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Evaluation of a multiplex panel of immune-related markers in cervical secretions: a methodologic study

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Abstract

While persistent carcinogenic human papillomavirus (HPV) infection is necessary for cervical carcinogenesis, the co-factors involved in HPV persistence and disease progression are poorly understood. Chronic cervical inflammation may increase risk, but few studies have measured immune markers (cytokines/chemokines/soluble receptors) in cervical secretions. We evaluated the performance of 74 multiplexed, bead-based immune markers in cervical secretions from three groups of women with biopsy evaluation of cervical intraepithelial neoplasia (CIN): 1) <CIN1, HPV-negative (n=24), 2) <CIN1, carcinogenic-HPV-positive (n=24), and 3) CIN2/3, carcinogenic-HPV-positive (n=48), matched on time since last period and smoking status. We considered markers with >25% detectability and >80% interclass correlation coefficients (ICC) acceptable for epidemiologic studies. Within-batch coefficients of variation (CV) of 25% indicated room for assay improvement. Secondly, we explored associations between marker levels and CIN/HPV status adjusted for matching variables, assay batch, age, and number of sexual partners. Sixty-two markers (84%) had >25% detectability and ICCs>80%. Of those, 53 (85%) had CVs<25%. Using these preliminary data, we found that HPV-positivity was associated with increased eotaxin-1 (OR: 15.63, 95% CI: 1.26–200.00) and G-CSF (OR: 12.99, 95% CI: 1.10–142.86) among CIN-negative women. There was suggestive evidence that higher chemoattractant marker levels were associated with CIN2/3 (e.g., MIP-1delta, OR: 4.48, 95% CI: 0.87–23.04 versus <CIN1/HPV-positive). Over 70 percent of markers were reliably measured. This assay may be used to evaluate associations of immune-related markers with CIN and HPV status.

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Background

Cervical cancer is the third most common cancer in women worldwide, accounting for 13% of all female cancers in developing countries.¹ Efficacious prophylactic vaccines are available against human papillomavirus (HPV) types 16 and 18, which cause about 70% of cervical cancers,² but do not protect women who have already been exposed to the virus.^{3, 4} Since many women will remain unprotected from infection, cervical cancer will likely remain a global health problem for decades to come. Thus, understanding factors that predispose some women to have persistent infection with carcinogenic HPV and progressive disease, while other women are able to clear such infections is of interest and might help identify clinically significant biomarkers for defining risk of progression to cervical cancer.

Several lines of evidence suggest that the interplay between HPV and the immune system determines whether a woman will develop cervical cancer. For example, women with persistent HPV infections may demonstrate a certain immune incompetence to clear infections effectively and thus are put at greatly increased risk of developing cervical precancer and cancer.^{5, 6} Factors such as smoking, which may act in part through local immune suppression,^{7–9} and HPV type,¹⁰ where some types like HPV16 might be better able to avoid immune detection,¹¹ may modify the immune response to infection.

Cervical inflammation has been hypothesized to act as a co-factor for HPV persistence and progression to disease, but cervical inflammation has been difficult to measure. Previous studies of cytokines in the context of HPV infection or cervical precancer or cancer typically have assessed only a few cytokines.^{12–16} New developments now make it feasible to measure many immune-related markers simultaneously in a high-throughput, multiplex format using small volumes of specimen. Measuring proteins that are secreted from immune cells like cytokines, chemokines, and soluble receptors, which affect cell-mediated immune response and can be altered in the presence of infection,^{17–19} may help detect early changes in the immune response that predict whether an HPV infection will progress to cervical cancer. However, it is important to formally evaluate reproducibility. Such studies are rarely performed.

Recently, a bead array-based, multiplexed panel of immune-related markers was shown to have acceptable performance using serum and plasma from 100 cancer-free subjects.²⁰ For a localized infection like HPV, markers measured in cervical secretions may better reflect the local cervical environment that regulates cervical infections and may affect development of cervical cancer. Thus, the purpose of this study was to evaluate the performance of a Luminex-based multiplex panel of immune-related markers in cervical secretions. As a secondary objective, we also explored differences in biomarker profiles in relation to cervical intraepithelial neoplasia (CIN) and HPV status.

Methods

Subjects

This methodologic study included samples from subjects enrolled in the Study to Understand Cervical Cancer Early Endpoints and Determinants (SUCCEED). Women who were referred to colposcopy at the University of Oklahoma Health Sciences Center (OUHSC) with abnormal cytological screening results or a biopsy diagnosis of CIN were recruited between November 2003 and September 2007.^{21–23} These women were representative of the catchment population with the exception of enrichment for invasive cancers. Written informed consent was obtained from all eligible women enrolled into the study, which was approved by the institutional review boards at the OUHSC and the National Cancer Institute. The participation rate was 55%. We excluded women who had

sex within three days prior to the study visit, reported an STI in the last year, or reported the start of their last period less than seven days prior to specimen collection. We randomly selected 24 women from those with a biopsy but no histologic evidence of CIN (<CIN1) and no HPV DNA (of the 37 HPV types detected by Linear Array) detected in their cytological specimens. Although these women were referred for colposcopy, the fact that there was no histologic evidence of CIN indicates that they were actually normal (i.e., the cytology that lead to referral was falsely positive). Because the cervical secretions from these normal women should reflect a normal microenvironment, we then matched 24 women with <CIN1 and carcinogenic HPV DNA detected (including HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) to those with no HPV DNA detected (i.e., normal women) by time since start of last menstrual period at sample collection (+/-5 days) and smoking status. To identify markers that may only be present in women with high grade cervical neoplasia, we selected 48 additional women with CIN2/3 and carcinogenic HPV, matched to the normal women (i.e., <CIN1/no HPV DNA) by time since last period and smoking status. Histology was determined by an experienced OUHSC pathologist.²¹

Specimens

Cervical secretions were collected using Merocel™ cervical sponges (Medtronic Xomed, Inc., Jacksonville, Florida) and then stored at -80 °C. HPV genotyping of liquid-based cervical cytology specimens was conducted for 37 HPV types using the Linear Array® HPV Genotyping Test (Roche Molecular Diagnostics, Pleasanton, CA).

