**Figure 1: Study design and flow diagram**

|  |  |  |  |
| --- | --- | --- | --- |
| **Enrolled in VMB sub-studies (N=448)** | | | |
| **VMB-hrHPV sub-study (N=304)** | **n** | **VMB-CIN sub-study (N=144)** | **n** |
| *≤CIN1 at both visits AND:* | *Regardless of hrHPV status* |
| Control group: No hrHPV at both visits | 37 | Control group: ≤CIN1 but same hrHPV at both visits2 | 92 |
| Incident hrHPV | 43 | Incident CIN2+ | 22 |
| Cleared hrHPV | 65 | Cured CIN2+ (after treatment) | 50 |
| Type swap | 67 | Cleared CIN2+ (spontaneously) | 14 |
| ≤CIN1 but same hrHPV at both visits2 | 92 | Persistent CIN2+ | 25 |
|  |  | [Prevalent CIN2+]3 | 33 |

Abbreviations: *CIN* cervical intraepithelial neoplasia, *hrHPV* high-risk human papillomavirus, *VMB* vaginal microbiota. N and n refer to the number of women.

1. One woman had had cervical cancer treatment prior to enrolment and was erroneously enrolled into the HARP study (N=623 correctly enrolled women). In addition, six women were treated for CIN2+ at baseline based on local histology results but their baseline histology was subsequently downgraded to ≤CIN1 by the HARP Endpoint Committee. This became clear after the samples had already been sequenced. Data from these seven women were included in the bioinformatics pipeline but excluded from all epidemiological analyses.
2. These are the same 92 women. In this figure, they are included in the sample size of the VMB-hrHPV sub-study but not the sample size of the VMB-CIN sub-study. The total sample size of the VMB-CIN sub-study is therefore 144+92=236.
3. We required two full assessments per person except for women with CIN2+ at one visit and a missing histology result at the other visit. These are referred to as prevalent CIN2+ cases (n=29 at the baseline visit, and n=4 at the endline visit). They were included in some but not all analyses. Of the 59 women with missing endline data, 21 were lost to follow up, 16 moved away, ten were withdrawn by the clinician (including referral for suspected cervical carcinoma or hysterectomy), seven withdrew due to personal circumstances and/or being unwilling to undergo further testing and five died (two from HIV-related causes and three from other causes). The other women had incomplete HPV and/or CIN results.

