Why STI-associated genital tract inflammation still matters in HIV transmission

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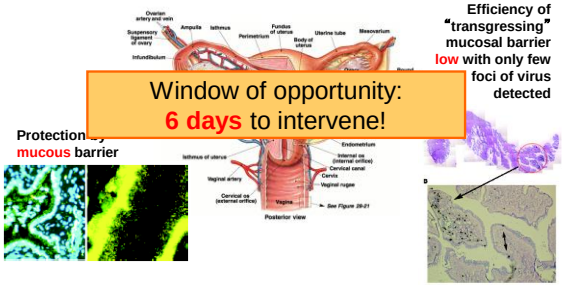
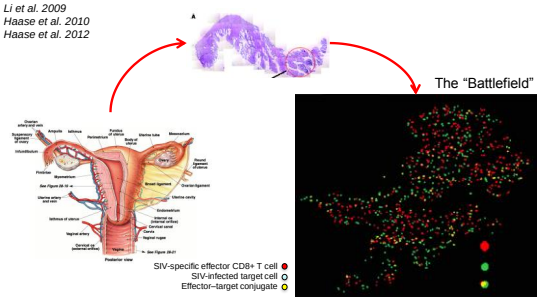
A healthy female genital mucosa presents a relatively effective barrier to HIV entry during sex

Miller et al. 2005



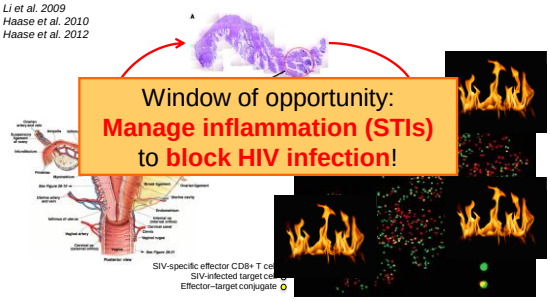
How does genital tract inflammation place women at higher risk for HIV infection?

Li et al. 2009
Haase et al. 2010
Haase et al. 2012



The battlefield is fuelled by inflammation allowing spread of local genital tract infections to draining lymph nodes and then, systemically

Li et al. 2009
Haase et al. 2010
Haase et al. 2012



Part 1

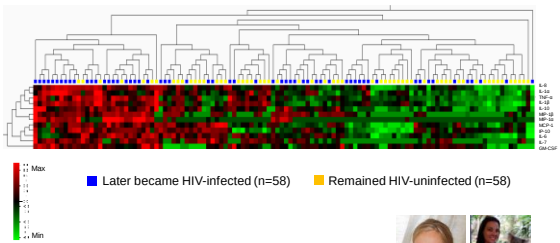
Women with **genital tract inflammation** (cytokines) prior to HIV infection were at increased risk for acquiring HIV

Genital Inflammation and the Risk of HIV Acquisition in Women

Lindi Masson,[1,2,a] Jo-Ann S. Passmore,[1,2,3,a] Lenine J. Liebenberg,[1,a] Lise Werner,[1] Cheryl Baxter,[1] Kelly B. Arnold,[4] Carolyn Williamson,[1,2] Fenzencea Little,[1] Leila E. Mansoor,[1] Vivek Nanmbhai,[1] Douglas A. Lauffenburger,[4] Katharina Ronacher,[5] Gerhard Walzl,[5] Nigel J. Garrett,[1] Brent L. Williams,[1] Mara Couto-Rodriguez,[1] Mady Hornig,[1] W. Ian Lipkin,[1] Anneke Grobler,[1] Guarraisha Abdool Karim,[1,3] and Salim S. Abdool Karim[1,3]

CID 2015 ePub 10.1093/cid/civ298

Women who later became HIV-infected had pre-infection genital inflammation



Dr Lindi Masson (UCT), Dr Lenine Liebenberg (CAPRISA)

Genital tract chemokines (MIP-1α, MIP-1β, IP-10, IL-8) were associated with HIV acquisition