Luminex-based MILLIPLEX MAP Multiplex Assays (Millipore Inc, Billerica, MA)

We evaluated 74 markers distributed between five separate panels (Supplementary Table 1). We grouped each protein in broad general functional biological categories (most proteins have roles in more than one category). To examine intra-plate and inter-plate reproducibility, each assay was run on two separate 96-well plates with 2 aliquots from each woman on one plate and 1 aliquot on a second plate (total of 282 aliquots). For each of the five panels we used a total of 75 µl of extracted specimen (described below, 25 µl per aliquot). These bead-based panels use polystyrene beads filled with fluorescent dye to distinguish each bead set, and bead set has a unique capture antibody bound to it, which along with a relevant detection antibody and streptavidin-PE, allows quantitation of a marker of interest.²⁴ The samples were analyzed following the manufacturer's instructions and using the BioPlex 100 Analyzer (Bio-Rad Laboratories, Inc., Hercules, California).²⁴

Cervical Sponge Extraction

Proteins were extracted from polyvinyl acetate-based Merocel™ cervical sponges using a previously established protocol.²⁵ First, the wet-weight of the sponge was recorded, and then each sponge was placed in a 2 ml Spin-X centrifuge filter tube (Corning Inc, Corning, NY) to which 300 µl of extraction buffer [PBS (Invitrogen, Grand Island, NY), 256 mM NaCl (Sigma-Aldrich, St. Louis, MO), and 100 µg/ml Aprotinin (Sigma-Aldrich, St. Louis, MO)] was slowly added. The sponges were incubated at 4 °C for 30 minutes and then centrifuged at 13,000 × g for 15 minutes at 4 °C. After centrifugation, 300 µl of additional extraction buffer was added to the sponge, which was then immediately centrifuged (as above) without incubation. Next, 10 µl of the resulting extract was removed for protein measurement using the Pierce Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific, Rockford, IL) according to the manufacturer's instructions. Lastly, 4 µl of fetal bovine serum was added to the extract, which was then briefly vortexed, aliquoted, and frozen at -80 °C until further testing.

We applied a dilution factor to account for variation in the amount of cervical secretions collected from each woman. The dilution factor was calculated as $[(x-y) + 0.6 \text{ g of buffer}]/(x$

–y), where x equals the weight of the sponge after collection, and y is the weight of the dry sponge, as previously described.²⁶ The weight of the sponge after specimen collection ranged from 0.06 to 0.36 grams (median: 0.10 grams). Normalization using total protein has been proposed as an alternative approach.²⁷ Thus, we also examined the results using total protein [(pg of immune markers)/(mg of total protein)] for normalization as a sensitivity analysis.

Statistical Analyses

The performance of each immune-related marker in cervical secretions was evaluated using three measures: 1) level of detection—the proportion of women with immune marker levels that were above the lower limit of detection (LOD) for each marker, 2) coefficients of variation (CVs) for within-batch and across-batch duplicate aliquots, and 3) intraclass correlation coefficients (ICCs) to assess the variability due to within-woman differences versus between-woman differences. Observed concentrations were log-transformed for each marker. CVs were estimated on the original scale²⁸, along with ICCs, using mixed linear models that were fit to all women combined, and also separately to women with CIN2/3 and women with <CIN1 using PROC MIXED (SAS 9.2). In the linear mixed models, person and batch were used as random effects and the duplicates on the same plates were accounted for in the “repeated” statement. For the few markers for which the mixed models gave values at the boundary, we combined batches one and two and three and four and adjusted the mixed models accordingly. For the mixed models to provide valid estimates, the logs of the marker concentrations need to be normally distributed. For markers that violated the normality assumption, we present results based on ANOVA-based models (PROC GLM, SAS 9.2), which is typically a somewhat less powerful approach to estimating CVs and ICCs but does not rely on normality.

We considered markers that were detectable in more than 25% of the samples and had an ICC greater than 80% to be acceptable for epidemiologic studies. Within-batch CVs less than 25% indicated markers that could be improved in terms of assay performance. While we present CVs of the markers on the original measurement scale, we also computed CVs for the markers on the log scale since the log scale is the scale that is used for analysis, and the previous methodologic study of the performance of this assay in serum and plasma presented CVs based on the log scale.²⁰

To evaluate the association of HPV status and cervical disease status with immune markers that had acceptable levels of detectability, we averaged the median fluorescence intensity (MFI) values for each marker from the two aliquots tested on the same plate for each woman and log-transformed the averaged value. Because conditional and unconditional logistic regression models produced very similar results when used to examine associations of immune markers with HPV positivity (<CIN1/HPV- positive versus <CIN1/HPV- negative) and high-grade CIN (CIN2/3 versus <CIN1/HPV- positive), we present odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional polytomous logistic models. In these models, immune markers were evaluated in categories defined as follows: 1) for markers detected in ≥75% of women, four categories were created based on quartiles of the detectable values with women with undetectable values included in the first quartile; 2) for markers detected in 50% to <75% of women, four categories were created with the first category including all women with undetectable values and the upper three categories based on tertiles of the detectable values; 3) for markers detected in <50% of women, three categories were created with the first category including all women with undetectable values and the upper two categories based on a median split of the detectable values.

All models were adjusted for matching factors [time since last period (continuous) and smoking status (never, former, current)], age at questionnaire (continuous), number of

sexual partners (tertiles), and batch. Additional factors were considered (race; education; body mass index; age began smoking and use of non-cigarette tobacco products; use of estrogen, estrogen patch or implant, estrogen shot, estrogen cream or suppository; use of a diaphragm, intrauterine device, spermicide, contraceptive film, Nuva Ring® (Merck & Co., Inc., Kenilworth, NJ), other types of contraceptives; douching; total protein) but were either present in a similar proportion of cases and controls or were extremely uncommon.

Results

Two women (one with <CIN1/HPV-positive and one with CIN2) had negative dilution factor values (i.e., the average dry sponge weight was greater than the weight of the sponge after collection) and were therefore dropped from the analyses, leaving 94 women for analysis. These 94 women were generally similar in terms of sociodemographic factors and other characteristics across the disease groups (Table 1). However, HPV-negative women with <CIN1 tended to be slightly older at enrollment (median age 31 years compared to 22 years for HPV-positive women with <CIN1 and 26 years for women with CIN2/3). The age at sexual debut was similar between the three groups (median age at sexual debut: 16.5, range: 13–26 for <CIN1, HPV-negative; median: 16, range: 10–25 for <CIN1, HPV-positive; median: 16, range: 13–22 for CIN2/3), as was the number of sexual partners (median: 4, range: 1–15 for <CIN1, HPV-negative; median: 5, range: 1–17 for <CIN1, HPV-positive; median: 5, range: 1–25 for CIN2/3). However, 30.4% of HPV-negative women with <CIN1 (7/23) reported having only one sexual partner versus 4.3% of HPV-positive women with <CIN1 and women with CIN2/3 (1/23 and 2/46, respectively).

Most immune markers were detectable in the cervical secretions from these 94 women (Figure 1). Only three of the 74 immune-related markers (4%), SCD30, sIL-1RI, and IL-3, were not detected in any of the samples. Sixty-two markers (84%) were detectable in 50% or more of women [including 31 (42%) that were detectable in all women], two (3%) were detectable in 25% to <50%, and 7 (9%) were detectable in <25% of women. Detectability was similar for women with CIN2/3 compared to women with <CIN1 (Supplemental Table 1). Because of concerns over potential interference of marker detectability by excess mucus, detection of each marker was also evaluated for women with (N=15) and without (N=79) excess mucus in their samples. Detection of the immune markers in samples with excess mucus was as good as or even better than that in samples without excess mucus (data not shown), so the presence of mucus was not considered further. A total of 64 markers (86%) met the >25% detectable criterion. Of the 71 detectable markers, the majority also had acceptable ICCs (Table 2). Only 5 markers (7%) had ICCs <80%; 62 of the 64 markers that were detectable in >25% of women also had ICCs >80% (Figure 1). The majority of markers had very similar ICCs when total protein was used for normalization instead of the dilution factor (Table 2). Overall, however, more markers (N=17) had ICCs <80% using total protein for adjustment.

