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Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection

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CONTRIBUTORSHIP STATEMENT:

LM developed the hypothesis, performed the laboratory work, analysed the data and prepared the manuscript; KBA, FL, KM, DAL, LJ and DAL analysed the data and prepared the manuscript; NM, HG and SN performed some of the laboratory work, analysed the data and contributed to manuscript preparation; QAK and SAK conceptualized the cohort and prepared the manuscript; JSP developed the hypothesis, analysed the data and prepared the manuscript.

POTENTIAL CONFLICTS OF INTEREST

The authors of this study do not have commercial or other associations that might pose a conflict of interest. JSP and LM, together with the University of Cape Town, have submitted a PCT International Patent application for IP-10 and IL-1α/β use for diagnosing an inflammatory condition in the female genital tract likely caused by an STI or BV (Patent Application No: PCT/IB2014/065740).

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Abstract

Background: Untreated sexually transmitted infections (STIs) and bacterial vaginosis (BV) cause genital inflammation and increased risk of HIV infection. WHO-recommended syndromic STI and BV management is severely limited as large numbers of women with asymptomatic infections go untreated. The purpose of this cross-sectional study was to evaluate genital cytokine profiles as a biomarker of STIs and BV to identify women with asymptomatic, treatable infections.

Methods: Concentrations of 42 cytokines in cervicovaginal lavages (CVL) from 227 HIV-uninfected women were measured using Luminex. All women were screened for BV by microscopy and STIs using molecular assays. Multivariate analyses were used to identify cytokine profiles associated with STIs/BV.

Results: A multivariate profile of seven cytokines [interleukin (IL)-1α, IL-1β, tumor necrosis factor (TNF)-β, IL-4, fractalkine, macrophage-derived chemokine (MDC), and interferon (IFN)-γ] most accurately predicted the presence of a treatable genital condition, with 77% classification accuracy and 75% cross-validation accuracy [sensitivity 72%; specificity 81%, positive predictive value (PPV) 86%, negative predictive value (NPV) 64%]. Concomitant increased IL-1β and decreased IP-10 concentrations predicted the presence of a treatable genital condition without a substantial reduction in predictive value (sensitivity 77%, specificity 72%, PPV 82% and NPV 65%), correctly classifying 75% of the women. This approach performed substantially better than clinical signs (sensitivity 19%, specificity 92%, PPV 79% and NPV 40%).

Conclusion: Supplementing syndromic management with assessment of IL-1β and IP-10 as biomarkers of genital inflammation may improve STI/BV management for women, enabling more effective treatment of asymptomatic infections and potentially reducing their risk of HIV infection.

INTRODUCTION

The prevalence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) in developing countries is unacceptably high, particularly in key populations at highest risk of HIV infection.[1,2] STIs and BV cause inflammation in the female genital tract [2] that potentially facilitates establishment of HIV infection by recruiting activated HIV target cells, [3,4] promoting HIV replication [5] and reducing epithelial barrier integrity.[6] Johnson et al. (2012) estimated that ~50% of new HIV infections in South African women were attributable to other STIs in 2010.

In resource-limited settings, STIs and BV are managed syndromically, according to the presence of clinical signs and symptoms, rather than by more costly laboratory-based diagnosis.[8] However, a large proportion of women who have STIs or BV are asymptomatic and are thus left untreated.[2,9] Women with asymptomatic STIs have comparable levels of genital inflammation to women with symptomatic infections, which are elevated compared to women without an STI or BV.[2] We found that women with chlamydia or gonorrhoea, who were mostly asymptomatic, had the highest genital cytokine concentrations, with 17/42 and 14/42 cytokines upregulated compared to women with no infection, respectively.[10] Women with BV had a mixed cytokine profile, with upregulated proinflammatory cytokine

concentrations, but also downregulated concentrations of chemokines and hematopoietic cytokines.[10] Many women in resource-limited settings are thus likely to have STI-related inflammation that remains unresolved, placing them at increased risk of HIV infection and reproductive complications.[11] There is thus an urgent need to improve STI management strategies for women, particularly in resource-limited settings, in order to identify commonly asymptomatic infections more effectively.

