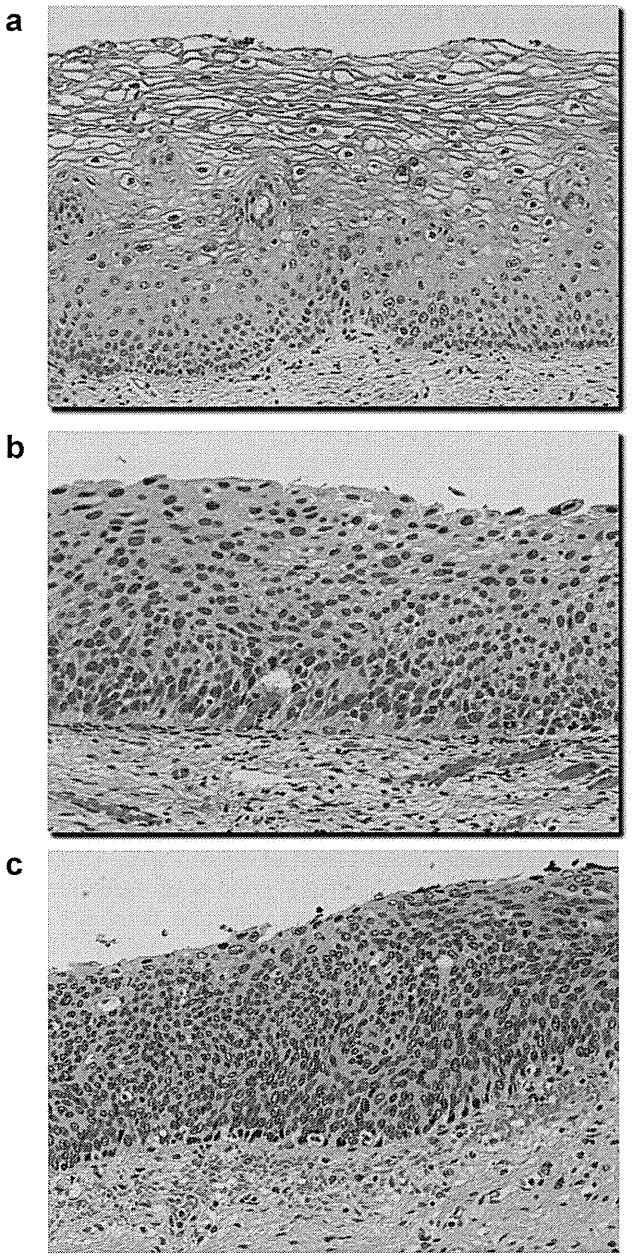

Fig. 1. Histopathological findings of cervical intraepithelial neoplasia in surface epithelium. **a**: Cervical intraepithelial neoplasia 1. The upper two-thirds of epithelium show maturation. **b**: Cervical intraepithelial neoplasia 2: nuclear abnormalities are more striking than in cervical intraepithelial neoplasia 1. The upper third of the epithelium shows maturation. **c**: cervical intraepithelial neoplasia 3: squamous epithelium entirely of atypical basaloid cells.

Test Kit (Roche Molecular Systems, Indianapolis, IN), which uses PGMY09/PGMY11 primers [Gravitt et al., 2000] to amplify the L1 conserved region. Following polymerase chain reaction (PCR) 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 the following 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, 16 HPV genotypes (16, 18, 31, 33, 35, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82) were considered as high-risk genotypes, which were related to cervical cancer in previous reports [Walboomers et al., 1999; Muñoz et al., 2003; Asato et al., 2004].

TaqMan Probes and Primers for HPV 52, HPV 16, and Human Albumin (ALB) Gene

Primers and probes for HPV 52 and 16 quantification were located on the E7 gene and those for the *ALB* gene were on exon 12. Primers and probes were chosen using the Primer express program (Applied Biosystems, Warrington, UK). Probes were labeled with FAM™ dye.