With respect to assay performance, 49 markers (69%) had within-batch dilution-factor-based CVs <20% on the original measurement scale, 10 (14%) had CVs between 20 and 25%, and 12 (17%) had CVs >25% (Table 2). Similarly, 49 markers (69%) had between-batch CVs less than 20%, 7 (10%) had CVs between 20 and 25%, and 15 (21%) had CVs >25% (Table 2). On the log scale, all markers had CVs less than 25%, and 58 markers (82%) had CVs less than 5% (data not shown). Of the 62 markers with ICCs >80% that were detectable in >25% of women, 53 (85%) had within-batch CVs <20% (Figure 1). Performance was generally similar for women with CIN2/3 and women with <CIN1, especially for markers with greater than 25% detectability (Supplementary Table 1). CV estimates were also quite similar if total protein normalization was used rather than normalization using the dilution factor (Table 2).

While the primary objective of this study was to evaluate the performance of the Luminex multiplex immune panel in cervical secretions, we also explored associations of immune markers with greater than 25% detectability with HPV and CIN status to provide preliminary data that might help inform future studies. Using dilution factor-adjusted immune marker values, eotaxin-1 and G-CSF levels were substantially higher in HPV-positive women with <CIN1 compared to HPV-negative women with <CIN1 (OR for highest versus lowest category: 15.63, 95% CI: 1.27–200.00 and OR: 12.99, 95% CI: 1.10–142.86, respectively), and there was some evidence of a dose response with increasing marker levels (Figure 2). Comparing categorical immune markers in HPV-positive women with CIN2/3 to HPV-positive women with <CIN1 to explore markers associated with progression, no markers were statistically significantly elevated or decreased (Table 3). However, the three markers with the most strongly increased ORs were all chemoattractant markers: MIP-3beta (OR: 3.23, 95% CI: 0.71–14.72), MIP-1delta (OR: 4.48, 95% CI: 0.87–23.04), and CTACK (OR: 6.85, 95% CI: 0.59–79.16).

The majority of results were similar if total protein was used for normalization instead of the sponge-weight-based dilution factor (Table 3). Only 3 of the <CIN1 HPV-positive versus HPV-negative ORs changed more than 3-fold (IL-8, sIL-1RII, and IL-12p70), and none of the CIN2/3 versus <CIN1/HPV-positive ORs changed more than 3-fold. ORs changed by less than 1.5-fold for the majority of markers. Among women with <CIN1, eotaxin-1 and G-CSF remained strongly elevated using total protein normalization (OR: 7.75, 95% CI: 0.91–66.67 and OR: 6.76, 95% CI: 0.97–47.62, respectively). While eotaxin-1 and G-CSF lost statistical significance using total protein normalization, sIL-1RII gained statistical significance (OR: 12.82, 95% CI: 1.31–125.00). Comparing women with CIN2/3 to those with <CIN1 who were HPV-positive, CTACK remained the most strongly elevated marker (OR: 7.22, 95% CI: 0.48–108.19). The ORs for MIP-3beta and MIP-1 delta remained elevated (OR: 1.93, 95% CI: 0.42–8.87 and OR: 1.60, 95% CI: 0.34–7.51, respectively), although not as strongly as with the dilution factor adjustment. Overall, dilution-factor-based adjustment and total-protein-based adjustment generally produced similar results, and where there were differences, the differences were not consistently in one direction or the other.

Discussion

Most markers on this large, multiplexed panel were reliably detected in cervical secretions. Eighty-six percent of markers were detectable in more than 25% of women (84% were detectable in more than 50%), and 89% of markers had ICCs over 80%. Of these, 85% of markers had within-batch CVs below 25%. Markers that have been problematic to measure in serum and plasma previously,²⁰ such as IL-4, IL-5, IL-12, and GM-CSF, were also challenging to quantify in cervical secretions. However, the large proportion of markers with ICCs over 80% is particularly important for epidemiologic studies since while the CV is a measure of assay reproducibility, the ICC is a measure of variability. The high ICCs mean that there is more variation in marker concentrations between people than there is within people and that the measured concentrations can therefore be used to evaluate associations with outcomes like HPV infection and CIN. Markers with good assay performance (low CVs) and low ICCs (more variation within people than between people) will have low utility in epidemiologic studies. In contrast, markers with poor assay performance (high CVs) and high ICCs may yet be useful, although assay performance can be improved. Thus, this assay appears to be a relatively robust analytical tool for the measurement of immune-related markers in cervical secretions. Given that this assay requires less specimen volume (approximately 50 µl per multiplexed panel since samples are normally run in duplicate versus approximately 200 µl per marker using enzyme-linked immunosorbent assays), less time, and less labor than traditional assays for individual immune markers, the multiplexed panel seems practically suited for successful application in future epidemiologic studies.

Assay performance results were generally similar when total protein was used to take into account the amount of sample collected instead of dilution factor adjustment. However, ICCs tended to be lower using total protein adjustment (only 76% of markers with ICCs>80% versus 93% using the dilution factor). When results are discrepant between these two normalization methods, we favor dilution-factor-based adjustment since total protein includes both human and non-human protein. Cervical secretions from women with STIs may not only include higher levels of total protein due to bacterial or other infections, but also may have higher levels of human proteins due to the human immune response to these infections. Thus, normalization using total protein could potentially skew the measurement of immune-related markers.

By applying a reliable plasma/serum-based multiplexed immune panel to cervical secretions using careful methodologic design, this study provides important preliminary data for evaluating local cervical immune responses. As such, it provides a new and powerful analytical tool for many valuable future studies. For example, the cervical secretion-specific panel could be used to characterize immune marker profiles across the spectrum of cervical disease (<CIN1, high-grade CIN, cancer) to identify profiles predictive of progression to cancer and to define cytokine profiles associated specifically with cancer. Additional analyses could evaluate differences by HPV type, variants, and viral load and define profiles associated with other sexually transmitted infections (STIs). A direct comparison of local immune response, as measured in cervical secretions, to systemic immune response, as measured in blood, using this panel in women with and without CIN and/or who had persistent HPV infection compared to those who cleared their HPV infection would also be an appealing and important addition to the literature.

