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Association of serum cytokines with oral HPV clearance

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Abstract

Background—Initial studies suggest higher serum levels of some pro-inflammatory cytokines may be associated with decreased cervical human papillomavirus (HPV) clearance. However, the relationship of cytokines with oral HPV clearance has not been explored.

Methods—From 2010 to 2014, oral rinse and serum samples were collected semi-annually from 1,601 adults. Oral rinse samples were tested for HPV DNA using PCR. Based on oral HPV results,

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931 serum samples were selected for cytokine evaluation to include a roughly equal number of prevalent (n=307), incident (n=313), and no oral HPV infections (n=311). Electrochemiluminescence multiplex assays were used to determine the concentrations of IL-6, IL-8, TNF-α, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12 and IL-13. The relationship between serum cytokine concentrations (categorized into quartiles) and oral HPV clearance was evaluated with Wei-Lin-Weissfeld regression models, adjusting for HPV infection type (prevalent vs. incident), age, HIV status, and CD4 T cell count.

Results—Higher TNF-α concentration was associated with decreased clearance in men (highest vs. lowest quartile, adjusted hazard ratio [aHR]=0.52, 95% CI=0.34-0.79) and women (aHR=0.76, 95% confidence interval [CI]=0.55-1.04), with stronger associations in men than women (p-interaction=0.049). Higher IL-2 concentration was associated with reduced clearance in men (aHR=0.69, 95% CI=0.50-0.95), but not women (p-interaction=0.058). Results were similar within CD4 T cell strata (CD4 ≥500 or CD4<500 cells/μl) among HIV-infected participants. No other cytokines were associated with clearance.

Conclusion—High serum TNF-α is associated with reduced clearance of oral HPV infection.

Keywords

oral HPV; persistence; TNF; natural history; infection

1. Introduction

Oral infection with human papillomavirus (HPV) is an established causal factor for oropharyngeal squamous cell carcinoma (OPSCC).(1) In the U.S., approximately 70% of OPSCC is caused by HPV, with higher incidence among men <60 years old and individuals with human immunodeficiency virus (HIV) infection.(1-4) Similar to HPV infection at other anatomic sites, most oral HPV infections clear in 1-2 years.(5) However, some individuals have persistently detected oral HPV infection, which is believed to be the precursor to HPV-related OPSCC.(6) Understanding key immune differences in individuals with persistent oral HPV may help explain the disproportionate burden of HPV-related OPSCC in population subgroups.

While the role of host immunity in oral HPV infection has not been explored, studies of cervical HPV infection have established that cell-mediated T cell responses are critical for viral clearance and lesion regression.(7, 8) Attempts to characterize these responses have found that women with persistent cervical HPV have distinct systemic and/or local cytokine profiles as compared to women who clear HPV or women without HPV infection.(9-13) In particular, higher circulating or local levels of interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) have been associated with reduced cervical HPV clearance,(9-11) suggesting that cytokines secreted may be predictive of disease progression and outcome, although results have been mixed.(14, 15) The relationships between cytokines and oral HPV infection, and any differences by biologic sex, have not been described. However, men have been noted to have a reduced inflammatory response to infection, compared to women.(16)

Given that innate and acquired immune responses effectively resolve most HPV infections, persistent oral infection may reflect disruption or alteration of host anti-viral or inflammatory responses.(17, 18) Alternatively, immunologic differences between individuals may influence oral HPV clearance. For example, women with persistent cervical HPV infection have reduced lymphoproliferative responses to HPV16.(19) HIV infection may further impact immune response, as HIV can alter cytokine secretion patterns, resulting in elevated circulating levels of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. (20-26) Furthermore, pro-inflammatory cytokines can modulate HPV transcription, suggesting that HIV-related immune perturbations at the systemic level could affect oral infection.(20)

Current technology allows simultaneous measurement of multiple immune markers in a single sample using a multiplex format. Characterizing the serum cytokine profiles associated with oral HPV clearance may provide insight on immune cofactors relevant to oral HPV infection. The goals of this study were to evaluate whether select serum cytokines associated with inflammation may be related to oral HPV clearance and to explore differences by sex and degree of HIV-related immunosuppression as measured by CD4 T cell count.