Laboratory-based nucleic acid amplification tests (NAATs) are the gold standard for STI diagnosis. Compared to syndromic management, these tests are costly and require experienced laboratory personnel and specialized equipment, which are often lacking in resource-limited settings.[8] In addition, these tests do not offer immediate results and transmission of STIs or acquisition of HIV may occur while patients wait for their results. Patients often do not return to the clinic for treatment, with a return rate as low as 37% reported in some studies.[12] Rapid point-of-care (POC) tests have been under development for several years. A rapid test for chlamydia with moderate sensitivity (63%) would result in more patients being treated than a laboratory test with a sensitivity of 94%, if the return rate were less than 65%.[13] However, several antigen-detecting rapid tests for STIs have yielded inconsistent predictive value.[14–18] Rapid NAATs for *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* have been developed and show comparable predictive value to laboratory NAATs.[19,20] Currently, however, rapid NAATs are likely to be too expensive for implementation in resource-limited settings.

Measurement of inflammatory cytokine biomarkers may prove useful to identify women with asymptomatic STIs or BV by means of an inflammatory cytokine test of their genital secretions that provides immediate results. The aim of this study was to evaluate the predictive value of potential cytokine biomarkers of STIs or BV measured in cervicovaginal lavage (CVL) samples.

METHODS

Study participants

This study included 227 high-risk HIV-uninfected women from Durban, South Africa.[2,10] In addition, to validate that this approach would be useful in HIV-infected individuals, 39 HIV-infected women from the same region were included.[21] All women provided informed consent and this study was approved by the University of KwaZulu-Natal and University of Cape Town Ethics Committees.

Screening for STIs and BV

Women were screened for STIs and BV as previously described.[2] A gynaecological examination was performed and two swabs were collected from the anterior and posterior fornices and lateral vaginal walls. Specimens were screened for bacterial STIs (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, and *Trichomonas vaginalis*) and herpes simplex virus (HSV) by PCR and BV using Nugent's criteria (women with a Nugent score ≥7 were considered to have BV). Blood was screened for IgG antibodies to HSV-2 gG-2 using ELISA (HerpeSelect®, Focus Diagnostics, USA). Exposure to

Treponema pallidum was detected serologically using the Becton Dickinson Macro-Vue™ Rapid Plasma Reagin card test and a haemagglutination test (ImmuTrep® TPHA, Omega Diagnostics LTD, UK). HSV-2 and *T. pallidum* both have long periods of latency, during which time they would likely be undetectable in the female genital tract by PCR or inflammatory biomarkers. Therefore, we aimed to identify women with one of the five treatable, bacterial conditions that were assessed (BV, *T. vaginalis*, *C. trachomatis, M. genitalium* or *N. gonorrhoeae*).

Cytokine measurements

CVLs were collected using sterile saline (10ml) as previously described.[2] Samples were centrifuged and the supernatant stored at −80°C. CVLs were not collected from menstruating participants. CVLs were pre-filtered by centrifugation using 0.2 μm cellulose acetate filters (Sigma, USA). The concentrations of 42 cytokines were measured using Human Cytokine LINCO*plex* kits (LINCO Research, USA): Epidermal growth factor (EGF), eotaxin/CCL11, FGF-2, fms-like tyrosine kinase-3 Ligand (FLT3L), fractalkine/ CX_3CL1, granulocyte colony stimulating factor (G-CSF), granulocyte macrophage (GM)-CSF, growth related oncogene (GRO) family (CXCL1-CXCL3), interferon (IFN)-α, IFN-γ, interleukin (IL)-1α, IL-1β, IL-1Ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8/CXCL8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IFN-γ-induced protein 10 (IP-10)/ CXCL10, monocyte chemotactic protein (MCP-1)/CCL2, MCP-3/CCL7, macrophage-derived chemokine (MDC)/CCL22, macrophage inflammatory protein (MIP-1α)/CCL3, MIP-1β/CCL4, platelet-derived growth factor (PDGF)-AA, PDGF-AB/BB, RANTES/ CCL5, soluble CD40 ligand (sCD40L), soluble IL-2 receptor α (sIL-2Rα), transforming growth factor (TGF)-α, tumour necrosis factor (TNF)-α, TNF-β, and vascular endothelial growth factor (VEGF). Cytokine lower limits of detection ranged between 0.01 and 27.65 pg/ml. Data was collected using a Bio-Plex™ Suspension Array Reader and a 5 PL regression formula was used to calculate cytokine concentrations from the standard curves using BIO-plex manager software (version 4; Bio-Rad Laboratories Inc®, USA). To confirm that the platform used to measure cytokine concentrations did not influence the reliability of this approach, the cytokines identified as the best biomarkers of STIs/BV were measured in the same samples using Human Inflammation and Chemokine Cytometric Bead Array (CBA) kits (BD Biosciences, Pharmingen, USA) according to the manufacturer's instructions. Cytokine concentrations below the lower limit of detection of the assays were reported as the mid-point between the lowest concentrations measured for each cytokine and zero.

