THE PERFECT STORM
Phylogenetics, age disparity, vaginal microbiome, and HIV risk in young women

11th International Workshop on HIV Treatment, Pathogenesis, and Prevention Research in Resource-Limited Settings (INTEREST Workshop)
Lilongwe, Malawi
17 May 2017
Session: HIV risk, prevention, and young African women in 2017

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Disclosure

I have no potential conflict of interest to report
Overview of Presentation

- Research priorities, studies and rationale
- Overview of new findings leading to “hyper-vulnerability”
  - “Mounting clouds”
    - Phylogenetic studies / HIV linked sequences / Sexual Network
  - “Threatening clouds”
    - Genital inflammation / Vaginal Microbiome
  - “Unforgiving cloudburst”
    - Vaginal microbiome undermining PrEp effectiveness in women
To undertake *globally relevant & locally responsive* research that contributes to understanding HIV pathogenesis, prevention & epidemiology as well as TB-HIV treatment.

Understand the epidemiology of HIV in young women.
HIV in South Africa
Disproportionate burden of HIV in young women

HIV infection and tuberculosis in South Africa: an urgent need to escalate the public health response

Salim S Abdool Karim, Gavin J Churchyard, Quarraisha Abdool Karim, Stephen D Lawn

Southern & eastern Africa have 70% of the world’s HIV burden


Young women have up to 8 times more HIV than men
Trends in HIV in pregnant women in rural KZN 2001-2013

Key findings

>30% of pregnant women <20 year age group
20% already HIV positive

Temporal trends in HIV prevalence consistently high between 30% and 40%

By age group

1 in 10 <16 year olds HIV positive
1 in 5 17-18 year olds HIV positive
1 in 2 by age 23 were HIV positive

Partner 4 years and older were 4 to 8 times more likely to be HIV positive.

HIV incidence: 11.2% per year

Trends in HIV in high school students in rural KZN

- **Key findings**

  1. In 4 learners were sexually active

  HIV consistently high across all ages groups higher burden in young girls compared to young boys.

  Young girls with older sex partners 3 to 4 times more likely to be HIV positive

  No obvious HIV transmission within schools

  Some evidence for spread between schools Possible introductions from community

Source:
Young women at high HIV risk: Who? Why? What works?

Who - source of infection? Why so vulnerable? What works for prevention?

Young women at high HIV risk
Challenges to understanding HIV acquisition

- Low sexual frequency but high risk sex
- Few have multiple concurrent partners
- Age disparate relationships – conflicting evidence
- Some indication – biased study design, reliability of self reported data

- Application of HIV Phylogenetic analyses
  - HIV linked sequences helpful to understand HIV transmission dynamics (used in medico-legal cases, outbreaks and communities)
Phylogenetics: Understanding HIV transmission dynamics

Lead: Ayesha Kharsany

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Tiago Gräf
Frank Tanser

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Sincere thanks to all the households and individual study participants, traditional and municipal leadership, field, office, laboratory and clinic staff
Community Based Surveillance

- Duration: 2014 - 2015
- Cross-sectional multi-stage random sampling of 15-49 year old
- 86% consent rate
- People tested for HIV: 9,812
- Viral load >1000: 47.1% (n=1,847)
- Phylogenetic linkage studies
  - Viruses sequenced (n=1,589)
  - 1700 bp pol gene sequences
  - Maximum likelihood tree
    Branch support > 90%
    Genetic diversity < 4.5%

Community findings

- HIV prevalence
  - HIV positive: 36.3% (CI: 35-38) (n=3,969)
  - Knew HIV+ status: 59.8% (n=2,337)
  - On ARVs: 42.3% (n= 1,590)
  - VL>50 000 copies/mL (7% males, 6% females)

- Phylogenetic linkages
  - 469 sequences - phylogenetically linked 202 individuals
  - 168 with 2 individuals
    - 22 with 3 individuals
    - 8 with 4 individuals
    - 1 with 5 individuals
    - 2 with 6 individuals
    - 1 with 18 individuals (large cluster)
"Mounting Clouds"
Phylogenetic linkages

90 clusters (Male-Female / heterosexual linkages) – 123 females linked to 103 males

<table>
<thead>
<tr>
<th>Community HIV prevalence</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>33</td>
<td>119</td>
</tr>
<tr>
<td>25-40 yrs (59.8%)</td>
<td>18</td>
<td>37</td>
</tr>
<tr>
<td>40-49 yrs (50.1%)</td>
<td>13</td>
<td>58</td>
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</table>