Cytokine	HIV Negative Women (n=58)	HIV Seroconvertors (N=58)	Pair-Matched Odds Ratio (95% CI)	P-value
	% detectable (n)	% detectable (n)	Per detection of cytokine	
MIP-1β	43·1% (25)	75·9% (44)	3·17 (1·49 - 6·77)	0·003*
MIP-1α	15·5% (9)	44·8% (26)	3·09 (1·38 - 6·92)	0·006*
GM-CSF	75·9% (44)	89·7% (52)	2·64 (0·87 – 8·05)	0·086
IL-7	58·6% (34)	60·3% (35)	1·06 (0·52 - 2·15)	0·874
IL-1α	1·97 (1·01 - 2·44)	2·22 (1·71 - 2·74)	1·65 (0·96 – 2·83)	0·067
MCP-1	1·31 (0·82 - 1·68)	1·43 (0·94 - 1·89)	1·47 (0·85 - 2·54)	0·164
IL-10	-0·37 (-1·70 - 0·33)	-0·14 (-0·89 - 0·55)	1·21 (0·91 - 1·60)	0·197
IL-6	0·79 (0·25 - 1·29)	0·87 (0·50 - 1·41)	1·23 (0·75 – 2·01)	0·419
IL-1β	0·27 (-0·42 - 1·02)	0·52 (-0·32 - 1·27)	1·22 (0·86 - 1·73)	0·265
TNF-α	-0·68 (-1·10 - 0·01)	-0·62 (-1·05 - 0·31)	1·16 (0·76 - 1·77)	0·498

Genital inflammation in individual women was relatively constant over time

Genital inflammation predicted HIV acquisition with an Odds Ratio of 3.2

	HIV+	HIV-	Total
Genital inflammation present*	**19**	**6**	25
Genital inflammation absent	**39**	**52**	91
Total	58	58	116

OR (95% CI) **3.2 (1·3 – 7.9)**
p-value 0·014

*Women with 5 or more **pro-inflammatory cytokines or chemokines** (MIP-1a, MIP-1b, IL-8, IP-10, TNF-a, MCP-1, IL-6, IL-1a, IL-1b) above the 75th percentile
Significant after adjusting for age, urban/rural, condom use, hormonal contraceptives, number of sex acts, number of returned used applicators, HSV-2 status

Proteomics data from CAPRISA004 CVLs is providing further confirmation that genital inflammation and barrier repair predict HIV infection risk in the trial *Lyle McKinnon (and Adam Burgener)*

Dr Lyle McKinnon, CAPRISA – **S14.3 11:30-11:45am** Rm M3 Mezzanine

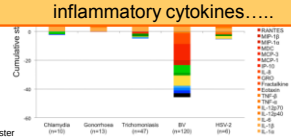
Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: a cross-sectional study

Lindi Masson,[1,2] Koleka Mlisana,[2,3,4] Francesca Little,[5] Lise Werner,[2] Nonhlanhla N Mkhize,[1,6] Katharina Ronacher,[7] Hoyam Gamieldien,[1] Carolyn Williamson,[1,2] Lyle R Mckinnon,[2] Gerhard Walzl,[7] Quarraisha Abdool Karim,[2,8] Salim S Abdool Karim,[2,8] Jo-Ann S Passmore[1,2,4]

STI 2014, 90 (8), 580-587

In the CAPRISA002 cohort, women infected with chlamydia, gonorrhoea or trichomonas had elevated genital tract pro-inflammatory cytokines…..

Dr Lindi Masson (UCT) - Poster



Potential causes of genital inflammation in CAPRISA004

Demographic and behavioral characteristics	Genital inflammation present (n=25)	Genital inflammation absent (n=91)	p-value
	% (n)	% (n)	
Age in years [median(IQR)]	22 (20-24)	22 (20-25)	0.40
Assigned to use tenofovir gel	36.0 (9)	40.7 (37)	0.82
Women who reside in a rural setting	64.0 (16)	69.2 (63)	0.63
Completed high school	44.0 (11)	40.7 (37)	0.82
Given birth previously	72.0 (18)	80.2 (73)	0.41
Married	8.0 (2)	4.4 (4)	0.61
Stable partner	92.0 (23)	92·3 (84)	1.00
Reported sexual intercourse per month [median(IQR)]	5 (4-6)	4 (3-7)	0.54
Number of sexual partners in lifetime [median(IQR)]	2 (2-3)	2 (1-3)	0.52
Reported always using a condom during sex	48·0 (12)	26.4 (24)	0.05
HSV-2 status during study†			
Baseline positive	56.0 (14)	58.9 (53)	0.50
Acquired new infection	20.0 (5)	11·1 (10)	
Remained negative	24.0 (6)	30.0 (27)	
Clinical signs of an STI	24·0 (6)	13·3 (12)	0.23
Genital discharge at sampled visit			
Genital ulcer at sampled visit	0·0 (0)	2·2 (2)	1.00
Contraceptive choice at sampled visit			
Injectable (Depo-Provera or Nur-Isterate)	80.0 (20)	82.4 (75)	0.73
Oral contraceptive pill	20.0 (5)	15·4 (14)	
Tubal ligation/hysterectomy	0·0 (0)	2·2 (2)	
Intravaginal insertions within 30 days of sampled visit	4.0 (1)	4.4 (4)	1.00
Intravaginal insertions at any point during trial	20.0 (5)	17.6 (16)	0.77