Statistical analysis

Statistical analyses were performed using STATA™ (StataCorp, USA) and Matlab (Mathworks, USA). Nonparametric receiver operating characteristic (ROC) curves were used to compare the predictive values of each cytokine. Youdin's Index was used to determine appropriate cutoffs for each cytokine, assuming that sensitivity and specificity are of equal importance. Logistic regression was used to determine the variables that were together most predictive of the presence of an STI/BV. Variables that were significantly associated with the presence of a STI/BV were added to the models in a stepwise manner. The likelihood ratio (LR) test was used to compare nested models.

Partial least squares discriminant analysis (PLSDA) [22] was used to determine cytokine profiles that best distinguished between individuals with and without a STI/BV. PLSDA uses an analysis of co-variance to identify combinations (latent variables) of independent variables (cytokines) that best differentiate individuals based on an assigned class (the presence or absence of a STI/BV). All data were normalized with mean centering and variance scaling. Cross-validation was performed by iteratively excluding random subsets of data during model calibration, and using excluded data to test model predictions. To identify the optimal minimum biomarker profile for STI/BV diagnosis and reduce the risk of over-fitting, we used the Least Absolute Shrinkage and Selection Operator (LASSO) method for regression and shrinkage.[23]

RESULTS

Description of study participants

Fifty-three percent of the 227 HIV-uninfected women had BV (Nugent score ≥7) and 29% were PCR positive for a bacterial STI (*C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *T. vaginalis*), while 2.6% were shedding HSV and 1.3% had a *T. pallidum* RPR titre >1:4 with a positive TPHA test (indicative of active syphilis, late-latent or serofast treated syphilis). Despite the high prevalence of STIs and BV, only 34/227 women (15%) had visible cervicovaginal discharge and none had genital ulceration (Table 1).

Genital cytokine profiles identify HIV-uninfected women with BV and/or bacterial STIs

We used PLSDA to determine cytokine signatures that distinguished women with BV and/or bacterial STIs. A model with all 42 cytokines distinguished between women with an STI or BV and women without STIs/BV with 78% classification accuracy, and 74% cross-validation accuracy (Figure 1A). Women with a STI/BV clustered in the positive region of latent variable 1 (LV1; Figure 1A), indicating their cytokine profiles were characterized by upregulation of cytokines positively loaded on LV1, and comparative downregulation of cytokines loaded negatively onto LV1 (Figure 1B). The model of all 42 cytokines classified women with 74% sensitivity, 80% specificity, 86% positive predictive value (PPV), and 65% negative predictive values (NPV; Table 2).

We then used LASSO to eliminate cytokines that did not contribute to group classification. A profile of seven cytokines remained, that classified with similar predictive strength (Table 2: 77% classification accuracy, 72% sensitivity, 81% specificity, 86% PPV, and 64% NPV), and slightly better classification performance on cross-validation compared to the model including all 42 cytokines (75%; Figure 1C). In this reduced model, TNF-β, IL-1α, and IL1β elevation with comparative reduction in IFN-γ, IL-4, MDC and fractalkine was associated with the STI/BV group (Figure 1D).