Although our analyses of potential associations of immune markers with HPV infection and CIN2/3 were exploratory, they produced some intriguing results. Using dilution-factor-adjusted immune marker values, eotaxin-1 and G-CSF levels were statistically significantly elevated in HPV-positive women with <CIN1 compared to HPV-negative women with <CIN1. While these two associations could be due to chance, especially given the number of comparisons made, they demonstrated dose-response patterns (Figure 2). In addition, Marks *et al.*²⁹ also found that carcinogenic HPV infection was associated with elevated eotaxin (P-value: 0.04). Likewise, carcinogenic HPV was associated with elevated IL-17 (P-value: 0.003) and GM-CSF (P-value: 0.01) in the Marks *et al.* study, and we found similar, although not statistically significant, patterns for IL-17 and GM-CSF. Taken together, these results may suggest that immune marker levels in cervical secretions vary by HPV status, although we cannot rule out that the effects may be due to co-infections with other STIs. The analysis of HPV-positive women with CIN2/3 compared to HPV-positive women with <CIN1 is of particular interest and relevance since it may help identify immune processes that affect progression to pre-cancer. Although no markers were significantly increased or decreased for this comparison in our study, it is interesting that levels of chemoattractant markers tended to be elevated in women with CIN2/3. Few studies have evaluated immune markers with CIN or HSIL,^{13–16} and those that did typically examined all CIN, rather than segregating into disease categories.^{15, 16}

It is also interesting to note that when total protein adjustment was used instead of dilution factor adjustment, results changed by less than 1.5 fold for the majority of markers. Given the small number of women included in this study and the resulting imprecision of the results, it is reassuring that total-protein-based and dilution factor-based adjustment produced broadly similar results. The occasions where there were larger discrepancies between total-protein-based and dilution factor-based results could reflect the imprecision in these estimates. Alternatively, normalization using total protein could potentially be skewed

by the presence of non-human protein and the immune system's response to those foreign proteins, as described above.

In either case, the findings from this study must be interpreted with caution. As a pilot study, it only included 48 women with CIN2/3 and 48 women with <CIN1 (24 with carcinogenic HPV and 24 with no HPV). Its primary purpose was to evaluate the performance of a commercially available multiplexed immune panel in the analysis of cervical secretions. Because this study was not specifically powered to make comparisons by HPV and CIN status, although its size is comparable to the majority of studies that have measured immune markers in cervical secretions,^{12–16} true differences may have been missed. Given the number of immune marker and CIN/HPV comparisons, some results may have been due to chance. In addition, although we excluded women who self-reported an STI in the last year, the presence of STIs was not directly measured in the cervical secretions. Thus, residual confounding by co-infections cannot be ruled out. However, the value of using a multiplexed assay of immune-related markers is that we can assess whether changes in immune processes are associated with cervical precancer, regardless of the source of those changes, which is particularly useful for studies of progression from HPV infection to CIN2/3. Another strength of this study is that we matched or adjusted for factors that could create fluctuations in the cervical microenvironment, such as smoking status,^{7, 9, 30} phase of the menstrual cycle,^{31–36} and patient age.³⁷ Previous studies of immune markers in cervical secretions rarely consider such factors.¹²

The biological processes and markers associated with infection may differ from the markers associated with disease. A variety of cells, including epithelial and immune cells, can produce these markers. These different cells likely make different contributions that may change with HPV infection and progression to high-grade neoplasia. Longitudinal studies are required to more directly evaluate the relation of these markers to in the development of cervical cancer or clearance of HPV infection. Such studies would provide a better understanding of the mechanisms of cervical carcinogenesis and the cells and pathways that determine the fate of an infection.

In conclusion, the results of this study suggest that measurement of multiple immune markers using Luminex-based technology can be reliably applied to cervical secretions. In addition, our findings, although preliminary, suggest that there may be differences in immune profiles by HPV and/or cervical disease status. These findings warrant the development of a larger study specifically designed to use this panel to address the relation of immune response to HPV-related carcinogenesis. By measuring immune markers in cervical secretions, we can clarify the local immune mechanisms associated with infection and cancer outcomes. In doing so, we may be able to address vital questions in the field, such as how to distinguish women who will clear their HPV infections from those who will develop persistent infections with progression to cervical cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Novelty and Impact

The multiplexed, bead-based immune-related marker assay performed well in cervical secretions and may be an important tool for expanding our understanding of the biological mechanisms of HPV-related carcinogenesis.

Figure 1.
Distribution of markers by performance characteristics.

Figure 2. Linear trend for selected immune markers and CIN or HPV status (versus <CIN1/HPV-positive)

*Adjusted for matching variables (time between cytokine sample and start of last period (+/-5 days) and smoking status), batch, age at entry, and number of sexual partners

†Q1: <264.59 pg/ml, Q2: 264.88–428.63 pg/ml, Q3: 451.12–714.80 pg/ml, Q4: 725.60–27,671.04 pg/ml

‡Q1: 5,730.96–51,714.18 pg/ml, Q2: 54,890.57–93,271.06 pg/ml, Q3: 96,101.65–195,761.28 pg/ml, Q4: 204,605.54–2,909,872.88 pg/ml

Distribution of selected characteristics by cervical intraepithelial neoplasia (CIN) and HPV status for all women included in the immune marker analyses*.

Table 1

Characteristic	CIN2/3		<CINI/HPV-positive		<CINI/HPV-negative	
	N (47)	%	N (23)	%	N (24)	%
Days since last period						
20	26	55.3%	11	47.8%	13	54.2%
>20	21	44.7%	12	52.2%	11	45.8%
Smoking status						
Never	21	44.7%	10	43.5%	11	45.8%
Former	6	12.8%	3	13.0%	3	12.5%
Current	20	42.6%	10	43.5%	10	41.7%
Age at enrollment (years)						
25	23	48.9%	16	69.6%	9	37.5%
>25	24	51.1%	7	30.4%	15	62.5%
Race [†]						
White	38	82.6%	16	80.0%	16	88.9%
Non-white	8	17.4%	4	20.0%	2	11.1%
Education [†]						
<High school	10	21.3%	5	22.7%	7	29.2%
Completed high school	15	31.9%	5	22.7%	9	37.5%
>High school	22	46.8%	12	54.5%	8	33.3%
Age at sexual debut (years)						
16.5	30	63.8%	12	52.2%	12	50.0%
>16.5	17	36.2%	11	47.8%	12	50.0%
Lifetime no. sexual partners						
1-3	12	26.1%	6	26.1%	10	43.5%
4-7	17	37.0%	9	39.1%	6	26.1%
7+	17	37.0%	8	34.8%	7	30.4%
Oral contraceptive use [†]						
Never	4	8.7%	1	4.3%	4	17.4%

Characteristic	CIN2/3		<CIN1/HPV-positive		<CIN1/HPV-negative	
	N (47)	%	N (23)	%	N (24)	%
Ever	42	91.3%	22	95.7%	19	82.6%

* Excludes two women with negative dilution factors.

[†] n's do not sum to total due to missing values

Table 2

Performance of 71 immune markers detectable in cervical secretions, sorted by within-batch CV.