Potential causes of genital inflammation in CAPRISA004

STI (by PCR)	Genital inflammation present (n=20)	Genital inflammation absent (n=68)	P-value
	% (n)	% (n)	
Trichomonas vaginalis	**40.0 (8)**	**20.6 (14)**	0.09
Chlamydia trachomatis	20.0 (4)	16.2 (11)	0.74
Neisseria gonorrhoeae	5.0 (1)	4.4 (3)	1.00
HSV-2	5.0 (1)	2.9 (2)	0.54
Any one of the above STIs	50.0 (10)	33.8 (23)	0.20

Only 20% of HIV infections could be attributed to (or were a result of) an STI

T. vaginalis was the most strongly predictive of genital inflammation

Microbiome analysis ongoing

Brent Williams
Ian Lipkin

Overlap between **genital** versus **plasma** cytokine signatures to predict HIV infection in CAPRISA004

Women with genital inflammation did not have similarly elevated plasma cytokine concentrations

Plasma cytokine concentrations (pg/ml)	Genital inflammation present (n=23) Median (IQR)	Genital inflammation absent (n=83) Median (IQR)	P-value
IL-1β	0.07 (0.01-0.23)	0.03 (0.01-0.14)	0.27
IL-1α	0.68 (0.68-0.68)	0.68 (0.68-0.68)	0.35
IL-6	0.86 (0.49-1.66)	0.73 (0.19-1.59)	0.42
IL-7	0.01 (0.01-0.08)	0.01 (0.01-0.31)	0.91
IL-8	1.73 (0.01-3.76)	1.87 (0.01-3.55)	0.90
IL-10	0.24 (0.24-8.12)	0.24 (0.24-5.88)	0.78
GM-CSF	0.89 (0.52-1.93)	0.86 (0.41-1.67)	0.51
TNF-α	3.72 (2.40-5.92)	3.62 (2.06-5.47)	0.73
IP-10	147.04 (99.85-246.13)	168.90 (114.26-253.56)	0.44
MCP-1	151.49 (122.68-218.63)	135.81 (86.01-201.64)	0.41
MIP-1β	15.63 (11.79-27.67)	24.69 (16.31-31.23)	0.20
MIP-1α	4.22 (0.21-8.40)	1.96 (0.21-5.31)	0.13

*Dr Lenine Liebenberg, CAPRISA; **S17.2 2.05-2.25pm** M1-2, Mezzanine*

No correlation between genital tract and plasma cytokines

Plasma cytokine concentrations (pg/ml)	Spearman Rho	*p*-value
IL-1β	-0.0084	0.93
IL-1α	0.1573	0.10
IL-6	0.0160	0.87
IL-7	-0.0717	0.46
IL-8	0.0954	0.33
IL-10	0.1272	0.19
GM-CSF	0.1020	0.29
TNF-α	0.0372	0.70
IP-10	-0.0969	0.32
MCP-1	0.0366	0.71
MIP-1β	0.0610	0.53
MIP-1α	0.0260	0.79

South African Press coverage about genital inflammation and HIV risk in women….

Lindi Masson handling some very awkward questions about genital inflammation from the South African public in interviews on several radio news shows in June….

Discussion (Part 1)

- **Women who had genital inflammation were at increased risk of HIV infection**
- Elevated concentrations of 4 chemokines (MIP-1α, MIP-1β, IP-10 and IL-8) were associated with increased risk of HIV infection
- These chemokines are likely to recruit potential HIV target cells
- MIP-1β, in particular, bind to the HIV co-receptor CCR5 and specifically recruit CCR5+ HIV target cells that potentially enhance HIV infection.
- In macaques, production of these and other inflammatory cytokines has been shown to be essential for recruitment of CD4+ T cell targets needed for SIV replication