IL-1β and IP-10 biomarkers are predictive of BV and bacterial STIs in HIV-uninfected women

Currently, a diagnostic test including seven biomarkers would be too expensive for implementation in resource-limited settings. We evaluated whether it would be possible to decrease the number of biomarkers included, without a substantial reduction in the

predictive value. The predictive value of each of the 42 cytokines was evaluated individually using nonparametric ROC analysis (Figure 2). IL-1α and IL-1β had the largest ROC areas, indicating that these cytokines had the highest predictive power. Of the cytokines that were inversely associated with the presence of an STI or BV, IP-10 had the best predictive power. IL-1α, IL-1β and IP-10 were therefore selected for further analysis. It was found that a marginally higher percentage of women were correctly classified using IL-1β (70.5%; cutoff ≥−0.37 $\log_{10}$ pg/ml) compared to IL-1α (69.6%; cutoff ≥1.96 $\log_{10}$ pg/ml) and IP-10 (63.9%; cutoff ≤1.30 $\log_{10}$ pg/ml; Supplementary Figure 1).

In order to determine whether inclusion of a second cytokine would improve the predictive value of IL-1β, a logistic regression model building procedure was used. Each of the other 41 cytokines was stepwise added to IL-1β and the predictive value of each model was compared. It was found that a model including IL-1β and IP-10 was best predictive of the presence of a bacterial STI/BV (Supplementary Figure 1), correctly classifying 75% of women with a sensitivity of 77%, specificity of 72%, PPV of 82% and NPV of 65% (Table 2). A model including a third cytokine, IL-1α, together with IL-1β and IP-10, correctly classified 76% of women (sensitivity 72%, specificity 81%, PPV 86% and and NPV 64%) and IL-1α significantly improved the fit of the model [Likelihood ratio (LR) χ^2: 14.61, p=0.0001], however this represented only a marginal improvement compared to IL-1β and IP-10 alone.

Upon evaluation of model performance by reapplication of the model to ten randomly chosen three-quarter subsets of the cohort, the relationships between IL-1β and IP-10 and the presence of a STI/BV remained statistically significant and the directionality of the relationships between the cytokines and STIs/BV remained constant, indicating that the model estimates are stable. When applied to each subset of women, the model correctly classified 72–80% of women, demonstrating that the biomarkers had similar predictive value in each resampled subset of women.

To evaluate whether the method of measuring cytokine concentrations influenced the results appreciably, the reproducibility of IL-1β and IP-10 measurements was evaluated by measuring the concentrations of these cytokines using CBA (Table 2). The predictive value of IL-1β and IP-10 was similar when these cytokines were measured using CBA (sensitivity 83%, specificity 61%, PPV 78%, NPV 69%; Table 2), compared to Luminex.

In order to determine whether the predictive value of IL-1β and IP-10 was influenced by factors other than STIs and BV that may affect genital cytokine concentrations, including age, injectable contraceptive use, yeast and seminal fluid,[24–27] these factors were each added to the model separately. It was found that inclusion of the age of participants (LR χ^2: 0.55, p=0.4601), injectable contraceptive use (LR χ^2: 0.15, p=0.6945), yeast infections (LR χ^2: 1.71, p=0.1912) or exposure to seminal plasma assessed by PCR of Y chromosome TSPY1 (LR χ^2: 2.93, p=0.0869) did not significantly influence the fit of the model.

Comparing cytokine biomarkers with clinical signs of an STI or BV

IL-1β and IP-10 biomarkers were found to predict the presence of an STI or BV with substantially better sensitivity compared to clinical signs (sensitivity 19%, specificity 92%,

PPV 79%, NPV 40%). Although addition of clinical signs to the IL-1β and IP-10 model significantly improved the fit [Likelihood ratio (LR) χ^2: 6.66, p=0.0099], this did not increase the percentage of women correctly classified [75% (sensitivity 74%, specificity 78%, PPV 86% and NPV 64%)]. It was further found that the model performed as well in asymptomatic women alone as it did in the entire cohort of both asymptomatic and symptomatic women, classifying 75% of women correctly (sensitivity 78%, specificity 70%, PPV 79%, NPV 69%).

IL-1β and IP-10 biomarkers are predictive of BV and bacterial STIs in HIV-infected women

As the HIV status of women utilizing a cytokine biomarkers test for bacterial STIs and BV may not necessarily be known, it is important that these biomarkers are also accurate in HIV-infected women, particularly since STIs and BV are prevalent in these women. Therefore, we investigated whether IL-1β and IP-10 would identify a bacterial STI or BV in HIV-infected women (median 6 weeks post-infection).[21] IL-1β and IP-10 were found to identify women with a bacterial STI or BV with similar sensitivity in HIV-infected women (80%), but improved specificity (100%), compared to HIV-uninfected women (Table 2). This indicates that IL-1β and IP-10 biomarkers are accurate predictors of bacterial STIs and BV, irrespective of the HIV status of the women being screened.