Measurement of Relative HPV DNA Loads by Quantitative Real-Time PCR

Genomic DNA was extracted from cervical cell samples using a DNA Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Genomic DNA samples were prepared at 5 ng/μl using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). For each TaqMan assay, genomic DNA from a case with single HPV 52 persistent infection and from a case with single HPV 16 persistent infection were diluted in sterile water to obtain a calibration curve from 10^6 to 10 copies/μl of solution by 10-fold serial dilution. Real-time PCR was performed using the LightCycler480 (Roche Molecular Systems). The primers and hybridization probe sequence for human albumin (*ALB*; GenBank accession no. M12523) were: forward primer: 5′-TTGGAAAAATCCCACTGCATT-3′, reverse primer: 5′-GAAGGCAAGTCAGCAGGCAT-3′, FAM reporter: 5′-CCGAAGTGGAAAATGATGA-3′. The equivalent sequences for HPV 52 E7 were: forward primer: 5′-CATTCATAGCACTGCGACGG-3′, reverse primer: 5′-CTTGTAATGTGCCCAACAGCA-3′, FAM reporter: 5′-CCTTCGTACTCTACAGCAA-3′, and those for HPV 16 E7 were: forward primer: 5′-TCAGAGGAGGAGGATGAAATAGATG-3′, reverse primer: 5′-AATGGGCTCTGTCCGGTTC-3′, FAM reporter: 5′-CCAGCTGGACAAGC-3′. All PCR mixtures were prepared automatically using QIAgility (Qiagen). Absolute quantitative real-time PCR (10 μl) were carried out in triplicate with 10 ng of unknown genomic DNA, 2× Quanti-Tect-Multiplex-PCR NoROX buffer (Qiagen), 0.125 μM of each primer and 0.0625 μM of each TaqMan probe. Thermal cycling was initiated with a 2-min incubation at 50°C, followed by a first denaturation step of 15 min at 95°C, and then by 60 cycles of 15 sec at 95°C and 1 min at 60°C, followed by one cycle at 5°C for 6 min.

To adjust for differences in the amount of genomic DNA between samples, estimates of the amount of the nuclear gene *ALB* were made. Relative HPV DNA load was expressed as the number of HPV DNA copies relative to *ALB* DNA, which was considered to reflect the total human cellular DNA in the sample. The following formula was used: viral load = number of HPV copies/μl/number of *ALB* copies/μl.

Statistical Analysis

Patient backgrounds were compared between the progression and non-progression groups using Student's *t*-tests and Fisher's exact tests for continuous and discrete variables, respectively. Regarding the relative HPV DNA loads, differences between the two groups were evaluated using Mann–Whitney *U*-tests. Statistical analyses were performed with SPSS software version 19 (IBM Japan, Tokyo, Japan). Significant differences were defined as P values <0.05.

RESULTS

Characteristics of the Progression and the Non-Progression Groups in Single HPV 16 or 52 Persistent Infection

Of the 24 cases of single HPV 52 persistent infection, 8 were classified in the progression and the remaining 16 in the non-progression group. Of the 24 cases with single HPV 16 persistent infection, 10 were classified in the progression and the remaining fourteen in the non-progression group. The characteristics of the progression and non-progression groups for single HPV 16 and 52 persistent infections are described in Tables I and II, respectively. Both groups were similar in terms of age, interval between cervical cytological tests, parity and body mass index. All study patients had engaged in sexual intercourse and were HIV negative. The cytological findings were consistent with the results obtained from the colposcopies and biopsies (Tables III and IV).

Relative HPV 52 DNA Loads at First Cytological Sampling and Changes in Cervical Cytological Findings

Relative HPV DNA loads were described as the median (minimum–maximum). The median relative HPV 52 DNA load in the progression group was significantly higher than in the non-progression group (2.211; 0.088–13.089 vs. 0.022; 0.001–0.618; Mann–Whitney *U*-test, $P = 0.003$; Table III and Fig. 2a).

Relative HPV 16 DNA Loads at First Cytological Sampling and Changes in Cervical Cytological Findings

Relative HPV DNA loads were described as the median (minimum–maximum). The median relative HPV 16 DNA load in the progression group was significantly higher than in the non-progression group (4.206: 0.407–38.999 vs. 0.103; 0.001–96.566; Mann–Whitney *U*-test, $P = 0.001$; Table IV and Fig. 2b).