**Table 1. Participant characteristics at baseline and endline (all groups combined)**

|  |  |  |
| --- | --- | --- |
| **Sociodemographic and behavioural characteristics** | **Baseline (N=448)** | **Endline (N=432)** |
| Age (median, IQR) | 34 (30-39) | NA |
| Current smoker (n %) | 24 (5.4) | 26 (6.0) |
| Has a current regular male sex partner (n %)   * Cohabiting | 362 (80.8)  182 (40.7) | NA  NA |
| Number of male sex partners last 3 (baseline) or 6 (endline) months (n %)   * 0 * 1 * 2+ | 77 (17.2)  345 (77.0)  25 (5.6) | 55 (12.7)  359 (83.1)  18 (4.2) |
| Ever used hormonal contraception (n %) | 386 (86.2) | 369 (82.4) |
| Current use of hormonal contraception (n %)1   * Combined pill (n %) * Combined patch (n %) * Progestin-only injectable (n %) | 113 (25.2)  23 (5.1)  6 (1.3)  84 (18.8) | 114 (27.1)  22 (5.2)  4 (1.0)  88 (20.9) |
| Any condom use in past two weeks (n %)   * Always * Sometimes * Never * No sex | 218 (48.7)  125 (27.9)  24 (5.4)  81 (18.1) | NA  NA  NA  NA |
| Practices vaginal cleansing at least weekly (n %) | 186 (41.5) | NA |
| **Clinical and laboratory results** |  |  |
| Antiretroviral therapy:   * On therapy at baseline and endline * Started therapy during the study * Not on therapy at all during the study |  | 300 (69.4)  23 (5.3)  109 (25.2) |
| CD4+ count in cells/μl (median, IQR)2 | 423 (317-566) | 426 (324-565) |
| HIV undetectable in plasma (n %)3 | 103 (23.4) | NA |
| HIV plasma viral load log10 copies/ml (median, IQR)3 | 2.6 (1.6-3.9) | NA |
| Any high-risk human papillomavirus by genotyping | 357 (79.7) | 306 (70.8) |
| Yeasts on vaginal Gram stain (n %) | 33 (7.5) | NA |
| *Trichomonas vaginalis* by NAAT (n %) | 70 (15.6) | NA |
| *Chlamydia trachomatis* by NAAT (n %) | 24 (5.4) | NA |
| *Neisseria gonorrhoeae* by NAAT (n %) | 10 (2.2) | NA |
| *Mycoplasma genitalium* by NAAT (n %) | 36 (8.0) | NA |
| Active syphilis by serology(n %) | 3 (0.7) | NA |
| Herpes simplex virus type 2 by serology(n %) | 425 (95.3) | NA |
| **VMB characteristics** |  |  |
| Bacterial vaginosis by Nugent 7-10 (n %) | 188 (42.9) | NA |
| Simpson diversity 1-D (mean, 95% CI)4 | 0.54 (0.51-0.57) | 0.54 (0.51-0.57) |
| Lactobacilli relative abundance (mean, 95% CI)4 | 0.48 (0.44-0.52) | 0.46 (0.42-0.50) |
| BV-anaerobes relative abundance (mean, 95% CI)4 | 0.49 (0.45-0.52) | 0.50 (0.46-0.54) |
| Pathobionts relative abundance (mean, 95% CI)4 | 0.03 (0.02-0.04) | 0.03 (0.02-0.04) |
| Other bacteria relative abundance (mean, 95% CI)4 | 0.01 (0.01-0.01) | 0.01 (0-0.02) |
| Vaginal microbiota type (n %):4   * *Lactobacillus crispatus*/*L. jensenii*-dominated (Lcj) * *L. iners*-dominated (Li) * *Bifidobacterium*-dominated (BD) * Lactobacilli + BV-anaerobes (LA) * BV-like (BV) * BV-anaerobe-dominated (AD) * Pathobiont-characterised (PB) * Did not align | 27 (6.2)  115 (26.3)  0  111 (25.3)  148 (33.8)  29 (6.6)  8 (1.8)  0 | 38 (9.1)  95 (22.8)  2 (0.5)  95 (22.8)  149 (35.8)  26 (6.3)  11 (2.6)  1 (0.2) |

Abbreviations: *BV* bacterial vaginosis, *CI* confidence interval, *IQR* inter-quartile range*, NA* not assessed, *NAAT* nucleic acid amplification test.

1. N=420 at endline.
2. N=381 at endline.
3. N=439 at baseline. If below the detection limit of 40 copies/ml, log10 of 40 was used.
4. N=438 at baseline and N=417 at endline.

**Figure 2. VMB types at baseline and endline by hrHPV and CIN status**

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Abbreviations: *AD* BV-anaerobe-dominated, *B* baseline visit, *BD* *Bifidobacterium*-dominated, BV bacterial vaginosis-like, *CIN* cervical intraepithelial neoplasia, *E* endline visit, *hrHPV* high risk human papillomavirus, *LA* lactobacilli and BV-anaerobes, *Lcj* *Lactobacillus crispatus* or *L. jensenii*-dominated, *Li* *L. iners*-dominated, *PB* pathobionts-characterised, *VMB* vaginal microbiota.

The number of women is equal at the baseline and the endline visit in all outcome groups (see Figure 1 for sample sizes), with the exception of the prevalent CIN2+ groep: 29 women were seen at baseline only and four women were seen at endline only.