DISCUSSION

The pro-inflammatory cytokine IL-1β and the chemokine IP-10 together were found to be the two most useful immunologic biomarkers that could be used to diagnose bacterial STIs and BV in HIV-uninfected South African women. These biomarkers identified 77% of the HIV-uninfected women who had a bacterial STI or BV, while only 19% of these women were identified by clinical signs alone. Using these cytokines, 75% of the HIV-uninfected women were correctly classified as either STI/BV-positive or negative, with a sensitivity of 77%, specificity of 72%, PPV of 82% and NPV of 65%. Similarly, in a cohort of HIV-infected women, with higher rates of BV and STIs, these two biomarkers correctly identified 80% of the women who had an STI or BV (sensitivity of 80%, specificity of 100%, PPV of 100%, NPV of 54%).

The LASSO cytokine model that most accurately classified women as STI/BV-positive or negative included seven cytokines (IL-1α, IL-1β, TNF-β, IL-4, fractalkine, MDC, IFN-γ) and correctly classified 77% of HIV-uninfected women, with a sensitivity of 72%, specificity of 81%, PPV of 86% and NPV of 64%. However, as reducing this model to only two cytokines, IL-1β and IP-10, only decreased the percentage of women correctly classified by 2%, the expense of including five additional biomarkers would likely outweigh the benefit.

We thus propose that IL-1β in combination with IP-10 would be the most cost-effective inflammatory cytokine biomarkers for a rapid POC test for identification of asymptomatic bacterial STIs and BV in resource-limited settings. IL-1β upregulation predicted the presence of a bacterial STI/BV, while IP-10 was inversely associated. This is due to IL-1β being significantly upregulated in CVL from women who had BV or a bacterial STI, while IP-10 was downregulated in women who had BV.[10] IL-1β allowed for the identification of

women with high levels of genital inflammation, while IP-10 differentiated women with BV who had more moderate levels of inflammation.

As only common bacterial STIs were evaluated, women who were "falsely" classified as positive may have had inflammation caused by other bacterial infections that were not assessed by laboratory methods in this study. These women may alternatively have viral infections such as human papillomavirus or genital parasitic infections such as schistosomiasis.[28,29] In this study, none of the bacterial STI/BV-negative women had active HSV or *T. pallidum* infections, therefore these potentially inflammatory infections were not the cause of any false-positive results in this cohort. Although reactivation of HSV-2 or *T. pallidum* may contribute to false positive results in other cohorts, these infections may be accompanied by genital ulcers and condylomata lata, respectively, which can be managed syndromically [8] and using an existing *T. pallidum* rapid test. In addition to infectious agents, other factors may influence genital inflammatory responses in women. We found that age, yeast infections, semen exposure, and injectable hormone contraception, each previously shown to influence genital cytokine concentrations,[24–27] did not influence the predictive value of the inflammatory cytokine model. In future, it would also be important to evaluate the impact of douching, and the use of broad-spectrum anti-inflammatories and antibiotics.

Women who had a bacterial STI or BV, but were classified as negative using IL-1β and IP-10 biomarkers were not identified because they had low levels of genital inflammation and may have recently acquired these infections, or have resolving or less severe infections. Measurement of inflammatory cytokine biomarkers likely identifies women with severe inflammatory infections, many of whom are asymptomatic,[2] who are at the greatest risk of reproductive complications and HIV acquisition. Although we found that IL-1β and IP-10 performed well in a cohort of HIV-uninfected women and a separate cohort HIV-infected women, it would be important to evaluate these biomarkers in a cohort of both HIV-infected and uninfected women together, as HIV-infection may influence genital cytokine concentrations and HIV status of women utilizing the test may not necessarily be known.