Relative HPV 52 and HPV 16 DNA Loads in Cervical Cytological Samples

The prevalences of abnormal cytological findings (atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion) were similar in patients with single HPV 16 persistent infection and those with HPV 52 persistent infection (Fisher's exact test, $P = 0.668$). In addition, both the relative HPV 52 and the relative HPV 16 DNA loads at entry were similar in cases with NILM and those with abnormal cytological findings (Mann–Whitney *U*-test, $P > 0.05$, respectively). However, the median relative HPV 16 DNA load in cases with single HPV 16 persistent infection was 0.636 (0.001–96.566), while the median relative HPV 52 DNA load in cases with single HPV 52 persistent infection was 0.065 (0.001–13.089) (Fig. 3), indicating that the relative HPV 52 DNA load was significantly lower than the HPV 16 DNA

TABLE I. Backgrounds of Patients With Single HPV 52 Persistent Infection

Characteristics	Progression group (n = 8)	Non-progression group (n = 16)	*P*-value
Age at first sampling (years)[a]	36.0 (14.5)	34.0 (7.0)	NS[b]
Interval between cytological tests (months)[a]	4.6 (1.2)	4.1 (1.2)	NS[b]
Parity			
Nulliparous	3	7	NS[b]
Parous	5	8	
Body mass index (kg/m^2)[a]	21.4 (3.5)	21.2 (3.7)	NS[b]
Sexual intercourse	8	16	NS[b]
HIV infection	0	0	NS[b]

[a]Mean (SD).
[b]NS indicates no significant difference between two groups (*t*-test and Fisher's exact test comparisons for continuous and discrete variables, respectively, in progression and non-progression groups). Significant differences were defined as $P < 0.05$.

TABLE II. Backgrounds of Patients With Single HPV 16 Persistent Infection

Characteristics	Progression group (n = 10)	Non-progression group (n = 14)	*P*-value
Age at first sampling (years)[a]	36.0 (10.5)	37.5 (12.6)	NS[b]
Interval between cytological tests (months)[a]	4.8 (1.6)	4.4 (1.5)	NS[b]
Parity			
Nulliparous	5	5	NS[b]
Parous	5	6	
Body mass index (kg/m^2)[a]	22.9 (3.2)	22.2 (3.1)	NS[b]
Sexual intercourse	10	14	NS[b]
HIV infection	0	0	NS[b]

[a]Mean (SD).
[b]NS indicates no significant difference between two groups (*t*-test and Fisher's exact test comparisons for continuous and discrete variables, respectively, in progression and non-progression groups). Significant differences were defined as $P < 0.05$.

TABLE III. HPV 52 DNA Load and Cervical Cytopathological Changes in Cases of Single HPV 52 Persistent Infection

Cases	Relative HPV virus load (HPV DNA/ALB DNA) at first sampling	Cytological findings		Cytological change	Results of colposcopy or biopsies	
		First sampling	Second sampling		First sampling	Second sampling
1	0.08782	LSIL	HSIL	Progression	CIN2	CIN3
2	0.29985	NILM	ASC-US	Progression	Not tested	CIN1
3	0.43585	LSIL	HSIL	Progression	CIN2	CIN3
4	0.51325	NILM	LSIL	Progression	Not tested	CIN1
5	2.21097	NILM	LSIL	Progression	Not tested	CIN2
6	6.42910	NILM	LSIL	Progression	Not tested	CIN2
7	7.75832	LSIL	HSIL	Progression	CIN1	CIN3
8	13.0894	NILM	HSIL	Progression	Not tested	CIN2
9	0.00125	NILM	NILM	Non-progression	Not tested	Not tested
10	0.00162	NILM	NILM	Non-progression	Not tested	Not tested
11	0.00379	HSIL	LSIL	Non-progression	CIN2	CIN1
12	0.00586	LSIL	NILM	Non-progression	NCF	Not tested
13	0.00706	NILM	NILM	Non-progression	Not tested	Not tested
14	0.00840	NILM	NILM	Non-progression	Not tested	Not tested
15	0.01412	NILM	NILM	Non-progression	Not tested	Not tested
16	0.01667	ASC-US	NILM	Non-progression	NCF	Not tested
17	0.02227	NILM	NILM	Non-progression	Not tested	Not tested
18	0.03270	NILM	NILM	Non-progression	Not tested	Not tested
19	0.03931	NILM	NILM	Non-progression	Not tested	Not tested
20	0.06276	LSIL	LSIL	Non-progression	CIN1	CIN1
21	0.06743	LSIL	NILM	Non-progression	CIN1	CIN1
22	0.07969	NILM	NILM	Non-progression	Not tested	Not tested
23	0.10207	LSIL	LSIL	Non-progression	CIN2	CIN2
24	0.61879	HSIL	ASC-US	Non-progression	CIN2	CIN1