Metabolite	% detectable in women	DF Adjusted*				TP Adjusted†				Mean value (pg/ml)				Metabolite Function
		ICC	CV			ICC	CV			Crude	DF Adjusted*	TP Adjusted†		
			Within	Between	Overall		Within	Between	Overall					
MIP-3alpha/CCL20	100.0	99.79	4.72	2.27	5.24	99.66	4.68	2.28	5.21	1036.03	39123.98	794.43	chemoattractant for lymphocytes, DC, neutrophils	
sTNFRII	100.0	99.73	4.99	4.49	6.71	99.36	4.98	4.66	6.82	1496.62	53664.37	890.60	inflammatory marker of chronic disease	
Eotaxin2/CCL24	100.0	99.68	3.81	3.37	5.09	99.47	3.78	3.50	5.16	424.51	13605.21	296.83	eosinophil>T cell>neutrophil chemoattractant	
MIG/CXCL9	100.0	99.63	3.48	7.28	8.07	99.55	3.45	7.31	8.09	1905.75	37017.80	1303.82	T cell chemoattractant	
IL-6	100.0	99.62	7.07	3.64	7.95	99.43	6.99	3.63	7.88	194.17	7636.94	133.68	acute phase protein/infection response	
IL-8	100.0	99.59	4.95	4.37	6.61	99.41	4.91	4.47	6.64	4921.29	177862.94	3252.70	innate immune inflammatory response, neutrophil chemoattractant	
MIP-1beta/CCL4	100.0	99.43	6.91	5.69	8.95	98.94	6.87	5.98	9.11	100.71	4159.00	74.11	granulocyte activation and induction of pro-inflammatory cytokines	
TRAIL	100.0	99.41	4.53	7.73	8.96	99.10	4.55	7.70	8.94	88.38	3774.22	65.99	induces apoptosis	
IL-1beta	100.0	99.14	13.43	4.58	14.19	99.21	13.35	4.59	14.11	101.43	1964.80	52.05	inflammation/cell proliferation, differentiation, apoptosis	
sgp130	100.0	98.91	7.06	6.83	9.82	98.96	7.02	6.92	9.85	25024.06	722936.14	17992.70	IL-6 antagonist	
IP-10/CXCL10	100.0	98.81	4.81	11.58	12.54	98.60	4.84	11.64	12.61	1103.68	29664.37	808.80	anti-tumor chemoattractant	
GCP2/CXCL6	100.0	98.71	6.42	7.44	9.83	97.01	6.37	7.51	9.85	772.64	30630.63	596.92	neutrophil chemoattractant	
sIL-6R	100.0	98.52	8.49	6.67	10.80	98.02	8.93	6.76	11.20	776.44	20071.01	482.34	trans-signaling of IL-6 via gp130	
MDC/CCL22	100.0	98.48	9.52	8.52	12.78	95.75	9.66	8.65	12.97	113.54	4947.81	75.99	chemoattractant for NK, monocyte, DC, activated T cells	
IL-16	100.0	98.44	7.71	11.13	13.53	99.12	7.76	0.00	7.76	965.34	30740.32	595.68	anti-viral chemoattractant	
sTNFRI	100.0	98.20	8.72	8.92	12.47	96.97	9.01	8.63	12.47	867.41	23301.82	529.45	inflammatory marker of chronic disease	
MCP-1/CCL2	100.0	98.19	3.16	16.05	16.36	97.75	3.13	15.91	16.21	848.96	21734.93	477.38	monocyte chemotactic protein	
IL-1alpha	100.0	98.03	8.60	14.81	17.12	97.98	8.57	15.10	17.36	375.88	10980.10	253.85	acute phase protein/infection response	
IL-1RA	100.0	97.82	7.12	16.75	18.20	96.48	7.09	16.79	18.23	3732.92	197918.71	3072.95	IL-1 (alpha, beta) antagonist	
TGF-alpha	100.0	97.35	9.17	8.70	12.64	96.31	9.21	6.82	11.45	16.41	504.94	11.44	EGF-like/wound healing/angiogenesis/angiogenesis	
ENA-78/CXCL5	100.0	96.97	4.71	16.73	17.38	96.14	4.66	16.58	17.22	6769.55	214679.80	5162.77	epithelial-derived neutrophil activating peptide	
VEGF	100.0	96.85	7.98	16.46	18.30	93.80	7.93	16.81	18.59	234.61	8077.91	153.55	growth factor/angiogenesis	
GRO1, 2, 3	100.0	96.79	7.15	11.80	13.80	96.12	7.09	11.72	13.69	7173.09	241556.15	5764.39	chemoattractant and adhesion of monocytes/tumorigenesis	
G-CSF	100.0	95.95	4.99	19.56	20.19	95.05	5.11	19.42	20.08	4824.53	164565.95	3566.44	stimulates production of granulocytes and stem cells for release into blood	
FGF-2	100.0	95.55	20.22	8.29	21.85	89.11	20.05	8.18	21.66	73.72	3790.83	56.93	fibroblast GF/angiogenesis	
sIL-1RII	100.0	93.45	23.16	13.91	27.02	88.65	22.98	15.12	27.51	1408.35	45999.79	938.16	IL-1beta antagonist	