An inexpensive biomarker rapid test could be used together with current syndromic management protocol, particularly in resource-limited settings where laboratory diagnosis is not feasible, in order to increase the number of women treated for genital infections. A reduction in the prevalence of bacterial STIs and BV may reduce the incidence of HIV infection and lead to improvements in reproductive health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY MESSAGES

- IL-1β and IP-10 concentrations in female genital secretions predicted the presence of a genital condition with 77% sensitivity, 72% specificity, 82% PPV and 65% NPV.
- These cytokine biomarkers performed substantially better than clinical signs (sensitivity 19%, specificity 92%, PPV 79% and NPV 40%).
- Supplementing syndromic management with assessment of IL-1β and IP-10 as biomarkers of genital inflammation may improve STI/BV management for women.
- This strategy may enable more effective treatment of asymptomatic infections and potentially reduce HIV incidence and reproductive complications in women.

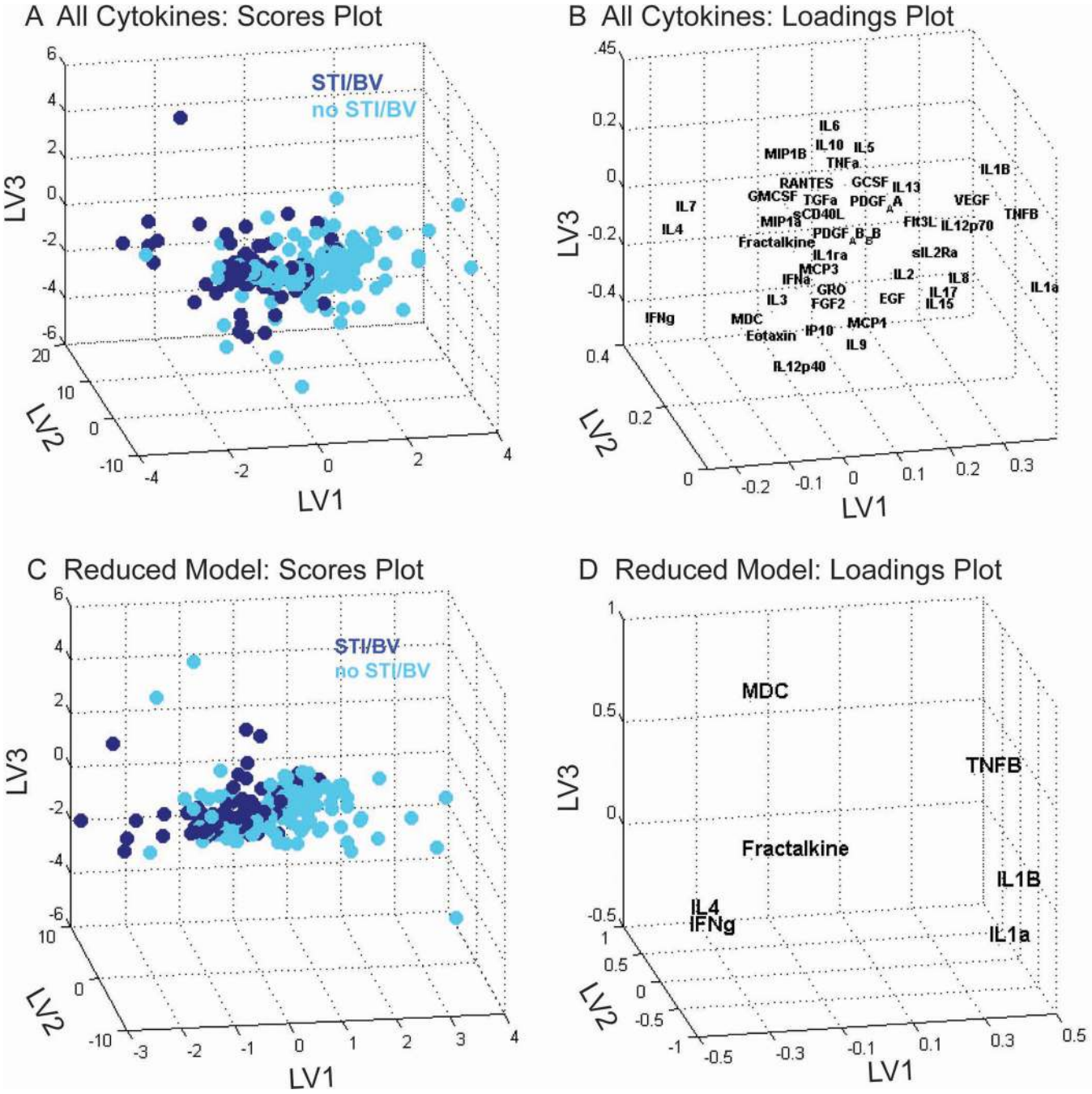

Figure 1.