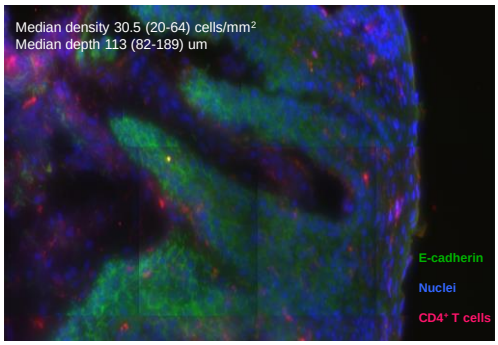
Part 2

Women who acquired HIV during CAPRISA004 had high frequencies of **CD68+ and CD4+ target cells** within the **stratified squamous epithelium** (from vaginal biopsies)

in collaboration with Prof Thomas Hope and Dr Ann Carias
Northwestern University, Chicago

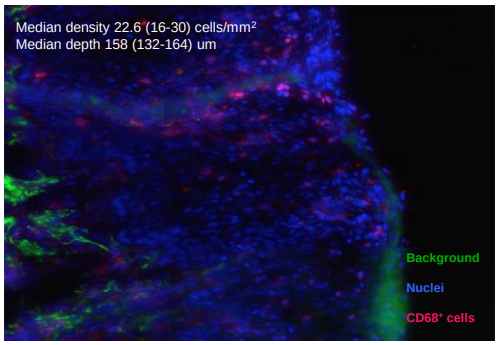
Sinaye Ngcapu, CAPRISA
Lenine Liebenberg, CAPRISA

CD4 target cell density in CAPRISA004 vaginal biopsies



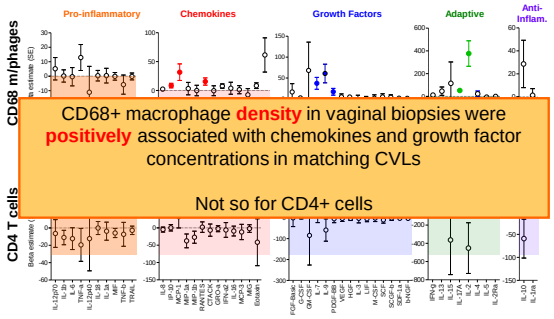
Tom Hope, Ann Carias (Northwestern)
Sinaye Ngcapu (CAPRISA)

CD68 cell density in CAPRISA004 vaginal biopsies



Tom Hope, Ann Carias (Northwestern)
Sinaye Ngcapu (CAPRISA)

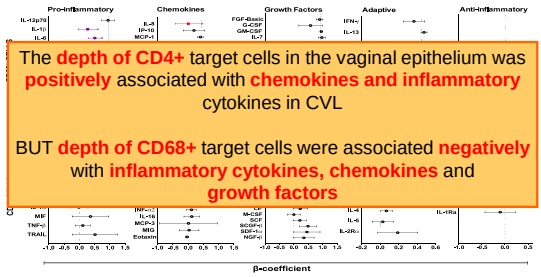
Relationship between target cell density in biopsies and genital tract (CVL) cytokines



CD68+ macrophage **density** in vaginal biopsies were **positively** associated with chemokines and growth factor concentrations in matching CVLs

Not so for CD4+ cells

Relationship between target cell depth in biopsies and genital tract (CVL) cytokines

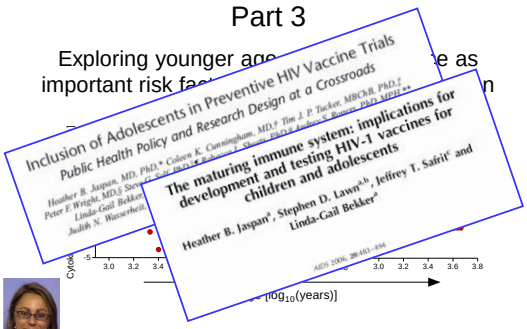


The **depth of CD4+** target cells in the vaginal epithelium was **positively** associated with **chemokines and inflammatory** cytokines in CVL

BUT **depth of CD68+** target cells were associated **negatively** with **inflammatory cytokines, chemokines** and **growth factors**

Part 3

Exploring younger age… as important risk fa… n



Heather Jaspan (UCT, UW)
Linda-Gail Bekker (DTHF)

Median age 23 (range 18 - 39)

CAPRISA

EDCTP Mucosal Primer

The WISH study
(Women's Initiative in Sexual Health)

Adolescent genital immune activation and inflammation

Study in 300 adolescent females (16-22 year olds) from Masipumelele, Cape Town (DTHF Youth Centre) and Soweto, Johannesburg (PHRU)