2. Methods

2.1. Study population

From 2010 to 2014, 1,601 participants were enrolled in the “Persistence of Oral Papillomavirus Study” (POPS), a longitudinal cohort for studying oral HPV natural history. POPS was nested within two ongoing observational studies of men and women with or at risk for HIV: the Multicenter AIDS Cohort Study (MACS) and the Women’s Interagency HIV Study (WIHS). POPS participants were enrolled at 5 study centers: Baltimore (MACS), Pittsburgh (MACS), Brooklyn (WIHS), Bronx (WIHS), and Chicago (MACS and WIHS). (27-29) Each study center’s Institutional Review Board approved the study. All participants provided written informed consent.

2.2. Data collection

At each semi-annual study visit, a 30-second Scope® oral rinse and gargle sample was collected and tested for 37 types of HPV DNA. A blood sample was also obtained, from which HIV status, CD4 T cell count, and cytokine profile were determined. Data on behavioral cofactors were collected by survey. Additional details of data collection methods have been previously described.(30)

2.3. Oral HPV detection and genotyping

DNA was extracted from oral exfoliated cells using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN). Extracted DNA samples were tested for HPV DNA using a polymerase chain reaction (PCR) assay targeting the L1 region of the viral genome using PGMY primers.(31) HPV-positive samples were genotyped using reverse line-blot hybridization (Roche Molecular Systems, Pleasanton, CA).(31) Each sample was evaluated for the presence of high-risk/oncogenic types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,

66) and low-risk/non-oncogenic types (6, 11, 26, 38, 40, 42, 53, 54, 55, 61, 62, 64, 67, 68, 69, 70, 71, 72, 73, 81, 83, 84, 89, IS39), classified according to criteria from the International Agency for Research on Cancer.(32-34) Participants with any of these HPV types were considered HPV-infected.

Prevalent oral HPV infection was defined as any type-specific oral HPV infection detected at study enrollment. Incident infection was defined as newly detected type-specific infection that was preceded by at least one HPV-negative oral rinse. Clearance was defined as two consecutive HPV-negative oral rinses, with time of clearance as the visit of the first negative oral rinse sample. A secondary less conservative definition of clearance was considered, requiring only one HPV-negative oral rinse sample.

2.4. Selection of serum samples for cytokine testing

We selected 931 serum samples for cytokine evaluation. Serum samples were selected based on oral rinse HPV results in order to include a roughly equal number of prevalent (n=307), incident (n=313), and no oral HPV infections (n=311). We included all prevalent HPV samples, a random selection of incident HPV samples (55% of all incident HPV samples), and a comparison group of samples with no oral HPV DNA at study enrollment, frequency matched to samples with prevalent HPV by study participant's HIV status and biologic sex. All of the serum samples selected for cytokine testing were from participants who contributed at least 3 oral rinses overall and at least 2 oral rinses after incident oral HPV detection.

Cytokines were evaluated using serum samples collected at the visit of incident HPV detection, or at study enrollment for participants with prevalent or no HPV infections. Some participants had prevalent infection at study enrollment and subsequent incident HPV that was also included in the study sample. For these cases, cytokines were determined separately in the serum sample corresponding to first detection of prevalent HPV and in the sample corresponding to incident HPV. Therefore, the 620 prevalent and incident serum samples selected for cytokine evaluation were obtained from 459 HPV-infected participants.

2.5. Serum cytokine testing

Serum cytokines were determined using the MSD V-PLEX Human Proinflammatory Panel 1 kit (Meso Scale Discovery −MSD Gaithersburg, MD), which consists of the following: TNF-α, IL-6, IL-8, IFN-γ, IL-1β, IL-2, IL-4, IL-10, IL-12 and IL-13. Cytokine testing was analyzed using the MSD 2400 Sector Imager using a standard protocol. The multiplex cytokine assay was previously validated and found to reliably quantify cytokine concentrations in both HIV-infected and HIV-uninfected individuals.(35)

To confirm acceptable assay performance, 10% of the samples were tested in duplicate and coefficients of variation (CV) were calculated for each duplicate test. Duplicated cytokine results were considered acceptable if CV≤20%. The primary analysis was restricted to the cytokines that could be reliably detected in ≥60% of the samples tested: TNF-α, IL-6, IL-8, IFN-γ, IL-2 and IL-10. The other cytokines tested (IL-1β, IL-4, IL-12 and IL-13) were excluded from this primary analysis because >40% of samples had cytokine concentrations

below the lowest detectable limit, but were evaluated based on presence or absence of detectable cytokine.