Identification of multivariate cytokine profiles associated with STIs/BV. A) PLSDA model of all 42 cytokines classified individuals with 78% overall accuracy for classification, and 74% accuracy for cross-validation (light blue: women with a STI/BV; dark blue: women with no STI/BV). B) Latent variable cytokine loadings indicate multivariate cytokines associated with STIs/BV. Since individuals with a STI/BV cluster in the positive region of LV1 (A), cytokines positively loaded on LV1 (B) are elevated in STI/BV profiles, while negative loadings are comparatively reduced. C) To avoid over-fitting, the LASSO method

for feature selection was used to eliminate cytokines that didn't contribute to classification, and resulted in a profile of seven cytokines that performed with 77% classification accuracy and 75% cross-validation accuracy. D) Loadings in the reduced model indicated that the STI/BV profile consists of elevated IL-1β, IL-1α, and TNF-β, with comparatively reduced IFN-γ, IL-4, MDC, and fractalkine.

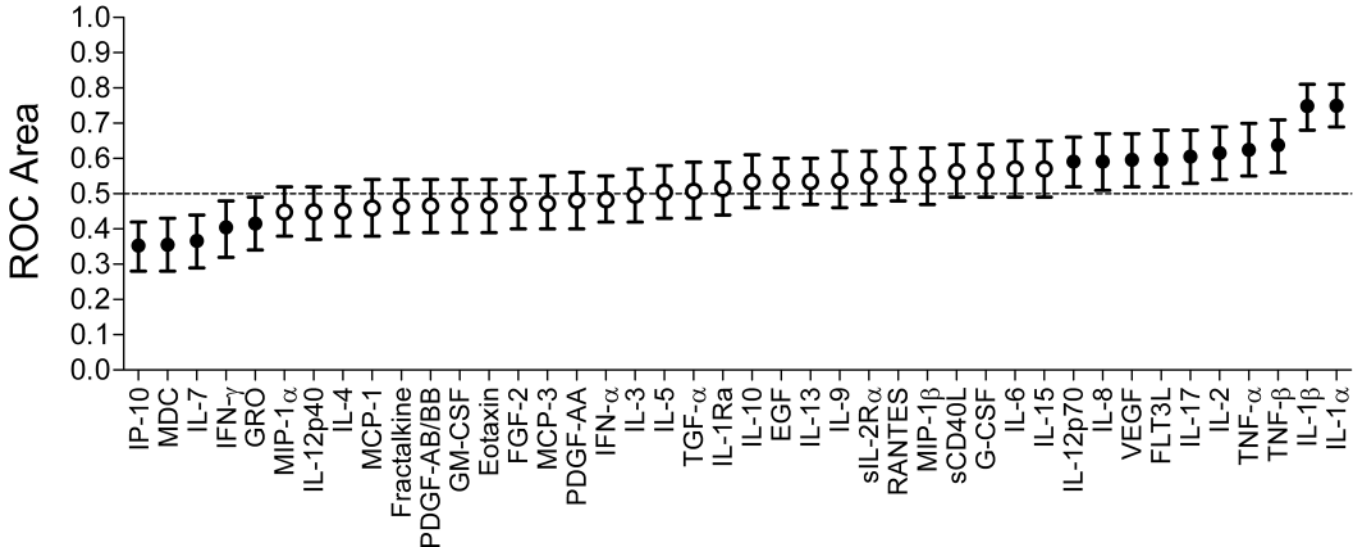

Figure 2.

Genital cytokine concentrations predict the presence of STIs and BV. Receiver Operating Characteristic (ROC) areas for each of the cytokines is shown. Cytokines with a ROC area of 0.5 have no predictive value. Cytokines with a ROC area >0.5 are positively associated with the presence of an STI or BV, whereas cytokines with a ROC area <0.5 are inversely associated. Cytokines indicated by dots were found to be significantly associated with the presence of a treatable bacterial STI or BV, whereas those indicated by circles were not significantly associated.