<table>
<thead>
<tr>
<th>Females age group</th>
<th>Median VL (c/ml)</th>
<th>on ARVs</th>
<th>Known HIV+</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25 yrs (7.6%)</td>
<td>16,629</td>
<td>4.1%</td>
<td>38.9%</td>
</tr>
<tr>
<td>25-40 yrs (40.3%)</td>
<td>31,000</td>
<td>4.9%</td>
<td>26.2%</td>
</tr>
<tr>
<td>40-49 yrs (47.2%)</td>
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<table>
<thead>
<tr>
<th>Age difference with male partners</th>
<th>Females</th>
<th>Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25: 8.7 yrs</td>
<td>11.5 yrs</td>
<td>&lt; 25:</td>
</tr>
<tr>
<td>21-25</td>
<td>7.0 yrs</td>
<td></td>
</tr>
<tr>
<td>26-30</td>
<td>1.5 yrs</td>
<td>≥ 25:</td>
</tr>
<tr>
<td>31-35</td>
<td>1.7 yrs</td>
<td></td>
</tr>
<tr>
<td>36-40</td>
<td>0.7 yrs</td>
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</tbody>
</table>
Cycle of HIV transmission

Schematic of sexual networks from clusters with heterosexual transmission

**Men 25-40 years (N=79)**
- Knew HIV status: 21.5%
- VL > 50,000: 37.1%

**Community HIV prevalence: 40.3%**

**Young women <25 years (N=43)**
- Knew HIV status: 23.3%
- 62% of male partners are 25-40 years

**Community HIV prevalence: 22.3%**

Most young women <25 years acquire HIV from older men (Mean age difference = 8.7 years)

39% of the men linked to a woman <25 are simultaneously also linked to a woman 25-40 years

**Women 25-40 years (N=56)**
- Knew HIV status: 42.6%
- 63% of male partners are 25-40 years

**Community HIV prevalence: 59.8%**

Most men & women 25-40 years acquire HIV from similarly aged partners (Mean age difference = 1.1 years)

When young women reach >25 years they continue the cycle

Genital inflammation and vaginal microbiome
Lead: Jo-Anne Passmore
Women who later became HIV-infected had pre-infection genital inflammation – what is the cause?

Only 20% of HIV infections could be attributed to an STI

*T. vaginalis* was the most strongly predictive of genital inflammation

Association between genital inflammation and HIV acquisition

<table>
<thead>
<tr>
<th></th>
<th>HIV+</th>
<th>HIV-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genital inflammation present*</td>
<td>19</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Genital inflammation absent</td>
<td>39</td>
<td>52</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>58</td>
<td>116</td>
</tr>
</tbody>
</table>

Odds Ratio 3.2  (95% CI: 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
Vaginal Microbiome

16s DNA sequencing of genital swab samples

Over 3 million sequences
1368 species identified

- Predominance of Cluster Community State Type (CST) 4 related organisms

- **Prevotella bivia**

- Linked with
  - genital inflammation
  - HIV acquisition
**Prevotella bivia** is strongly associated with genital inflammation and HIV acquisition

<table>
<thead>
<tr>
<th></th>
<th>P. bivia+ OR*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td>19.2</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>(95% CI: 4.0-92.4)</td>
<td></td>
</tr>
<tr>
<td>HIV+</td>
<td>12.7</td>
<td>p=0.006</td>
</tr>
<tr>
<td></td>
<td>(95% CI: 2.1-77.8)</td>
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*adjusted odds ratio

22 women were HIV positive & had inflammation – 9/22 (41%) had **P. bivia**

Women with **P. bivia** were **19 times** more likely to have genital inflammation and **13 times** more likely to acquire HIV
Potential mechanism: $\uparrow P. bivia \Rightarrow \uparrow$ potent LPS

Women with microbiome cluster (CST4) and women heavily populated with $P. bivia$ have significant enrichment of Lipopolysaccharide (LPS)
“Threatening Clouds”

Summary

- Increased vulnerability
  - Genital inflammation

- *Prevotella bivia* was associated with genital inflammation leading to increased HIV vulnerability

- Potent LPS response - potential mechanism of action
Vaginal microbiome undermining PrEp effectiveness in women

Lead: Adam Burgener
Effectiveness of Tenofovir / Truvada antiretroviral pills & gels in women

