analyzed samples from patients with CIN3 and invasive SCC histologically and counted the number of patients in each age group, 20–29, 30–39, 40–49, 50–59, 60–69 and 70-plus. The most prevalent and dangerous HPV genotypes appeared to be HPV 16 and HPV 18, but the degree of risk of HPV 16/18 remained to be quantified. The histogram in Figure 2 shows the number of individuals who had a single infection with HPV 16, HPV 18 or HPV 52. The mean age and distribution of ages among HPV 16 and 52 types was different (paired t-test, $P = 0.07$), but HPV 52 appeared to be associated with slower progression of carcinogenesis, and HPV 16 and 18 with faster progression. The differences in HPV genotypes may be related not only to development of persistent infection, but also to the speed of progression to SCC.

In Japan, one commercial CC vaccine became available in December 2009. Although this study has some limitations because we included data only from SIL/CC women, our pre-vaccination data on the distribution of genital HPV infections in a region where HPV 52 and 58 are prevalent is valuable to determine the potential usefulness of a bivalent HPV vaccine. Paavonen et al. reported an estimated cross-reactivity against CIN2+ lesions with non-vaccine oncogenic HPV types of 37–54%¹³. Further study of the distribution of HPV genotypes in a SIL/CC population and the transition of pathological changes in patients according to HPV genotype is warranted.

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Figure legends

Figure 1 Prevalence of human papillomavirus (HPV) genotype among participants who were diagnosed with NILM (A), participants who were diagnosed with LSIL (B), participants who were diagnosed with HSIL (C) and with SCC (D). Closed boxes show infection with a single type of HPV DNA, and open boxes show multiple infections with two or more HPV-DNA types.

NILM: negative for intraepithelial lesion or malignancy; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; SCC: squamous cell carcinoma. CP: CP6108 (HPV 89)

Figure 2 Distribution of ages in CIN3 and SCC patients who were infected with one HPV type, HPV 16, HPV 18 or HPV 52. Open boxes show the number of patients infected with only HPV 16, closed gray boxes show the number of patients infected with only HPV 18 and closed black boxes show the number of patients infected with only HPV 52.

20: 20–29 years; 30: 30–39 years; 40: 40–49 years; 50: 50–59 years; 60: 60–69 years; 70+: ≥ 70 years; CIN3: cervical intraepithelial lesions grade 3.

Table. Multiple HPV infection status by cytologic diagnosis. The table shows the percentage and the number of participants who were infected with a single HPV genotype, and two, three or more than four HPV genotypes.

Cytology	n	Single type	Two types	Three types	More than four types
LSIL	119	53.8% (64)	24.4% (29)	10.1% (12)	11.8% (14)
HSIL	127	67.7% (86)	22.8% (29)	5.5% (7)	3.9% (5)
SCC	67	86.6% (58)	10.4% (7)	3.0% (2)	0