2.6. Statistical analyses

Demographic and health characteristics of men and women in the study were compared using χ^2 tests. Median cytokine concentrations were compared in serum samples that had prevalent, incident and no oral HPV DNA using a nonparametric test for trends across ordered groups.

The analysis included 917 type-specific oral HPV infections from 620 samples among 459 participants (some participants were infected with multiple HPV types at the same visit and the cytokine values at that visit were applied to clearance analysis of all of those infections). Concentrations of each cytokine were categorized into quartiles (see Table 4 footnote for quartile cutoffs). Alternative analyses considered cytokine quartiles based on sex-specific cytokine distributions and continuous cytokine concentrations (i.e. the per natural log increase in cytokine concentration), with similar results. Cytokines with low detectability were categorized as detectable vs. non-detectable).

Predictors of type-specific oral HPV clearance were explored using Wei-Lin-Weissfeld regression models, accounting for within-participant clustering of HPV infections.(36) The effect of each cytokine was modeled separately in unadjusted models, and then in multivariable models adjusting for prevalent vs. incident infection type, age, HIV status, and CD4 T cell count at time of oral HPV detection, based on prior evidence that these factors are associated with oral HPV clearance. Stata 12 statistical software package was used for statistical analyses.(37) Statistical significance was defined by a two-sided p-value <0.05.

3. Results

Characteristics of study participants are described in Table 1. Among the 766 participants, half were men (52%) and the median age was 50 years. Approximately 74% of participants were HIV-infected, and 82% of these reported current use of antiretroviral therapy (ART). Compared to men, women were younger, more likely to be Black, HIV-infected, and current smokers (p-values<0.05, Table 1). Among HIV-infected participants, women had higher current HIV RNA viral load (p<0.0001), but similar CD4 T cell count, as compared to men (p=0.17).

Median follow-up time for infections was 16.7 months (interquartile range, IQR: 6.1-36.6). For infections that cleared, the median time to clearance was 6.5 months, with longer clearance time for prevalent infections (11.7 months; IQR 6.0-21.6) than incident infections (6.2 months; IQR 5.9-11.9).

3.1. Comparison of serum cytokine concentrations by HPV status

Median serum concentrations of TNF-α, IL-8, IFN-γ, IL-10 and IL-2 increased by HPV infection status (each p-trend<0.05, Table 2). The lowest median concentrations of each of these cytokines were in samples from participants with no oral HPV infection, followed by samples from participants with incident HPV, and the highest concentrations were in

samples from participants with prevalent HPV. When comparing samples with any oral HPV infection (prevalent or incident) to samples without oral HPV infection, the median serum concentrations of TNF-α, IL-8, IFN-γ, IL-10 and IL-2 were significantly higher (each $p<0.05$) in samples from HPV-infected participants (data not shown). Results were similar when restricted to only HIV-infected, or only HIV-uninfected participants (results not shown).

3.2. Association of cytokines with oral HPV clearance

Of the cytokines included in the analysis, TNF-α was most strongly associated with oral HPV clearance (Table 3). In unadjusted analyses, the highest quartile of TNF-α was significantly associated with reduced oral HPV clearance in both men (HR=0.50, 95% CI=0.35-0.72) and women (HR=0.67, 95% CI=0.48-0.94), as compared to the lowest TNF-α quartile (p for interaction=0.24). The second and third quartiles of TNF-α concentration were also associated with reduced oral HPV clearance in men but not in women (each p for interaction<0.05, Table 3).

In unadjusted analyses, the highest quartile of IL-2 concentration was associated with significantly reduced oral HPV clearance among men (HR=0.69, 95% CI=0.50-0.96), but not among women (HR=1.10, 95% CI=0.80-1.50), p for interaction=0.047. This finding should be interpreted with caution as IL-2 had the most values (35%) below the lower limit of detection, of all the cytokines included in the analysis. IL-6, IL-8, IFN-γ, and IL-10 concentration were not strongly associated with oral HPV clearance; marginal associations were observed in single quartiles for some of these cytokines in unadjusted analyses (Table 3), but none remained significant predictors of oral HPV clearance in adjusted analyses (results not shown).