Effect size (CI)
- TDF2 - daily Tenofovir-Emtricitabine (Women & Men - Botswana)
  - Effect size: 75%* (24; 94)
- Partners PrEP - daily oral Tenofovir (Discordant couples – Kenya, Uganda)
  - Effect size: 71%* (37; 87)
- Partners PrEP - daily Tenofovir-Emtricitabine (Discordant couples – Kenya, Uganda)
  - Effect size: 66%* (28; 84)
  - Effect size: 6% (-52; 41)
- MTN003/VOICE - daily Tenofovir-Emtricitabine (Women – South Africa, Uganda, Zimbabwe)
  - Effect size: -4% (-49; 27)
- MTN003/VOICE - daily Tenofovir (Women - South Africa, Uganda, Zimbabwe)
  - Effect size: -49% (-129; 3)
- CAPRISA 004 – coital Tenofovir gel (Women – South Africa)
  - Effect size: 39% (6; 60)
- MTN003/VOICE – daily Tenofovir gel (Women – South Africa, Uganda, Zimbabwe)
  - Effect size: 15% (-21; 40)
- FACTS 001 – coital Tenofovir gel (Women – South Africa)
  - Effect size: 0% (-40, 30)

Varying outcomes from PrEP trials - attributed to adherence

What biological factors affect PrEP?

?? Why

?? Role of vaginal dysbiosis

*(Study population and countries where the study was conducted)
*Effect size calculated from the incidence rate ratio for women only
Metaproteomic analysis of bacteria

Method
- CVL samples processed using Tandem mass spectrometry
- Identifies Major Phyla
- 188 species (3,334 unique proteins)

Mass spectrometry based proteomics

Method:
- CVL samples processed using Tandem mass spectrometry
- Identifies Major Phyla
- 188 species (3,334 unique proteins)
Identification of Vaginal microbial groups

G. vaginalis dominant

Lactobacillus dominant

Community groups

Community groups

Proportion

Shannon H

<50% Lactobacillus

>50% Lactobacillus

L. crispatus (13.8%)

L. iners (63.4%)

Gardnerella vaginalis (59.0%)

Lactobacillus (7.1%)

Mobiluncus (5.7%)

Prevotella (9.0%)

Pseudomonas (5.5%)

Lactobacillus (94%)

Lactobacillus spp. (15.8%)

Vaginal pH

% Lactobacillus

P=0.001

5.4 4.0

n=423

n=265

(61%) (61%)

(39%)
Metaproteomic analysis of bacteria

688 Cervicovaginal lavage samples

Mass spectrometry based proteomics

Bacterial peptide library

Compares well with 16s rRNA sequencing

Identifies major phyla
188 species (3,334 unique proteins)

Mass spectrometry based proteomics

Bacterial peptide library

Compares well with 16s rRNA sequencing

688 Cervicovaginal lavage samples
Effectiveness of Tenofovir gel against HIV amongst women with Lactobacillus dominance

**Lactobacillus dominant**

Effectiveness: 61%  95%
CI: 11 to  84%

Effectiveness: 18%
95% CI: -77 to  63%

**Non- Lactobacillus dominant**

HR = 0.39 (95% CI: 0.20; 0.83)
P = 0.013

HR = 0.82 (95% CI: 0.40; 1.65)
P = 0.644
**Tenofovir highly protective with *Lactobacillus* dominance**

60% of *Lactobacillus* dominant women compared to 61.4% of non-*Lactobacillus* dominant women had >50% gel adherence (monthly empty applicator returns)

Baseline characteristics, sexual behaviour and gel adherence across microbial groups and gel assignment

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>Lactobacilli dominant</th>
<th>non-Lactobacilli dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td># HIV-1 infections</td>
<td>Tenofovir Placebo</td>
<td>Tenofovir Placebo</td>
<td>Tenofovir Placebo</td>
</tr>
<tr>
<td>HIV-1 incidence per /100 py</td>
<td>13 26</td>
<td>4 15</td>
<td>9 11</td>
</tr>
<tr>
<td>HIV-1 protection effectiveness</td>
<td>3.7 8.6</td>
<td>1.9 8.5</td>
<td>6.4 8.6</td>
</tr>
<tr>
<td>95% CI, P-value</td>
<td>56% (12, 79), p=0.013</td>
<td>78% (29, 95), p=0.003</td>
<td>26% (-98, 73), p=0.558</td>
</tr>
</tbody>
</table>
Potential mechanism: lack of tenofovir efficacy in non-Lactobacillus women

Tenofovir has metabolizable phosphate group

Hypothesis: that the drug may be biodegraded by bacteria


To assess bio-degradation of tenofovir by bacteria

1) Inoculate NYClIII medium with or without TFV, and abiotic controls

$L. \text{ iners (x15)}$  $G. \text{ vaginalis (x15)}$  Abiotic (x9)

+TFV  -TFV  +TFV  -TFV  +TFV

2) Sample. Separate cells from culture supernatant by centrifugation. Extract TFV into acetonitrile and analyze on MS

(Supe)  MS

3) Track biomass growth and intracellular TFV levels

Culture sample  Acetonitrile  Intensity

△ = Tenofovir
Tenofovir rapidly depleted by *Gardnerella* but not by *Lactobacillus*.