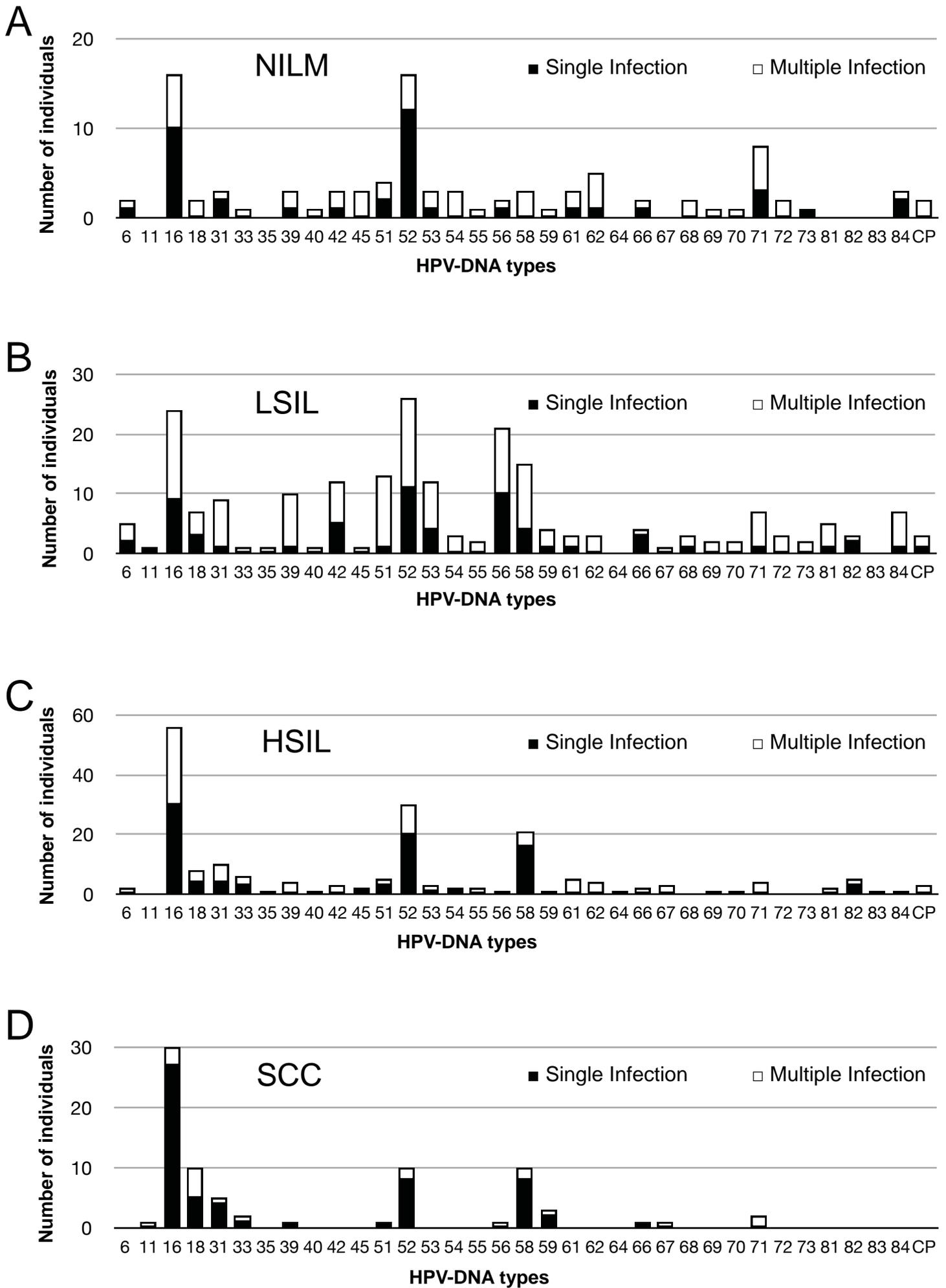


Figure 1. Prevalence of HPV genotype among participants who were diagnosed with cytology.

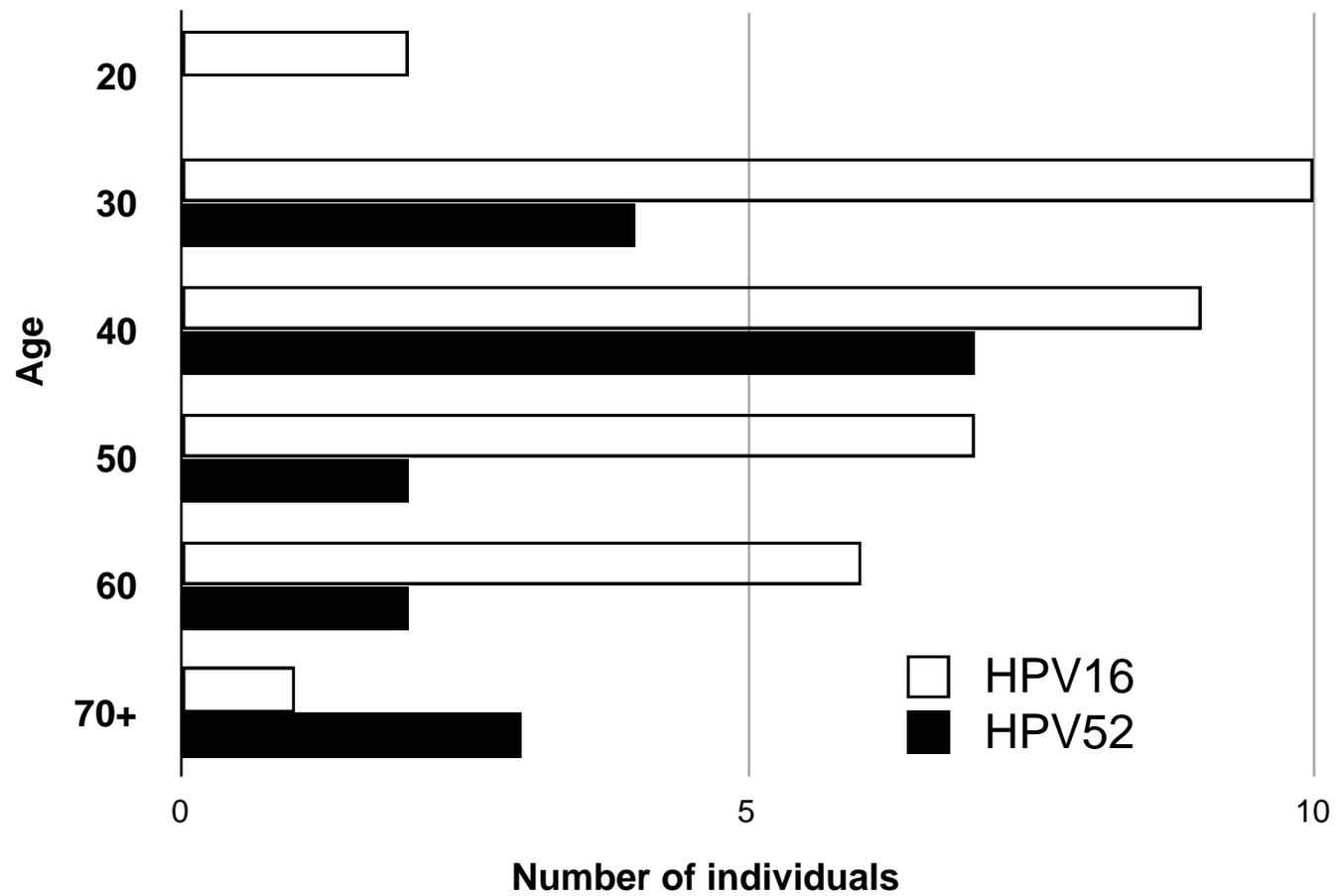


Figure2. Distribution of ages in CIN3 and SCC patients who were infected with single HPV type, HPV 16 or HPV 52.