After adjusting for the effects of HPV infection type (prevalent vs. incident), age, HIV status and CD4 T cell count in a multivariable model, serum TNF-α concentration remained significantly associated with reduced oral HPV clearance in all quartiles for men (each $p<0.01$) and remained marginally associated with reduced clearance in the highest quartile for women (p for interaction=0.049, Table 4). IL-2 also remained associated with decreased oral HPV clearance in all quartiles in men only (each $p<0.05$). The associations of higher TNF-α and IL-2 concentrations on oral HPV clearance were stronger in men than women (TNF-α, p for interaction=0.049; IL-2, p for interaction=0.058).

Results were similar when restricted to HIV-infected participants with baseline CD4 ≥500 or CD4<500 cells/μl (results not shown) and when evaluating TNF-α and IL-2 concentrations as continuous variables instead of quartiles (Supplemental Table S1). When using sex-specific cytokine quartile cutoffs, results were also similar but the highest TNF-α quartile in women remained significantly associated with reduced clearance in the adjusted model (p=0.02, Supplemental Table S2). When using a less strict clearance definition of 1 HPV-negative oral rinse, similar patterns were observed, but associations were attenuated (data not shown). When considering cytokines with low detectability (IL-1β, IL-4, IL-12 and IL-13), detection of these cytokines was not associated with reduced oral HPV clearance (Supplemental Table S3).

4. Discussion

We found that while most cytokines were not associated with oral HPV clearance, the highest serum TNF-α concentration quartile in our study population (4.94-19.33 pg/ml) was associated with decreased oral HPV clearance in both sexes, with stronger associations in men than women. IL-2 concentration above the first quartile (≥0.21 pg/ml) was associated with reduced oral HPV clearance in men only. These results suggest that higher levels of some inflammatory cytokines may decrease oral HPV clearance and thus contribute to explaining why some individuals may be at increased risk for persistent oral HPV infection.

Our finding that higher TNF-α was associated with reduced oral HPV clearance is consistent with previous studies of cervical HPV, which found that women with persistent cervical HPV have higher serum TNF-α at their final study visit, as compared to women who cleared infection.(9, 10) In addition, our results show that higher serum TNF-α at first detection of oral HPV infection was associated with longer time to clearance, suggesting that TNF-α may be relevant in early stages of oral HPV infection, in addition to being a marker of long-term infection. Furthermore, while previous studies restricted the study population to HIV-uninfected participants,(9-11, 14) the association between TNF-α and clearance in our study remained similar after adjusting for HIV infection and CD4 T cell count, suggesting that the relationship between TNF-α and oral HPV may be independent of HIV-related immunosuppression.

TNF-α plays an important role in inflammatory reactions and viral clearance. Since HPV infection of epithelial cells is sequestered from systemic immunity, and typically does not cause a highly inflammatory immune response,(38, 39) the reason why higher concentrations of TNF-α are associated with reduced clearance remains unclear. It is possible that these participants had higher systemic TNF-α resulting from other pro-inflammatory co-factors, such as concurrent illness or chronic conditions, which may promote or facilitate HPV persistence. Previous research suggests that although initial HPV infection does not cause an inflammatory reaction,(38, 39) persisting infection can activate inflammatory pathways, including ones with established roles in cancer promotion.(40) Repeated longitudinal cytokine measurements, or cytokine measurements taken directly from the oropharynx, may show a decrease in TNF-α following HPV clearance, and may help clarify temporal patterns of immune response over the course of oral HPV infection.