**Tenofovir (supernatant)**

- **Abiotic**
- **L. iners**
- **G. vaginalis**

4 hours:
- G. vag vs. L. iners: $P=0.002$
- G. vag vs Abiotic: $P=0.005$

24 hours:
- G. vag vs. L. iners: $P<0.001$
- G. vag vs Abiotic: $P<0.001$

**Tenofovir (intracellular)**

- **L. iners**
- **G. vaginalis**

4 hours:
- G. vag vs. L. iners: $P<0.001$

24 hours:
- G. vag vs. L. iners: $P<0.001$

Tenofovir conc declined by 50.6% within 4 hrs.
“Unforgiving cloudburst”

Summary

- Signatures associated vaginal PrEP effectiveness
  - Tenofovir gel effective - 3-fold higher (>78%) in women with vaginal *Lactobacillus* dominance
  
  - Tenofovir gel not effective (26%) in women with non-*Lactobacillus* communities containing *G. vaginalis*

- Tenofovir is rapidly depleted by *G. vaginalis* but not by *L. iners*

- Importance of vaginal health for PrEP effectiveness against HIV in women

- Considerations for other trials, bacteria undermining PrEP effectiveness and possible other delivery routes
Implications of the new evidence

Why such high risk of HIV acquisition following exposure?
- Genital inflammation
- LPS released by *Prevotella bivia*

Why such high variability in the efficacy of PrEP?
- Tenofovir absorption by Gardnerella

Who is exposing young women to HIV?
- Men with high viral loads in their thirties

Technologies to empower women to protect themselves from HIV infection

Young women at high HIV risk
Targeted combination prevention to break the Cycle of HIV transmission

Men 25-40 years (N=79)
Knew HIV status: 21.5%
VL > 50,000: 37.1%

Medical male circumcision & Antiretroviral therapy for HIV positive men

Innovative forms of HCT

PrEP

Changing community norms on age-disparate sex & patriarchy

Women 25-40 years

Test & Treat

Women <25 years

Potential - Incorporate into SRH services – screening and Rx of STIs

Innovative forms of HCT

Innovative forms of HCT
Transmission networks and risk of HIV infection in KwaZulu-Natal, South Africa: a community-wide phylogenetic study


UNAIDS AIDS Update 2016
Get on the Fast-Track
The Life-cycle approach to HIV

Important public health benefit
LET OUR ACTIONS COUNT
SOUTH AFRICA’S NATIONAL STRATEGIC PLAN ON HIV, TB and STIs 2017-2022

Goal 1: 
Accelerate prevention in order to reduce new HIV and TB infections and new STIs.

- Breaking the cycle of transmission

The NSP sets out intensified prevention programmes that combine biomedical prevention methods, such as medical male circumcision (MMC) and the preventive use of antiretroviral drugs (ARVs) and TB medication, with communication designed to educate and encourage safer sexual behaviour in the case of HIV and STIs, and environmental interventions to control TB infection.

Transmission Pathways
Next steps

- Replicate findings elsewhere

- Future trials to assess the *Prevotella* sub-groups
  - BV is a risk for HIV but trials have not shown that treating BV reduces HIV acquisition
  - Possible use of probiotics (eg. Osel’s tampon *L. crispatus*) to enhance *Lactobacilli* dominance

- Future trials to assess whether BV screening & treatment would improve PrEP efficacy

- Our findings highlight the need to continue for a better understanding of HIV acquisition in young women.
Final acknowledgements

- Funders
- Collaborators
- Many women over the many years who through their participation in our research studies allowed us to better understand the HIV epidemic.
- Many men who we recognize as the important “other half” in the epidemic
- All our CRSG, community members, traditional and municipal leadership, KZN provincial Departments of Health and Education and PHC clinic staff
- All the learners from the participating schools, SGBs, educators and Principals.