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; NCF, normal colposcopic findings.

load in the cervix in patients with single HPV infections (Mann–Whitney *U*-test, $P = 0.019$).

DISCUSSION

This preliminary study demonstrated a relationship between a high viral load in single HPV 16 persistent infection and the progress of cervical cytopathological findings, as observed previously [Lo et al., 2005; Carcopino et al., 2006], and also found a similar relationship for single HPV 52 persistent infection. The relative HPV 52 DNA load was significantly lower than the HPV 16 DNA load in cervical cells with single oncogenic HPV infection.

These results suggest that the initial viral load in cases with single HPV 52 or 16 persistent infection might be a predictive marker of later progression of cytopathological findings in the uterine cervix. The importance of the immune reaction in HPV infection is supported by the association of persistent infection with an increased risk of cervical cancer [Ho et al., 1998], and the amount of oncogenic HPV DNA may also reflect inherent differences between individuals in terms of their response to HPV 52 or 16 persistent infection. Systematic detection and quantification of oncogenic HPV DNA is also important not only for the screening of infection, but also for post-therapeutic follow-up [Carcopino et al., 2006], and high HPV DNA loads may have a critical value in predicting the evolution toward cytological abnormalities in women with no detectable cytological abnormalities in the uterine cervix [Carcopino et al., 2006].

TABLE IV. HPV 16 DNA Load and Cervical Cytopathological Changes in Cases of Single HPV 16 Persistent Infection

Cases	Relative HPV virus load (HPV DNA/ALB DNA) at the first sampling	Cytological findings		Cytological change	Results of colposcopy or biopsies	
		First sampling	Second sampling		First sampling	Second sampling
1	0.40754	NILM	HSIL	Progression	Not tested	CIN2
2	2.15193	LSIL	HSIL	Progression	CIN1	CIN3
3	2.21686	NILM	HSIL	Progression	Not tested	CIN3
4	4.08485	LSIL	HSIL	Progression	CIN1	CIN3
5	4.12118	ASC-US	HSIL	Progression	CIN1	CIN2
6	4.29190	ASC-US	LSIL	Progression	NCF	CIN1
7	6.70919	LSIL	HSIL	Progression	CIN1	CIN3
8	8.49491	ASC-US	HSIL	Progression	Chronic cervicitis	CIN3
9	22.40488	LSIL	HSIL	Progression	CIN2	CIN3
10	38.99907	ASC-US	LSIL	Progression	Chronic cervicitis	CIN2
11	0.00060	LSIL	LSIL	Non-progression	CIN1	Chronic cervicitis
12	0.01041	HSIL	HSIL	Non-progression	CIN3	CIN3
13	0.01485	LSIL	LSIL	Non-progression	CIN3	CIN3
14	0.02130	NILM	NILM	Non-progression	Not tested	Not tested
15	0.02540	HSIL	HSIL	Non-progression	CIN3	CIN3
16	0.70239	HSIL	HSIL	Non-progression	CIN3	CIN3
17	0.07359	HSIL	HSIL	Non-progression	CIN3	CIN3
18	0.13196	NILM	NILM	Non-progression	Not tested	Not tested
19	0.15679	NILM	NILM	Non-progression	Not tested	Not tested
20	0.40484	LSIL	LSIL	Non-progression	CIN2	CIN2
21	0.57038	HSIL	HSIL	Non-progression	CIN3	CIN3
22	0.07042	HSIL	HSIL	Non-progression	CIN3	CIN2
23	0.86522	HSIL	HSIL	Non-progression	CIN3	CIN3
24	96.56597	HSIL	HSIL	Non-progression	CIN3	CIN3

NILM, negative for intraepithelial lesion or malignancy; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; CIN, cervical intraepithelial neoplasia; NCF, normal colposcopic findings.