Some sex differences in the association of cytokines with oral HPV clearance were observed in this study. Genetic or hormonal differences that influence immune responses and HPV infection may explain the difference between men and women in strength of association of TNF-α and IL-2 with reduced clearance. Sex differences in cellular and humoral immune responses, as well as cytokine activity, have been consistently documented in previous research (16, 41, 42), (16, 41, 42), and findings from several studies suggest that men have weaker immune responses to HPV infection as compared to women.(43) This may due to previous cervical HPV infection prompting a stronger immune response when exposed to oral HPV infection. It is also possible that immune differences may be influenced by other unmeasured sex-related factors. Higher incidence of HPV-associated oropharyngeal cancer has consistently been reported in men compared to women,(5) Reasons for this difference in

cancer rate are not fully understood, and may be explained, in part, by sex-based differences in immune response. Given that our study population was primarily middle-aged, it is also possible that hormonal changes related to oral contraceptive use and menopause in women might influence immune responses.(45, 46)

Previous clinical studies have not found evidence that serum IL-2 may be related to clearance of HPV infection. However, a previous study that measured IL-2 reactivity when stimulated with HPV E6/E7 oncoproteins in culture supernatants of peripheral blood mononuclear cells found that IL-2 responsiveness was strongly associated with cervical HPV16 persistence.(47) Other studies have shown that plasma IL-2 levels can differ depending on cervical HPV positivity and/or disease stage, although results have varied.(13) Our finding that IL-2 is related to decreased clearance in men may have been affected by its lower detectability, and may not represent true sex differences in IL-2. Therefore, the association of IL-2 with oral HPV clearance observed in this study warrants confirmation from other studies.

In contrast to previous studies on cervical HPV,(9-11) IL-6 and IL-8 were not strongly associated with oral HPV clearance in this study. This may be due to differences in the biology of HPV infection in the oropharynx and cervix. It is also possible that these cytokines were expressed at time points not captured by the testing schedules, that concentrations differed between studies or that they may be involved in oral HPV clearance, but not at concentrations detectable in serum. Two previous analyses, which reported associations between IL-8 and reduced cervical HPV clearance were conducted in a weighted subpopulation of women with type-specific HPV infection persistently detected over a 9-year interval.(9, 10) Therefore, it is possible that IL-8 is associated with HPV infection at a later stage, or may be more relevant for established infections. We found that the concentrations of nearly all cytokines were indeed different across participants by HPV infection status, suggesting a linkage between cytokine profile and presence or duration of HPV infection. While the current analysis controlled for incident vs. prevalent infection type, longitudinal repeated measurements may be more informative of differences between short- and long-term persistence.

Our study had several strengths, including: enrollment of a large study population with high oral HPV prevalence, including men and women at multiple U.S. study sites; racial and ethnic diversity; inclusion of HIV-infected and HIV-uninfected participants; ability to control for biological and behavioral risk factors; evaluation of oral HPV infection in a prospective manner; and, centralized testing of cytokines using a validated multiplex assay. This study also had several limitations. Cytokine levels were only measured in serum, not saliva, although results of a recent study indicate that serum cytokine measurement may reflect markers detectable in oral samples.(48) It remains uncertain how cytokine concentrations in the periphery may be related to viral clearance at the local site of infection, or how clearance may be affected by the contributing effects of the mucosal immunologic environment. Cytokines were measured at the time of first oral HPV detection so subsequent changes in cytokine concentration during infection could not be evaluated. We did not have data on concurrent genital HPV infections in the study participants. Finally, the 6-month study visit

intervals limited our ability to identify time of infection and time of clearance with greater precision.

To our knowledge, this is the first study to explore the association of circulating immune markers with oral HPV clearance. While the majority of cytokines were not related to oral HPV clearance, these results suggest higher TNF-α concentration may be associated with reduced clearance of oral HPV infection, and that the strength of association may be stronger in men than women. Future studies, which include longitudinal cytokine evaluation, are needed to clarify the role of TNF-α in oral HPV natural history.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Article highlights

- Higher serum TNF-α concentration was associated with reduced oral HPV clearance
- The association of TNF-α with oral HPV clearance was stronger among men than women
- Results are consistent with studies of TNF-α and decreased cervical HPV clearance

Table 1

Description of study participant characteristics at baseline, overall and by sex[a]