Table 1

Demographic and clinical characteristics of women participating in study

Demographic and clinical characteristics	**HIV-uninfected**	**HIV-infected**
Age [median (range)]	**36 (18–58)**	**24 (18–59)**
	n/total (%)	**n/total (%)**
Black African race	224/227 (98.7)	38/38 (100)
Oral contraception	5/226 (2.2)	0/38 (0)
Injectable hormone contraception	63/226 (27.9)	13/38 (34.2)
No active STI or bacterial vaginosis	86/227 (37.4)	7/38 (18.4)
Any active STI or bacterial vaginosis	141/227 (62.1)	31/38 (81.6)
Trichomonas vaginalis (PCR+)	47/227 (20.7)	4/38 (10.5)
Chlamydia trachomatis (PCR+)	10/227 (4.4)	5/38 (13.2)
Neisseria gonorrhoeae (PCR+)	13/227 (5.7)	6/38 (15.8)
Mycoplasma genitalium (PCR+)	3/227 (1.3)	4/38 (10.5)
HSV–2 IgG seropositive	198/227 (87.2)	35/38 (92.1)
HSV (PCR+)	6/227 (2.6)	2/38 (5.3)
Treponema pallidum (RPR>1:4, TPHA positive)	3/227 (1.3)	2/38 (5.3)
Bacterial vaginosis (Nugent score ≥7)	120/227 (52.9)	28/38 (73.7)
Yeast hyphae [n/total (%)]	19/173 (11.0)	ND
Cervicovaginal discharge	34/227 (15.0)	7/38 (18.4)
Genital ulceration	0/227 (0.0)	4/38 (10.5)
Y chromosome detected	42/185 (22.7)	ND

* Intact BV slides were available for 173/227 HIV-uninfected women for retrospective analysis for yeast hyphae. PCR: Polymerase Chain Reaction; ND: Not done

Table 2

Genital cytokine concentrations as biomarkers of STIs and BV in HIV-uninfected and HIV-infected women

Selection of biomarkers by partial least squares discriminant analysis (PLSDA)									
Participants	**Biomarker**	**Cytokine measurement**	**Model Classification**	**True STI*/BV diagnosis (n)**		**Sensitivity**	**Specificity**	**PPV**	**NPV**
				Pos	**Neg**	**(%)**	**(%)**	**(%)**	**(%)**
HIV−	All cytokines	Luminex	Pos	105	17	74	80	86	65
			Neg	36	68				
HIV−	IL-1α, IL-1β, TNF-β, IL-4, fractalkine, MDC, IFN-γ	Luminex	Pos	102	16	72	81	86	64
			Neg	39	69				
Selection of biomarkers by logistic regression									
Participants	**Biomarker**	**Cytokine measurement**	**Model Classification**	**True STI*/BV diagnosis (n)**		**Sensitivity**	**Specificity**	**PPV**	**NPV**
				Pos	**Neg**	**(%)**	**(%)**	**(%)**	**(%)**
HIV−	IL-1β + IP-10	Luminex	Pos	108	24	77	72	82	65
			Neg	33	62				
HIV−	IL-1α + IP-10	Luminex	Pos	100	22	71	74	82	61
			Neg	41	64				
HIV−	IL-1β + IP-10	CBA[Ŧ]	Pos	115	33	83	61	78	69
			Neg	23	51				
HIV+	IL-1β + IP-10[#]	Luminex	Pos	25	0	80	100	100	54
			Neg	5	7				
HIV−	Discharge or ulceration	N/A	Pos	27	7	19	92	79	40
			Neg	115	78				
HIV+	Discharge or ulceration	N/A	Pos	10	0	32	100	100	25
			Neg	21	7				

[*] *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis* were assessed by PCR.

[Ŧ] Cytokine concentrations were measured for a subset of 222/227 women using CBA.

[#] IP-10 data was missing for one HIV positive participant who only had sufficient sample available for a high sensitivity Luminex assay. CBA: Cytometric Bead Array; PPV: Positive predictive value; NPV: Negative predictive value