However, the estimated positive predictive value of HPV DNA load was too low for the test to be directly applicable as a single test for predicting cancer risk, except in women with the highest levels of HPV DNA [Josefsson et al., 2000]. Testing for oncogenic HPV DNA levels may thus be a useful addition to cytological screening for identifying women at high risk [Sun et al., 2002].

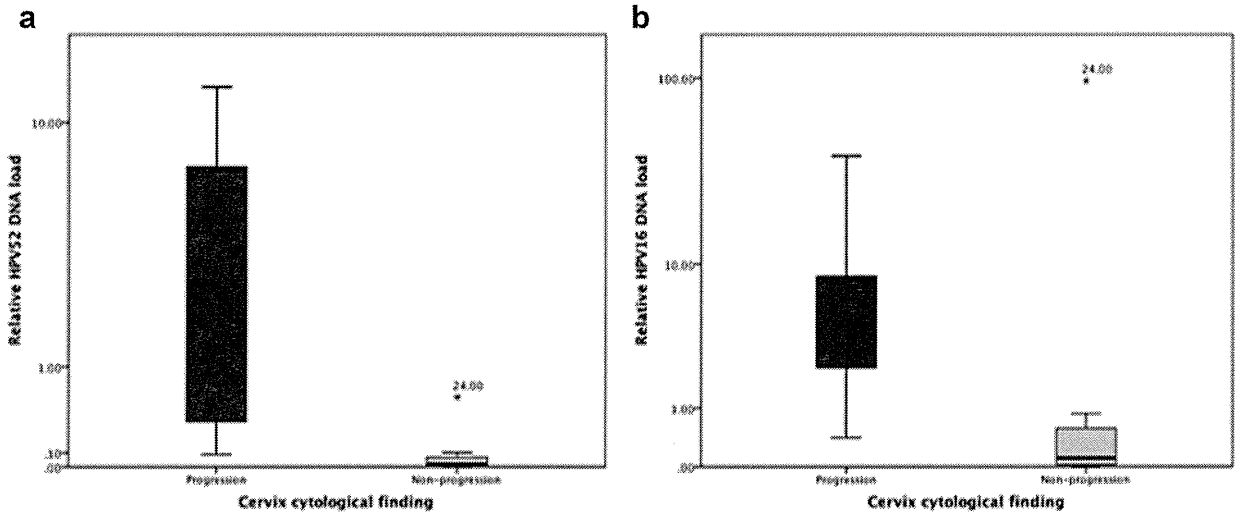

Fig. 2. Relative HPV DNA loads at first cytological sampling and changes in cervical cytology. **a**: Relative HPV 52 DNA loads and changes in cervical cytology. **b**: Relative HPV 16 DNA loads and changes in cervical cytology. Vertical axis indicates relative HPV DNA loads expressed as $\log_{10}$. Relative HPV 52 and 16 DNA loads were both significantly higher in the progression group than in the non-progression group (Mann–Whitney U-tests, $P = 0.003$ and 0.001, respectively).