Characteristic	n (%) Overall (N=766)	Men (N=402)	Women (N=364)	p-value
Infection type				0.88
Incident only	152 (19.8)	77 (19.2)	75 (20.6)	
Any prevalent	307 (40.1)	163 (40.5)	144 (39.6)	
None	307 (40.1)	162 (40.3)	145 (39.8)	
Age (years)				**<0.0001**
<45	200 (26.1)	58 (14.5)	142 (39.0)	
45-54	328 (42.9)	171 (42.6)	157 (43.1)	
≥55	238 (31.1)	173 (43.0)	65 (17.9)	
Race/Ethnicity				**<0.0001**
White, non-Hispanic	254 (33.2)	238 (59.2)	16 (4.4)	
Black, non-Hispanic	413 (53.9)	144 (35.8)	269 (73.9)	
Other race or Hispanic	99 (12.9)	20 (5.0)	79 (21.7)	
Study site				
Baltimore, Maryland	140 (18.3)	140 (34.8)	NA	
Bronx, New York	116 (15.1)	NA	116 (31.9)	
Brooklyn, New York	134 (17.5)	NA	134 (36.8)	
Chicago, Illinois	253 (33.0)	139 (34.6)	114 (31.3)	
Pittsburgh, Pennsylvania	123 (16.1)	123 (30.6)	NA	
HIV status				**0.02**
Uninfected	197 (25.7)	117 (29.1)	80 (22.0)	
Infected	569 (74.3)	285 (70.9)	284 (78.0)	
Current CD4 T cell count (cells/μL)				0.17
≥500	313 (55.0)	165 (57.9)	148 (52.1)	
<500	256 (45.0)	120 (42.1)	136 (47.9)	
Current HIV RNA viral load (copies/mL)				**<0.0001**
<200	415 (73.3)	228 (80.9)	187 (66.5)	
200-19,999	94 (16.7)	32 (11.3)	62 (22.1)	
≥20,000	54 (9.6)	22 (7.8)	32 (11.4)	
Current ART use				0.43
No	101 (17.8)	47 (16.5)	54 (19.0)	
Yes	468 (82.2)	238 (83.5)	230 (81.0)	
Smoked cigarettes in the last 6 months				**<0.0001**
No	439 (57.8)	271 (68.3)	168 (46.3)	
Yes	321 (42.2)	126 (31.7)	195 (53.7)	

[a]For HPV-uninfected participants and participants with prevalent HPV, baseline was defined as POPS enrollment. For participants with incident HPV, baseline was defined as visit of incident HPV detection.

Table 2

Comparison of baseline serum cytokine concentrations (pg/ml) in 931 samples, by oral HPV status[a]

	Median serum concentration at baseline (IQR)			
Cytokine	**No Oral HPV n=311**	**Incident Oral HPV n=313**	**Prevalent Oral HPV n=307**	**p-trend**
TNF-α	**3.1 (2.4-4.3)**	**3.3 (2.5-4.5)**	**3.5 (2.6-4.8)**	**0.009**
IL-6	1.0 (0.7-1.8)	1.1 (0.7-1.7)	1.1 (0.7-1.7)	0.41
IL-8	**15.2 (10.7-38.1)**	**16.4 (11.4-37.5)**	**19.5 (13.0-62.3)**	**0.001**
IFN-γ	**8.0 (5.2-14.1)**	**8.2 (5.4-15.7)**	**9.4 (5.7-16.5)**	**0.03**
IL-10	**0.47 (0.27-0.76)**	**0.55 (0.32-0.89)**	**0.60 (0.37-0.94)**	**0.001**
IL-2	**0.27 (0-0.55)**	**0.34 (0-0.55)**	**0.38 (0-0.59)**	**0.007**

[a]For samples with no oral HPV or prevalent HPV, baseline was defined as POPS enrollment. For samples with incident HPV, baseline was defined as visit of incident HPV detection. Unadjusted (no log transformation) median cytokine concentrations and interquartile ranges (IQR, 25th to 75th percentiles, in parentheses) are shown. Bolding indicates values with $p<0.05$ in tests for trend. Results were similar when restricted to only HIV-infected, or only HIV-uninfected participants (results not shown).