Metabolite	% detectable in women	DF Adjusted*			TP Adjusted†			Mean value (pg/ml)			Metabolite Function		
		ICC	CV		ICC	CV		Crude	DF Adjusted*	TP Adjusted†			
			Within	Between		Overall	Within					Between	Overall
IFN-gamma	100.0	91.87	20.94	22.74	30.91	89.67	20.74	24.09	31.79	23.45	923.73	17.29	adaptive immune mediator for bacterial/viral infections and tumors
Flt3L	100.0	90.59	12.51	27.14	29.88	83.28	12.40	27.06	29.77	23.77	1158.60	19.32	growth factor/DC development and maturation
TARC/CCL17	100.0	89.71	16.84	28.92	33.47	93.89	16.92	7.48	18.49	7.90	323.60	5.32	T cell chemotaxis (thymal expression)
sCD40L	100.0	85.90	23.20	36.86	43.55	80.30	23.01	36.52	43.17	55.03	1743.25	26.76	induces pro-inflammatory tumoricidal cytokines/B cell activation
Fractalkine/CXCL1	100.0	79.01	13.88	44.08	46.21	69.05	13.78	44.01	46.12	52.16	2701.93	45.54	T cell and monocyte chemoattractant
I-TAC/CXCL11	98.9	99.12	6.05	10.64	12.24	98.82	5.99	10.79	12.34	37.54	929.10	24.88	chemoattractant for activated T cells
BCA-1	98.9	99.11	5.86	13.49	14.71	98.63	6.59	13.12	14.68	74.20	2799.95	52.26	B cell chemoattractant
TNF-alpha	98.9	98.89	13.03	8.10	15.34	98.58	13.07	9.33	16.06	22.72	673.23	15.00	acute phase protein/infection response
Eotaxin-1/CCL11	98.9	94.66	13.57	16.98	21.73	87.64	13.42	16.83	21.53	26.64	1257.89	21.39	eosinophil chemoattractant
IFN-alpha2	98.9	88.53	20.27	24.35	31.68	85.88	20.04	24.77	31.86	17.83	812.49	15.77	viral defense
IL-10	97.9	98.05	11.73	20.78	23.86	97.23	11.74	20.69	23.79	63.69	1924.23	37.51	anti-inflammatory cytokine
sIL-2Ralpha	97.9	81.51	19.66	40.32	44.86	72.17	19.49	40.25	44.72	14.16	661.21	11.21	IL-2 antagonist
IL-7‡	96.8	97.03	24.40	23.48	51.73	98.92	16.83	17.33	16.61	4.98	197.30	4.63	B cell maturation, T and NK cell survival, development and homeostasis
IL-15	96.8	92.67	19.98	14.08	24.44	88.08	20.35	13.90	24.65	2.61	103.03	1.99	regulates T cell and NK cell activation and proliferation
sEGFR	95.7	97.65	15.89	3.57	16.29	95.98	16.04	1.83	16.14	1367.55	35120.98	821.11	cell migration/adhesion/proliferation/mutations of receptor in cancer
IL-12p40	95.7	86.32	29.14	25.07	38.44	78.48	28.64	26.15	38.78	24.44	1102.54	19.42	dimers are agonistic to biologically active IL-12p70(p35p40)
MCP-2/CCL8	94.7	99.52	4.72	5.39	7.16	98.89	4.72	5.60	7.32	30.89	1505.19	25.31	chemoattractant for immune cells, especially allergy
MCP-3/CCL7	94.7	95.86	12.93	14.83	19.67	89.33	13.00	14.58	19.53	31.75	1533.94	25.18	anti-tumor macrophage/T cell/granulocyte chemoattractant in infection/metastasis
MIP-1a/CCL3	93.6	92.20	16.93	29.88	34.34	89.72	16.95	29.93	34.40	80.25	2565.19	56.95	acute inflammatory protein/granulocyte recruitment
IL-29	91.5	94.50	15.38	18.72	24.22	85.51	15.33	18.32	23.89	80.75	3344.61	65.53	microbial/viral defense
sVEGFR1	90.4	97.93	11.72	9.45	15.06	96.18	11.64	9.56	15.07	604.06	18484.14	447.56	VEGF antagonist/anti-angiogenesis
IL-2	90.4	92.64	23.69	16.61	28.93	94.58	23.94	16.14	28.87	5.93	143.92	6.37	T cell growth and function
TSLP	90.4	89.56	24.59	19.78	31.56	77.74	24.45	19.71	31.40	5.40	200.42	3.99	DC maturation
IL-17‡	89.4	93.89	44.40	66.00	92.40	93.41	21.79	20.95	22.50	4.30	176.85	3.38	pro-inflammatory, induces other cytokines, chemokines prostaglandins
IL-12p70	89.4	80.68	40.96	31.78	51.84	68.98	41.68	30.89	51.87	4.01	259.99	3.53	biologically active IL-12, innate immune activation, T cell differentiation
SCF	88.3	88.17	27.95	15.57	32.00	69.55	28.16	14.56	31.70	7.08	231.08	5.14	hematopoiesis and mast cell chemoattractant
MIP3-beta/CCL19	87.2	97.97	11.36	9.13	14.58	95.79	11.42	9.13	14.62	8.11	269.65	5.58	T and B cell chemoattractant (lymph nodes)
MIP1delta/CCL15	86.2	96.68	11.51	9.53	14.94	96.08	11.44	10.52	15.54	289.83	6399.35	199.69	chemoattractant for T cell and monocytes

Metabolite	% detectable in women	DF Adjusted*			TP Adjusted†			Mean value (pg/ml)				Metabolite Function	
		ICC	CV		ICC	CV		Crude	DF Adjusted*		TP Adjusted†		
			Within	Between		Overall	Within		Between	Overall			
MCP-4/CCL13	79.8	95.97	18.87	11.38	22.04	91.36	19.11	10.73	21.91	75.65	4051.13	69.07	monocyte and T cell chemoattractant
sVEGFR2	79.8	91.74	18.02	23.46	29.58	78.89	17.97	23.05	29.22	218.12	11182.43	169.64	inhibits lymphangiogenesis
GMCSF	75.5	84.43	39.80	16.39	43.04	71.71	39.06	17.34	42.73	2.98	163.70	2.27	stimulates production of granulocytes and monocytes/infection
EGF	70.2	87.48	28.41	18.75	34.04	82.01	28.37	18.11	33.66	15.96	375.41	11.75	cell growth, proliferation, and differentiation
CTACK	64.9	78.92	34.94	13.88	37.59	56.61	33.43	18.00	37.97	8.84	194.91	5.12	T cell-mediated skin inflammation, T-cell chemoattractant
SDF-1/CXCL12	63.8	80.30	31.45	22.36	38.59	66.62	32.42	15.38	35.88	206.98	5814.86	160.81	chemoattractant for lymphocytes
IL-33	54.3	90.15	29.06	25.27	38.51	82.65	28.90	24.24	37.72	42.05	1257.52	29.13	induces production of allergic inflammation
TNF-beta/LT-alpha	51.1	85.02	21.68	36.09	42.10	75.64	21.79	24.72	32.95	1.28	54.49	1.05	immunostimulatory inflammatory cytokine/anti-viral
sIL-4R	45.7	92.72	12.65	11.79	17.29	87.25	12.63	12.52	17.79	85.06	1636.69	37.71	IL-4 antagonist/anti-inflammatory
TPO	44.7	90.23	28.34	12.72	31.06	78.67	27.11	16.29	31.62	108.69	3773.86	84.70	platelet production
LIF	24.5	84.53	15.14	41.94	44.59	79.11	15.16	40.30	43.06	120.91	2320.21	91.08	inhibits cell differentiation
IL-4	24.5	55.17	22.83	41.08	47.00	58.60	22.80	39.33	45.46	3.13	46.37	2.27	allergic inflammation
IL-5	14.9	95.90	14.01	3.99	14.21	84.84	14.00	19.96	24.38	1.03	18.97	0.70	eosinophil activation, B cell stimulation/Ig production
sVEGFR3	9.6	96.23	13.93	9.38	16.80	95.98	13.78	9.76	16.88	231.02	4225.77	163.73	lymphangiogenesis/wound healing/tumor
IL-11	7.5	80.24	53.81	22.22	58.21	23.26	51.24	42.15	66.35	18.80	741.40	14.19	hematopoiesis, lymphocyte growth
6ckine/CCL21	7.5	14.43	31.48	50.68	59.66	69.33	34.76	9.21	35.96	192.96	2913.13	125.09	T cell chemoattractant/adhesion
sRAGE	3.2	61.31	0.08	71.16	71.16	0.03	0.00	38.97	38.97	18.63	2026.55	13.06	anti-inflammatory/RAGE antagonist

*Dilution factor (DF) applied to account for variation in the amount of cervical secretions collected from each woman

†Total protein (TP) applied to account for variation in the amount of cervical secretions collected from each woman

‡CVs and ICCs calculated using ANOVA-based models (PROC GLM, SAS 9.2) due to non-normal distribution

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) for the association of immune marker concentration (highest versus lowest category for 64 markers detectable in >25% of samples) with cervical intraepithelial neoplasia (CIN) and HPV.