EDCTP

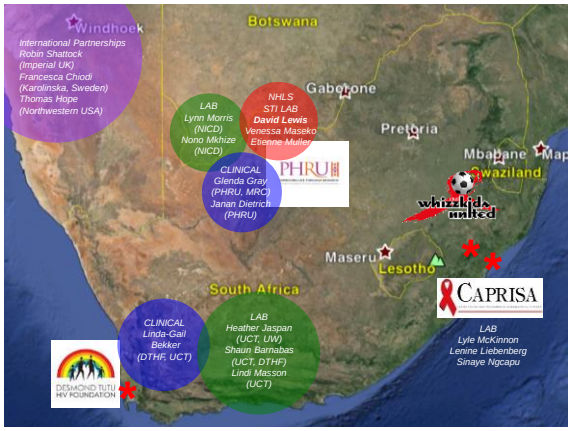
Wish

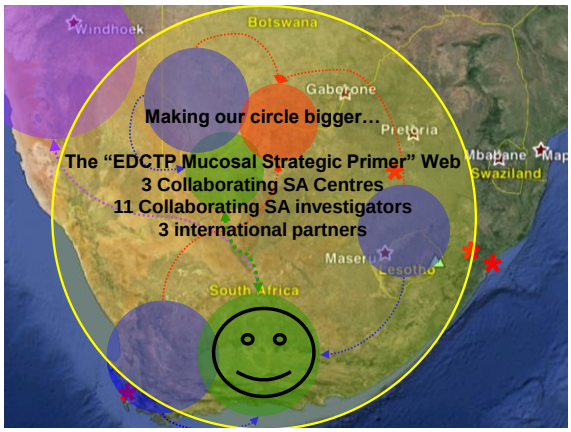
EDCTP Mucosal Primer

The WISH study
(Women's Initiative in Sexual Health)

- Cytokines (luminex, 48, Biorad)
- T cell activation (cytobrush CD38, HLA-DR, CCR5, Ki67 on CD4+ and CD8+ by FACS, *ex vivo*)
- Microbiome (16S)
- Culture

*Dr Shaun Barnabas (DTHF, UCT) – **Oral presentation O18.3 11:45-12:00, Door 8 Mezzanine***
Ms Smritee Dabee (UCT) – Poster P15.04; Katie Viljoen (UCT); Heather Jaspan (UCT, SHC)



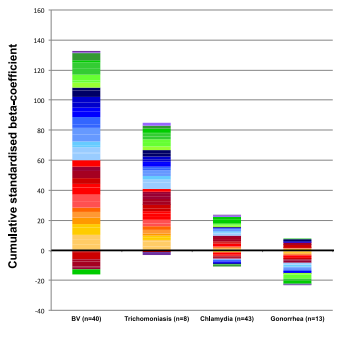


Prevalence of STIs (particularly CT) and BV higher in adolescent women in Cape Town compared to Johannesburg, South Africa

	Masi Cape Town	Soweto Johannesburg	P-value
Chlamydia trachomatis	**41.6%** (62/149)	**15% (15/100)**	**<0.0001**
Trichomonas vaginalis	**7.4%** (11/149)	4% (4/100)	0.2507
Neisseria gonorrhoea	**11.4%** (17/149)	4% (4/100)	0.0140
HSV-2	**4.7%** (7/149)	0% (0/100)	0.0289
Mycoplasma genitalium	**4.0%** (6/149)	2% (2/100)	1.0000
Bacterial Vaginosis (Nugent >7)	**48.0%** (71/148)	31% (31/100)	0.0793
Total	150/150	100/150	

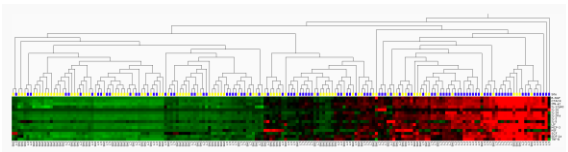
*Dr Shaun Barnabas (DTHF, UCT) – **Oral presentation O18.3 11:45-12:00, Door 8 Mezzanine***

Concentrations of cytokines in mucosal softcup secretions



Ms Smritee Dabee (UCT) – Poster P15.04

Comparing the genital cytokines between Cape Town and Johannesburg



Cape Town Johannesburg

Unsupervised hierarchical clustering was used to visualize the variation in genital tract cytokine concentrations in individual women and to cluster women across 2 sites in South Africa according to the similarities of their cytokine expression profiles (using Qlucore Omics Explorer).