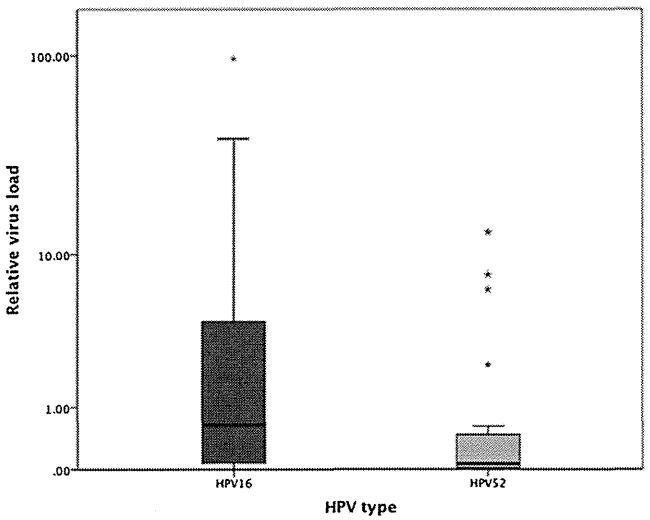

Fig. 3. Relative HPV 52 and HPV 16 DNA loads in cervix cytological samples. The median relative HPV 16 DNA load in cases with single HPV 16 persistent infection was 0.636 (0.001–96.566), while the median relative HPV 52 DNA load in cases with single HPV 52 persistent infection was 0.065 (0.001–13.089). The relative HPV 52 DNA load was significantly lower than the HPV 16 DNA load in the cervix in cases with single HPV infection (Mann–Whitney U-test, $P = 0.019$).

Persistent infection with oncogenic HPV types was found to be a key prognostic variable for risk of cytopathological progression [Moscicki et al., 2001; Schlecht et al., 2001; Dalstein et al., 2003], and the risk of cervical intraepithelial neoplasia 2/3 was increased in line with high initial oncogenic HPV loads. In this study, the influence of sexual intercourse and HIV infection on progression was identical between progression and non-progression groups (Tables I and II). The results demonstrate that the DNA load of single oncogenic HPV 52 or 16 infection may have a significant influence on the progression of cytopathological findings. Although higher viral loads may result from an increased rate of viral replication and may sustain viral persistence [Yoshida et al., 2008], this study demonstrated that the viral loads of HPV 52 and 16 at entry were not associated with the degree of cytologic abnormalities, supporting the idea that HPV DNA load seems to predict the risk of developing cervical carcinoma before any cytological alterations are visible [Ylitalo et al., 2000]. This result is not in accordance with previous studies [Carcopino et al., 2012; Al-Awadhi et al., 2013], and this discrepancy may reflect the small sample size used in the current study. Further studies using a greater number of patients are required to establish the reason for this discrepancy. It is interesting and necessary to monitor these women to identify any changes in cytology, HPV genotype, or viral loads. Thus patients with normal cytology who present with high viral loads of HPV 52 or 16 should be monitored because of their risk of developing dysplastic lesions.

Multiple oncogenic HPV types are more common in cases of normal, atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion and high-grade squamous intraepithelial lesion than in cases of invasive cervical carcinoma [Yamasaki et al., 2011a,b]. To exclude the possibility of a high virus load being the result of infection with multiple HPV types, the current study estimated viral loads only in cases with single HPV 52 or 16 persistent infections, and samples with multiple HPV types including HPV 52 or HPV 16 were excluded. As in a previous study that found lower viral loads for HPV 18 (7.93×10^4 copies/μl) compared with HPV 16 (5×10^{13} copies/μl) [Botezatu et al., 2009], the results of this study also showed that the relative HPV 52 DNA load was significantly lower than the HPV 16 DNA load in the cervix in single HPV infections. In cases of multiple oncogenic HPV infections including HPV 16, it is difficult to estimate the risk of cervical cancer using oncogenic HPV DNA load itself because the viral load of HPV 16 may mask the influence of other oncogenic HPVs. For many other oncogenic HPV types, the causal attribution regarding the progression of later cytopathological findings is less clear, and future studies using multiple parallel evaluations of viral loads will be needed to understand the role of viral load in other carcinogenic types [Wentzensen et al., 2012]. The values of the viral load for samples collected 3 months later were not shown in this study. Future studies may investigate changes in HPV viral loads between initial and 3 months in an effort to identify factors which may affect progression of cytological findings.

In conclusion, the results of this study confirmed the key roles of HPV 52 and 16 DNA load in the progression of cytopathological findings. HPV 52 and 16 DNA loads measured by real-time quantitative methods may be useful as short-term markers for identifying women at high risk for progression of cervical cytopathology.

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REFERENCES

Al-Awadhi R, Chehadeh W, Al-Jassar W, Al-Harmi J, Al-Saleh E, Kapila K. 2013. Viral load of human papillomavirus in women with normal and abnormal cervical cytology in Kuwait. J Infect Dev Ctries 7:130–136.

