Inflammatory cytokine biomarkers to identify women with asymptomatic STIs and BV who are at high risk of HIV infection

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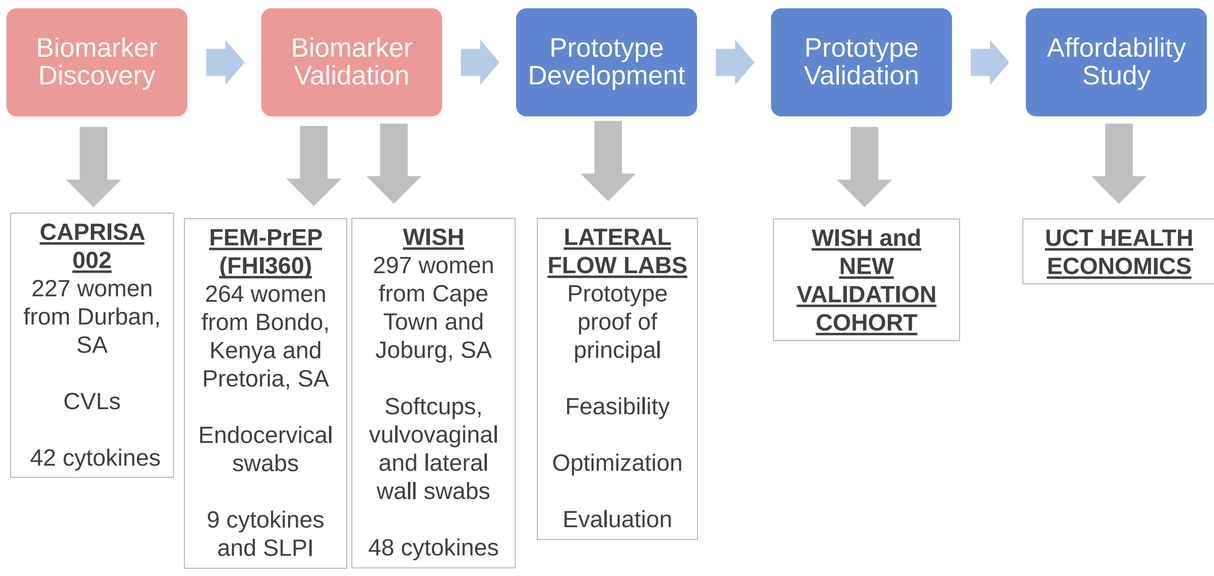
Introduction

Sexually transmitted infections (STIs) and bacterial vaginosis (BV) are associated with increased risk of HIV acquisition (Mlisana et al., 2012). An estimated **23%** of new HIV infections in women in 2010 could be attributed to curable STIs (chlamydia, gonorrhea, trichomoniasis), BV and candida (Johnson et al., 2012). In South Africa (and other resource-limited settings), women are only treated for STIs/BV if they visit a clinic with signs or symptoms (syndromic management). However, STIs are often asymptomatic in women (Wilkinson et al., 1999; Mlisana et al., 2012), with vaginal discharge evident in only 11.8% of women with a discharge-causing STI. Women with STIs who do not have clinical signs have the same level of genital inflammation as women with clinical signs which is elevated compared to STI/BV-negative women (Mlisana et al., 2012). A large proportion of women who have inflammatory infections are not being treated. Because of the relationship between STIs/BV and HIV risk (as well as reproductive complications), it is important to develop new, inexpensive strategies to better manage these conditions.

We aimed to (i) investigate whether cytokines in the female genital tract can be used to identify women with any one of several common asymptomatic STIs and BV and (ii) use cytokine biomarkers to develop an inexpensive, easy-to-use point-of-care test for STIs/BV that can be used together with syndromic management protocol to improve STI/BV management in resource-limited settings.

Study design



Results

Biomarker Discovery

CAPRISA 002 cohort:
HIV-uninfected women: 141/227 had a treatable discharge-causing STI (trichomoniasis, gonorrhoea, chlamydia, *Mycoplasma genitalium*) or BV.