Table 3

Unadjusted association of cytokine concentration (by quartile) with oral HPV clearance among 459 participants with 917 infections, by sex.[a]

	Hazard ratio (95% CI)					
	Infections in Men, n=472			Infections in Women, n=445		
Cytokine	**Quartile 2**	**Quartile 3**	**Quartile 4**	**Quartile 2**	**Quartile 3**	**Quartile 4**
TNF-α	**0.55 (0.40-0.76)**	**0.56 (0.40-0.78)**	**0.50 (0.35-0.72)**	0.93 (0.67-1.30)	0.97 (0.68-1.39)	**0.67 (0.48-0.94)**
IL-6	**0.72 (0.53-0.99)**	0.71 (0.49-1.02)	0.83 (0.61-1.12)	1.41 (0.97-2.05)	1.04 (0.71-1.52)	1.05 (0.71-1.54)
IL-8	0.88 (0.64-1.20)	0.74 (0.51-1.07)	1.05 (0.75-1.48)	0.83 (0.58-1.20)	0.95 (0.71-1.29)	**0.73 (0.53-1.00)**
IFN-γ	0.97 (0.70-1.34)	0.98 (0.69-1.40)	0.79 (0.53-1.17)	0.79 (0.54-1.14)	0.86 (0.63-1.17)	0.84 (0.61-1.16)
IL-10	0.78 (0.58-1.06)	0.81 (0.58-1.14)	0.82 (0.58-1.15)	0.89 (0.63-1.24)	0.88 (0.61-1.25)	**0.74 (0.55-1.00)**
IL-2	**0.63 (0.42-0.93)**	**0.73 (0.54-1.00)**	**0.69 (0.50-0.96)**	0.80 (0.52-1.22)	0.98 (0.71-1.36)	1.10 (0.80-1.50)

[a]Reference is quartile 1. Bolding indicates hazard ratios with $p<0.05$

Table 4

Multivariable risk factors for oral HPV clearance among 459 participants with 917 oral HPV infections, by sex[a]

Characteristic at visit of first oral HPV detection	Infections in Men, n=472			Infections in Women, n=445		
	No. of cleared infections	Person-months	aHR (95% CI)	No. of cleared infections	Person-months	aHR (95% CI)
TNF-α concentration (pg/ml) [b]						
Quartile 1	115	2,557	Ref	59	1,443	Ref
Quartile 2	77	3,593	**0.56 (0.40-0.79)**	69	1,869	0.97 (0.70-1.34)
Quartile 3	62	2,713	**0.60 (0.44-0.82)**	84	2,392	1.07 (0.76-1.49)
Quartile 4	46	2,432	**0.52 (0.34-0.79)**	81	3,264	0.76 (0.55-1.04)
			p for interaction=0.049			
IL-2 concentration (pg/ml) [c]						
Quartile 1	124	3,612	Ref	101	3,053	Ref
Quartile 2	39	1,868	**0.66 (0.45-0.96)**	33	1,243	0.90 (0.58-1.37)
Quartile 3	69	2,703	**0.70 (0.52-0.93)**	79	2,442	1.09 (0.82-1.47)
Quartile 4	65	2,906	**0.69 (0.50-0.95)**	79	2,223	1.15 (0.85-1.57)
			p for interaction=0.058			
Infection type						
Incident	140	2,908	Ref	148	2,480	Ref
Prevalent	160	8,388	**0.52 (0.40-0.67)**	146	6,494	**0.45 (0.34-0.59)**
Age (years)						
<45	63	1,995	Ref	107	2,929	Ref
45-54	124	4,423	0.83 (0.61-1.12)	137	4,036	0.95 (0.74-1.22)
≥55	113	4,877	**0.67 (0.48-0.92)**	50	2,009	0.82 (0.58-1.15)
HIV and current CD4 cell count (cells/μL)						
HIV uninfected	74	2,326	Ref	53	1,370	Ref
HIV infected, CD4≥500	124	4,386	0.89 (0.66-1.21)	93	3,157	0.78 (0.56-1.08)
HIV infected, CD4<500	102	4,584	0.87 (0.64-1.19)	148	4,447	0.92 (0.68-1.25)

[a] TNF-α and IL-2 were each analyzed in separate multivariable models adjusting for infection type, age, and HIV status/CD4 count. Covariates shown are from the model with TNF-α. Bolding indicates adjusted hazard ratios (aHR) with p<0.05.

[b] TNF-α quartiles: 0.0-2.56 (Q1), 2.57-3.58 (Q2), 3.59-4.93 (Q3), 4.94-19.33 (Q4).

[c] IL-2 quartiles: 0.0-0.0 (Q1), 0.21-0.37 (Q2), 0.38-0.58 (Q3), 0.59-20.62 (Q4).