Asato T, Maehama T, Nagai Y, Kanazawa K, Uezato H, Kariya K. 2004. A large case-control study of cervical cancer risk associated with human papillomavirus infection in Japan, by nucleotide sequencing-based genotyping. J Infect Dis 189:1829–1832.

Berger A, Preiser W. 2002. Viral genome quantification as a tool for improving patient management: The example of HIV, HBV, HCV and CMV. J Antimicrob Chemother 49:713–721.

Botezatu A, Socolov D, Goia CD, Iancu IV, Ungureanu C, Huică I, Anton G. 2009. The relationship between HPV16 and HPV18 viral load and cervical lesions progression. Roum Arch Microbiol Immunol 68:175–182.

Carcopino X, Henry M, Benmoura D, Fallabregues AS, Richet H, Boubli L, Tamalet C. 2006. Determination of HPV type 16 and 18 viral load in cervical smears of women referred to colposcopy. J Med Virol 78:1131–1140.

Carcopino X, Henry M, Mancini J, Giusiano S, Boubli L, Olive D, Tamalet C. 2012. Two years outcome of women infected with high risk HPV having normal colposcopy following low-grade or equivocal cytological abnormalities: Are HPV16 and 18 viral load clinically useful predictive markers? J Med Virol 84: 964–972.

Dalstein V, Riethmuller D, Prétet JL, Le Bail Carval K, Sautière JL, Carbillet JP, Kantelip B, Schaal JP, Mougin C. 2003. Persistence and load of high-risk HPV are predictors for development of high-grade cervical lesions: A longitudinal French cohort study. Int J Cancer 106:396–403.

Gravitt PE, Peyton CL, Alessi TQ, Wheeler CM, Coutlée F, Hildesheim A, Schiffman MH, Scott DR, Apple RJ. 2000. Improved amplification of genital human papillomaviruses. J Clin Microbiol 38:357–361.

Gravitt PE, Burk RD, Lorincz A, Herrero R, Hildesheim A, Sherman ME, Bratti MC, Rodriguez AC, Helzlsouer KJ, Schiffman M. 2003. A comparison between real-time polymerase chain reaction and hybrid capture 2 for human papillomavirus DNA quantitation. Cancer Epidemiol Biomarkers Prev 12:477–484.

Ho GY, Bierman R, Beardsley L, Chang CJ, Burk RD. 1998. Natural history of cervicovaginal papillomavirus infection in young women. N Engl J Med 338:423–428.

Inoue M, Okamura M, Hashimoto S, Tango M, Ukita T. 2010. Adoption of HPV testing as an adjunct to conventional cytology in cervical cancer screening in Japan. Int J Gynaecol Obstet 111:110–114.

Josefsson AM, Magnusson PK, Ylitalo N, Sørensen P, Qwarforth-Tubbin P, Andersen PK, Melbye M, Adami HO, Gyllensten UB. 2000. Viral load of human papillomavirus 16 as a determinant for development of cervical carcinoma in situ: A nested case-control study. Lancet 355:2189–2193.

Lo KW, Yeung SW, Cheung TH, Siu NS, Kahn T, Wong YF. 2005. Quantitative analysis of human papillomavirus type 16 in cervical neoplasm: A study in Chinese population. J Clin Virol 34:76–80.

Marks M, Gravitt PE, Utaipat U, Gupta SB, Liaw K, Kim E, Tadesse A, Phongnarisorn C, Wootipoom V, Yuenyao P, Vipupinyo C, Rugpao S, Sriplienchan S, Celentano DD. 2011. Kinetics of DNA load predict HPV 16 viral clearance. J Clin Virol 51:44–49.

Moberg M, Gustavsson I, Gyllensten U. 2003. Real-time PCR-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. J Clin Microbiol 41:3221–3228.

Moberg M, Gustavsson I, Wilander E, Gyllensten U. 2005. High viral loads of human papillomavirus predict risk of invasive cervical carcinoma. Br J Cancer 92:891–894.

Monnier-Benoit S, Dalstein V, Riethmuller D, Lalaoui N, Mougin C, Prétet JL. 2006. Dynamics of HPV16 DNA load reflect the natural history of cervical HPV-associated lesions. J Clin Virol 35:270–277.