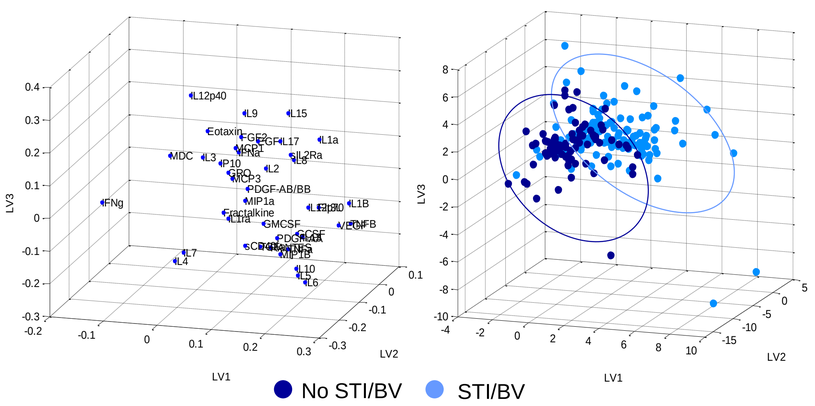


FIGURE 1
Partial Least Squares Discriminant Analysis (PLSDA) was used to distinguish between women with a STI/BV and women without an infection or BV. This approach classified women with 78% classification accuracy, and 74% cross-validation accuracy.

TABLE 1 Selection of biomarkers using logistic regression

Biomarker	HIV status	Model classification	STI/BV diagnosis (n)		Sensitivity	Specificity	PPV	NPV	Correctly classified
			Pos	Neg	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
IL-1β + IP-10	HIV negative	Pos	108	24	77 (69-83)	72 (61-81)	82 (74-88)	65 (55-75)	75 (69-80)
		Neg	33	62					
IL-1α + IP-10	HIV negative	Pos	100	22	71 (63-78)	74 (64-83)	82 (74-88)	61 (51-70)	72 (66-78)
		Neg	41	64					
IL-1α + IL-1β + IP-10	HIV negative	Pos	102	16	72 (64-80)	81 (72-89)	86 (79-92)	64 (54-73)	**76 (70-81)**
		Neg	39	70					
Cervicovaginal discharge/ulceration	HIV negative	Pos	27	7	19 (13-26)	92 (84-97)	79 (62-91)	40 (33-48)	46 (40-53)
		Neg	115	78					
IL-1α + IL-1β + IP-10	HIV positive	Pos	24	0	80 (61-92)	100 (59-100)	100 (86-100)	54 (25-81)	**84 (68-94)**
		Neg	6	7					

Semen contamination, yeast, injectable contraceptive use and age did not influence the model.

Biomarker Validation

FEM-PrEP (FHI360) cohort:
HIV-uninfected women: 165/264 had a treatable discharge-causing STI (trichomoniasis, gonorrhoea, chlamydia), BV or intermediate flora.

TABLE 2 Classification of FEM-PrEP participants using logistic regression

Biomarker	Model Classification	True STI*/BV diagnosis (n)		Sensitivity	Specificity	PPV	NPV	Correctly classified
		Pos	Neg	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
IL-1α + IL-1β+ IP-10	Pos	100	10	61 (53-68)	90 (82-95)	91 (84-96)	58 (50-66)	**72 (66-77)**
	Neg	65	89					
Clinical signs	Pos	47	22	29 (22-36)	78 (68-86)	68 (56-79)	39 (33-47)	47 (41-53)
	Neg	118	77					

Controlling for semen contamination marginally improved the model (75% correctly classified); controlling for candidiasis did not influence the accuracy.

WISH cohort:
HIV-uninfected young women (aged 16-22): 205/285 had a STI (chlamydia, *M. genitalium*, gonorrhea, trichomoniasis), BV or intermediate flora.

TABLE 3 Classification of WISH study participants using logistic regression

Biomarker	Sample	Model classification	True STI/BV diagnosis		Sensitivity	Specificity	PPV	NPV	Correctly classified
			Pos	Neg	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
IL-1α + IL-1B + IP-10	Softcup	Pos	152	18	74 (68-80)	78 (67-86)	89 (84-94)	54 (44-63)	75 (70-80)
		Neg	53	62					
	Vulvovaginal swab	Pos	152	23	76 (69-82)	71 (60-81)	87 (81-91)	54 (44-64)	75 (69-80)
		Neg	48	56					
	Lateral wall swab	Pos	154	23	78 (72-84)	70 (58-80)	87 (81-92)	55 (45-65)	**76 (70-81)**
		Neg	43	53					

The influence of factors including candidiasis, semen contamination, blood contamination, hormone contraceptive use, HSV-2, HPV, behavioural factors and residence (Cape Town or Joburg) is being investigated.

Conclusions

Across three cohorts of women residing in different regions in sub-Saharan Africa, genital IL-1α, IL-1β and IP-10 together was the best immunological predictor of the presence of an STI or BV.

Adjusting for number of sexual partners, injectable hormone contraceptive use, age, candida and semen contamination did not materially influence the accuracy of the model. Lateral wall swabs performed marginally better than other genital sample types.

Supplementing syndromic management with point-of-care assessment of cytokine 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.

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