b-NGF, CTACK, IFN-a2, IL-12p40, IL-16, IL-18, IL-2Ra, IL-3, IL-5, LIF, MCP-3, MIF, SCF, SDF-1a, TNF-b

Comparing the **vaginal microbiome** between Cape Town and Johannesburg in STI/BV negative adolescents

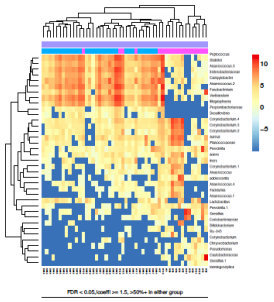
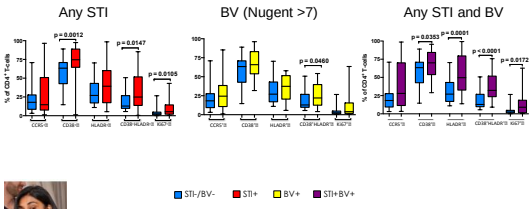


Fig 4: Differentially abundant OTUs between Masiphumelele and Soweto in women with Nugent scores<4 with adjusted p values of <0.05 by FDR.

Dr Katie Viljoen (UCT)

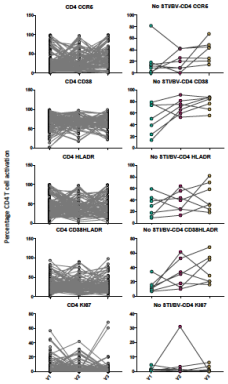
Having an **STI or BV** (Nugent >7) increases the frequency of activated CD4 T cells in the female genital tract (cytobrush)



Ms Smritee Dabee (UCT) – Poster P15.04

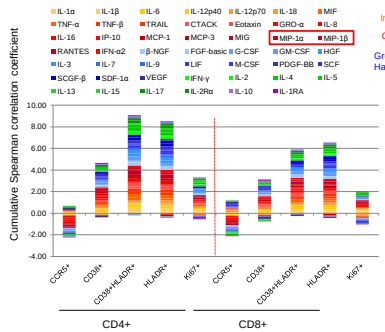
Longitudinal cytobrush CD4 T cell activation

T cell activation did not differ significantly between time points (spanning 4 months)
T cell activation did not correlate significantly between visits



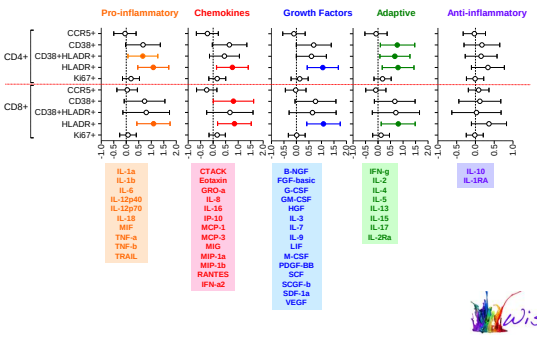
Smritee Dabee (UCT)

Relationship between genital tract cytokines and cervical T cell activation

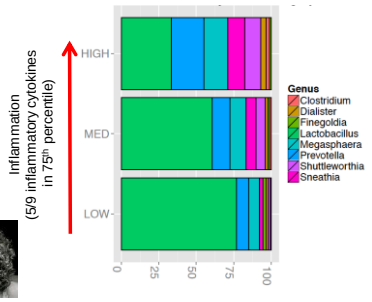


Ms Smritee Dabee (UCT) – Poster P15.04

Relationship between genital tract cytokines and cervical T cell activation



Vaginal microbiome according to genital inflammatory cytokine score



Dr Katie Viljoen (UCT)

Discussion (Part 2)

- A collaborating network of South African clinical and laboratory investigators has been established, with specific expertise in mucosal assessment for conducting HIV prevention research

Discussion (Part 2)

- A collaborating network of South African clinical and laboratory investigators has been established, with specific expertise in mucosal assessment for conducting HIV prevention research
- **Young women, particularly in Cape Town, had unacceptably high rates of asymptomatic STIs and BV, calling for an urgent re-evaluation of how appropriate our current STI surveillance and testing guidelines are for sub-Saharan Africa**
- Both having an STI and/or BV was associated with significant increases in both inflammatory cytokines and chemokines in genital secretions and frequencies of activated CD4+ HIV target cells at the cervix
- **This study provides an important link between genital cytokine markers of inflammation and cellular activation**

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Kelly Arnold

Imperial College London:
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