Metabolite	% detectable in women	ICC	Within-batch CV	Dilution factor-adjusted values				Total protein-adjusted values			
				<CIN1/HPV+ vs. <CIN1/HPV-		CIN2/3 vs. <CIN1/HPV+		<CIN1/HPV+ vs. <CIN1/HPV-		CIN2/3 vs. <CIN1/HPV+	
				OR*	95% CI	P-trend	OR*	95% CI	P-trend	OR*	95% CI
MIP-3alpha/CCL20	100.0	99.79	4.72	1.38	0.16-11.90	0.92	2.41	0.55-10.61	0.60	1.09	0.15-7.69
sTNFRII	100.0	99.73	4.99	2.60	0.89-12.23	0.39	0.36	0.06-2.36	0.44	2.38	0.35-16.13
Eotaxin-2/CCL24	100.0	99.68	3.81	0.90	0.15-5.32	0.78	2.15	0.46-10.02	0.38	0.86	0.15-5.13
MIG/CXCL9	100.0	99.63	3.48	0.81	0.12-5.32	0.98	0.77	0.16-3.65	0.61	1.15	0.18-7.63
IL-6	100.0	99.62	7.07	1.50	0.24-9.26	0.77	0.66	0.15-2.93	0.74	1.24	0.23-6.58
IL-8	100.0	99.59	4.95	7.41	0.89-62.5	0.08	0.58	0.12-2.92	0.69	1.92	0.29-12.82
MIP-1beta/CCL4	100.0	99.43	6.91	2.97	0.48-18.18	0.37	0.44	0.10-1.92	0.63	1.11	0.17-7.35
TRAIL	100.0	99.41	4.53	0.86	0.11-6.99	0.58	1.05	0.22-4.97	0.47	0.88	0.14-5.71
IL-1beta	100.0	99.14	13.43	1.61	0.28-9.35	0.85	0.74	0.16-3.34	0.67	1.49	0.24-9.26
sgp130	100.0	98.91	7.06	2.65	0.37-19.23	0.27	0.54	0.10-3.03	0.19	2.58	0.38-17.54
IP-10/CXCL10	100.0	98.81	4.81	1.16	0.16-8.40	0.92	0.72	0.16-3.20	0.76	1.22	0.20-7.41
GCP2/CXCL6	100.0	98.71	6.42	1.93	0.34-10.87	0.28	1.55	0.35-6.83	0.46	1.73	0.27-11.11
sIL-6R	100.0	98.52	8.49	1.22	0.15-9.90	0.95	1.10	0.18-6.77	0.90	1.74	0.25-12.05
MDC/CCL22	100.0	98.48	9.52	1.04	0.18-6.06	0.93	1.07	0.22-5.22	0.87	1.45	0.20-10.53
IL-16	100.0	98.44	7.71	0.71	0.12-4.23	0.80	0.89	0.21-3.74	0.53	0.56	0.10-3.26
sTNFR1	100.0	98.20	8.72	1.86	0.30-11.63	0.61	1.05	0.21-5.25	0.83	2.55	0.38-17.24
MCP-1/CCL2	100.0	98.19	3.16	0.75	0.10-5.49	0.78	0.60	0.13-2.86	0.56	1.10	0.13-8.93
IL-1alpha	100.0	98.03	8.60	3.02	0.49-18.87	0.31	1.64	0.37-7.26	0.44	3.98	0.57-27.78
IL-1RA	100.0	97.82	7.12	1.96	0.34-11.24	0.41	0.46	0.10-2.13	0.51	2.03	0.31-13.51
TGF-alpha	100.0	97.35	9.17	1.83	0.27-12.50	0.43	2.43	0.56-10.65	0.72	1.94	0.31-12.05
ENA-78/CXCL5	100.0	96.97	4.71	6.06	0.91-40.00	0.19	0.40	0.09-1.82	0.60	2.34	0.36-15.15
VEGF	100.0	96.85	7.98	2.79	0.42-18.52	0.31	0.53	0.12-2.38	0.53	3.76	0.52-27.03
GRO1, 2, 3	100.0	96.79	7.15	5.49	0.77-38.46	0.08	0.92	0.22-3.80	0.82	2.35	0.31-17.86
G-CSF	100.0	95.95	4.99	12.99	1.10-142.86	0.04	0.81	0.18-3.72	0.82	6.80	0.97-47.62
FGF-2	100.0	95.55	20.22	2.84	0.44-18.50	0.17	1.01	0.22-4.73	0.92	2.56	0.39-16.95
										0.52	0.09-2.89
										0.26	0.07-1.61
										0.35	0.07-1.61
										0.63	0.32-8.26
										0.70	0.14-2.78
										0.82	0.18-3.69
										0.64	0.14-2.85
										0.94	0.19-4.16
										0.76	0.16-4.02
										0.76	0.16-2.99
										0.24	0.06-1.85
										0.98	0.15-3.18
										0.51	0.54-12.42
										0.81	0.15-2.64
										0.77	0.09-2.55
										0.56	0.14-2.64
										0.55	0.11-2.48
										0.60	0.09-2.50
										0.21	0.11-3.63
										0.29	0.07-1.62
										0.61	0.34-7.62
										0.34	0.13-3.01
										0.11	0.12-3.78
										0.21	0.22-4.97
										0.08	0.08-2.00
										0.26	0.09-2.89