Moscicki AB, Palefsky J, Smith G, Siboshski S, Schoolnik G. 1993. Variability of human papillomavirus DNA testing in a longitudinal cohort of young women. Obstet Gynecol 82:578–585.

Moscicki AB, Hills N, Shiboski S, Powell K, Jay N, Hanson E, Miller S, Clayton L, Farhat S, Broering J, Darragh T, Palefsky J. 2001. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA 285:2995–3002.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ, International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348:518–527.

Schlecht NF, Kulaga S, Robitaille J, Ferreira S, Santos M, Miyamura RA, Duarte-Franco E, Rohan TE, Ferenczy A, Villa LL, Franco EL. 2001. Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. JAMA 286:3106–3114.

Shimada T, Miyashita M, Miura S, Nakayama D, Miura K, Fukuda M, Masuzaki H. 2007. Genital human papilloma virus infection in mentally-institutionalized virgins. Gynecol Oncol 106:488–489.

Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM. 2007. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: A meta-analysis update. Int J Cancer 121:621–632.

Sun CA, Liu JF, Wu DM, Nieh S, Yu CP, Chu TY. 2002. Viral load of high-risk human papillomavirus in cervical squamous intraepithelial lesions. Int J Gynaecol Obstet 76:41–47.

Tavassoli FA, Devilee P. 2003. World Health Organization Classification of Tumours. Pathology & Genetics of Tumours of the Breast and Female Genital Organs. Lyon: International Agency for Research on Cancer (IARC) Press. 270 p.

van Duin M, Snijders PJ, Schrijnemakers HF, Voorhorst FJ, Rozendaal L, Nobbenhuis MA, van den Brule AJ, Verheijen RH, Helmerhorst TJ, Meijer CJ. 2002. Human papillomavirus 16 load in normal and abnormal cervical scrapes: An indicator of CIN II/III and viral clearance. Int J Cancer 98:590–595.

Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N. 1999. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19.

Wentzensen N, Gravitt PE, Long R, Schiffman M, Dunn ST, Carreon JD, Allen RA, Gunja M, Zuna RE, Sherman ME, Gold MA, Walker JL, Wang SS. 2012. Human papillomavirus load measured by Linear Array correlates with quantitative PCR in cervical cytology specimens. J Clin Microbiol 50:1564–1570.

Woodman CB, Collins S, Winter H, Bailey A, Ellis J, Prior P, Yates M, Rollason TP, Young LS. 2001. Natural history of cervical human papillomavirus infection in young women: A longitudinal cohort study. Lancet 357:1831–1836.

Xi LF, Koutsky LA, Castle PE, Wheeler CM, Galloway DA, Mao C, Ho J, Kiviat NB. 2009. Human papillomavirus type 18 DNA load and 2-year cumulative diagnoses of cervical intraepithelial neoplasia grades 2–3. J Natl Cancer Inst 101:153–161.

Yamasaki K, Miura K, Shimada T, Ikemoto R, Miura S, Murakami M, Sameshima T, Fujishita A, Kotera K, Kinoshita A, Yoshiura K, Masuzaki H. 2011a. Pre-vaccination epidemiology of human papillomavirus infections in Japanese women with abnormal cytology. J Obstet Gynaecol Res 37:1666–1670.

Yamasaki K, Miura K, Shimada T, Miura S, Abe S, Murakami M, Sameshima T, Fujishita A, Kotera K, Kinoshita A, Yoshiura K, Masuzaki H. 2011b. Epidemiology of human papillomavirus genotypes in pregnant Japanese women. J Hum Genet 56:313–315.

Ylitalo N, Sørensen P, Josefsson AM, Magnusson PK, Andersen PK, Pontén J, Adami HO, Gyllensten UB, Melbye M. 2000. Consistent high viral load of human papillomavirus 16 and risk of cervical carcinoma in situ: A nested case-control study. Lancet 355:2194–2198.

Yoshida T, Sano T, Kanuma T, Owada N, Sakurai S, Fukuda T, Nakajima T. 2008. Quantitative real-time polymerase chain reaction analysis of the type distribution, viral load, and physical status of human papillomavirus in liquid-based cytology samples from cervical lesions. Int J Gynecol Cancer 18:121–127.