Metabolite	% detectable in women	ICC	Within-batch CV	Dilution factor-adjusted values					Total protein-adjusted values				
				<CIN1/HPV+ vs. <CIN1/HPV-	OR*	95% CI	P-trend	CIN2/3 vs. <CIN1/HPV+	<CIN1/HPV+ vs. <CIN1/HPV-	OR*	95% CI	P-trend	CIN2/3 vs. <CIN1/HPV+
				OR*	95% CI	P-trend	CIN2/3 vs. <CIN1/HPV+	OR*	95% CI	P-trend	CIN2/3 vs. <CIN1/HPV-	OR*	95% CI
sIL-IRII	100.0	93.45	23.16	3.65	0.53–25.64	0.23	0.70	0.13–3.81	12.82	1.31–125.00	0.11	0.25	0.04–1.63
IFN-gamma	100.0	91.87	20.94	2.52	0.27–23.81	0.54	1.20	0.24–6.08	2.84	0.46–17.54	0.25	1.11	0.23–5.41
Fit3L	100.0	90.59	12.51	6.29	0.79–50.0	0.09	0.42	0.07–2.35	6.37	0.72–55.56	0.11	0.69	0.14–3.40
TARC/CCL17	100.0	89.71	16.84	1.21	0.20–7.19	0.70	0.90	0.22–3.72	0.72	0.11–4.63	0.85	1.00	0.24–4.18
sCD40L	100.0	85.90	23.20	9.26	0.84–100.00	0.09	1.56	0.26–9.41	6.58	0.75–58.82	0.09	0.73	0.15–3.57
Fractalkine/CXCL1	100.0	79.01	13.88	4.22	0.51–34.48	0.12	0.63	0.14–2.77	3.58	0.50–25.64	0.10	0.42	0.10–1.89
I-TAC/CXCL11	98.9	99.12	6.05	1.92	0.22–16.95	0.76	0.93	0.22–3.93	1.77	0.23–13.51	0.69	0.83	0.16–4.32
BCA-1	98.9	99.11	5.86	0.60	0.09–3.89	0.81	1.78	0.40–7.99	0.27	0.03–1.89	0.67	1.18	0.24–5.89
TNF-alpha	98.9	98.89	13.03	0.67	0.11–4.27	0.97	1.09	0.23–5.12	1.16	0.22–6.21	0.88	0.78	0.18–3.43
Eotaxin-1/CCL11	98.9	94.66	13.57	15.63	1.26–200.00	0.02	0.67	0.14–3.18	7.75	0.91–66.67	0.02	0.40	0.06–2.61
IFN-alpha2	98.9	88.53	20.27	2.33	0.33–16.13	0.27	1.52	0.33–7.13	2.46	0.40–15.38	0.23	0.71	0.16–3.26
IL-10	97.9	98.05	11.73	0.96	0.15–5.95	0.76	0.50	0.12–2.12	0.83	0.14–4.93	0.59	0.43	0.10–1.88
sIL-2Ra1pha	97.9	81.51	19.66	2.25	0.23–21.28	0.20	1.06	0.18–6.34	2.23	0.30–16.39	0.39	1.07	0.20–5.74
IL-15	96.8	92.67	19.98	4.78	0.58–40.00	0.27	2.14	0.47–9.88	1.66	0.30–9.17	0.46	2.32	0.49–10.95
IL-7 [†]	96.8	97.03	24.40	3.13	0.46–21.28	0.29	0.77	0.17–3.38	1.65	0.28–9.90	0.29	1.36	0.32–5.77
sEGFR	95.7	97.65	15.89	0.64	0.11–3.57	0.65	1.03	0.24–4.53	0.33	0.05–2.19	0.22	2.01	0.44–9.15
IL-12p40	95.7	86.32	29.14	ND			0.98	0.20–4.91	3.46	0.50–24.39	0.09	1.95	0.33–11.41
MCP-2/CCL8	94.7	99.52	4.72	3.58	0.49–26.32	0.10	0.76	0.17–3.44	5.29	0.70–40.00	0.06	0.60	0.11–3.41
MCP-3/CCL7	94.7	95.86	12.93	2.02	0.34–12.05	0.52	0.51	0.11–2.40	1.27	0.21–7.75	0.69	0.33	0.07–1.55
MIP-1a/CCL3	93.6	92.20	16.93	1.18	0.18–7.58	0.71	0.92	0.21–4.03	1.65	0.28–9.62	0.88	0.62	0.13–2.95
IL-29	91.5	94.50	15.38	6.90	0.61–76.92	0.29	1.48	0.36–6.06	6.67	0.96–45.45	0.03	1.03	0.21–5.02
sVEGFR1	90.4	97.93	11.72	2.04	0.27–15.15	0.61	0.34	0.07–1.58	1.67	0.27–10.53	0.49	0.88	0.22–3.58
IL-2	90.4	92.64	23.69	2.72	0.44–16.67	0.23	1.33	0.30–5.83	1.98	0.36–10.87	0.22	2.56	0.52–12.49
TSLP	90.4	89.56	24.59	3.08	0.26–37.04	0.37	2.62	0.63–10.86	2.21	0.34–14.29	0.47	1.03	0.22–4.78
IL-17 [†]	89.4	93.89	44.40	7.87	0.58–111.11	0.27	0.73	0.14–3.76	2.65	0.34–20.83	0.34	0.49	0.09–2.84
IL-12p70	89.4	80.68	40.96	3.47	0.47–25.64	0.18	1.58	0.37–6.78	10.53	0.79–142.86	0.42	0.97	0.21–4.38
SCF	88.3	88.17	27.95	0.37	0.06–2.30	0.28	2.11	0.47–9.43	1.05	0.18–6.10	0.85	1.99	0.45–8.86
MIP3-beta/CCL19	87.2	97.97	11.36	0.12	0.01–1.12	0.08	3.23	0.71–14.72	0.22	0.03–1.40	0.09	1.93	0.42–8.87

Metabolite	% detectable in women	ICC	Within-batch CV	Dilution factor-adjusted values					Total protein-adjusted values				
				<CIN1/HPV+ vs. <CIN1/HPV-		P-trend	OR*	95% CI	<CIN1/HPV+ vs. <CIN1/HPV-		P-trend	OR*	95% CI
				OR*	95% CI				OR*	95% CI			
MIP1delta/CCL15	86.2	96.68	11.51	0.26	0.03–1.90	0.12	4.48	0.87–23.04	0.49	0.08–2.87	0.67	1.60	0.34–7.51
MCP-4/CCL13	79.8	95.97	18.87	1.56	0.25–9.80	0.45	1.28	0.29–5.57	0.82	0.13–5.29	0.39	1.14	0.26–4.97
sVEGFR2	79.8	91.74	18.02	1.67	0.22–12.66	0.41	1.23	0.27–5.67	4.22	0.47–37.04	0.40	0.52	0.12–2.27
GMCSF	75.5	84.43	39.80	3.17	0.36–27.78	0.17	1.11	0.23–5.48	2.92	0.42–20.41	0.29	0.81	0.18–3.75
EGF	70.2	87.48	28.41	0.84	0.14–5.10	0.97	1.34	0.31–5.67	0.97	0.18–5.08	0.98	0.83	0.20–3.33
CTACK	64.9	78.92	34.94	0.37	0.02–5.99	0.80	6.85	0.59–79.16	0.32	0.01–3.66	0.34	7.22	0.48–108.19
SDF-1/CXCL12	63.8	80.30	31.45	4.85	0.59–40.00	0.15	0.42	0.10–1.82	ND			0.47	0.11–2.04
IL-33	54.3	90.15	29.06	0.58	0.10–3.23	0.61	1.32	0.28–6.26	1.11	0.19–6.49	0.57	1.42	0.33–6.17
TNF-beta/LT-alpha	51.1	85.02	21.68	ND			1.05	0.26–4.25	3.83	0.30–50.00	0.21	1.94	0.39–9.60
sIL-4R	45.7	92.72	12.65	ND			ND		ND			ND	
TPO	44.7	90.23	28.34	ND			ND		10.20	0.91–111.11	0.14	1.27	0.35–4.65

* Adjusted for matching variables (time between cytokine sample and start of last period (+/–5 days) and smoking status), batch, age at entry, and number of sexual partners

† CVs and ICCs calculated using ANOVA-based models (PROC GLM, SAS 9.2) due to non-normal distribution

ND=not determined