**Table 2: Bivariable multinomial logistic regression models with hrHPV and CIN outcomes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **hrHPV outcome categories**  *All ≤ CIN1; control group: no hrHPV at both visits* | **Baseline VMB** | **RRR** | **p** | **Endline VMB** | **RRR** | **p** |
| Incident hrHPV | Lcj vs Li | 0.196 | 0.171 | Lcj vs Li  BV vs Li | 0.125  0.455 | **0.019**  0.187 |
|  |  |  |  |  |  |  |
| Cleared hrHPV | None |  |  | Simpson  BV+AD+PB vs Li  Lactobacilli RA  BV-anaerobes RA | 3.856  2.250  0.386  2.331 | **0.034**  0.146  0.057  0.092 |
|  |  |  |  |  |  |  |
| hrHPV type-swap | Nugent  Simpson  AD vs Li  BV+AD+PB vs Li  Lactobacilli RA  BV-anaerobes RA | 1.183  3.818  2.316  2.423  0.277  3.246 | **0.016**  **0.042**  0.136  0.090  **0.014**  **0.026** | Lcj vs Li | 0.397 | 0.174 |
|  |  |  |  |  |  |  |
| Type-specific hrHPV persistence | BV-anaerobes RA | 1.936 | 0.188 | Simpson  Lcj vs Li | 2.716  0.315 | 0.184  0.074 |
|  |  |  |  |  |  |  |
| Any hrHPV at one visit | None |  |  | Simpson  Lcj vs Li | 2.395  0.321 | 0.132  0.072 |
|  |  |  |  |  |  |  |
| Any hrHPV at both visits | Nugent  Simpson  Lactobacilli RA  BV-anaerobes RA | 1.126  2.066  0.412  2.386 | **0.050**  0.198  0.055  0.065 | Simpson  Lcj vs Li | 2.165  0.348 | 0.162  0.069 |
| **CIN outcome categories**  *Control group: ≤CIN1 and hrHPV-positive at both visits* | **Baseline VMB** | **RRR** | **p** | **Endline VMB** | **RRR** | **p** |
| Incident CIN2+ | None |  |  | Simpson  LA vs Li  BV vs Li  BV+AD+PB vs Li  Lactobacilli RA  BV-anaerobes RA | 7.352  3.751  4.297  4.167  0.335  2.828 | **0.028**  0.129  0.073  0.075  0.086  0.101 |
|  |  |  |  |  |  |  |
| Cured CIN2+ | Lcj vs Li | 2.836 | 0.139 | LA vs Li | 2.187 | 0.144 |
|  |  |  |  |  |  |  |
| Cleared CIN2+ | AD vs Li | 8.662 | **0.021** | Simpson  LA vs Li | 3.597  3.123 | 0.198  0.200 |
|  |  |  |  |  |  |  |
| Persistent CIN2+ | None |  |  | Simpson  BV vs Li  BV-anaerobes RA | 4.239  2.343  2.291 | 0.068  0.181  0.149 |
|  |  |  |  |  |  |  |
| CIN2+ at one or two visits | Nugent | 1.087 | 0.170 | Simpson | 5.981 | **0.003** |
| *Control group: no hrHPV at both visits* | AD vs Li  Lactobacilli RA  BV-anaerobes RA | 4.259  0.433  2.561 | 0.187  0.075  **0.049** | LA vs Li  BV vs Li  BV+AD+PB vs Li  Lactobacilli RA  BV-anaerobes RA | 3.094  2.404  2.210  0.352  2.412 | 0.064  0.101  0.122  **0.025**  0.060 |

Abbreviations: *BV* bacterial vaginosis-like, *CIN* cervical intraepithelial neoplasia, *hrHPV* high-risk human papillomavirus, *LA* lactobacilli and BV-anaerobes, Lcj *Lactobacillus crispatus* or *L. jensenii*-dominated, Li *L. iners*-dominated, *RA* relative abundance, *RRR* relative risk ratio, *VMB* vaginal microbiota. Each model includes one multi-category outcome and one VMB independent variable. The VMB independent variable in each hrHPV outcome category is compared to that in the persistent hrHPV-negative control group. VMB variables tested: Nugent score (baseline only), Simpson diversity index, VMB type, and relative abundance of lactobacilli, BV-anaerobes, pathobionts, and other bacteria. Only results with p<0.2 are shown in the table: see Table S4 in Supplement 1 for full results including 95% confidence intervals. ‘None’ means that none of the VMB variables were associated with the outcome listed in the first column at p<0.2. Statistically significant results (p<0.05) are shown in bold.

**Table 3. Multinomial logistic regression models with combined hrHPV/CIN outcome**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Bivariable models1** | **Combined hrHPV/CIN outcome2** | | | | | | | |
| All ≤CIN1; hrHPV at one visit vs no hrHPV at both visits | | All ≤CIN1; hrHPV at both visits vs no hrHPV at both visits | | CIN2+ at one or both visits vs ≤CIN1 and no hrHPV both visits | | CIN2+ at one or both visits vs ≤CIN1 and hrHPV at both visits | |
| **RRR** | **p** | **RRR** | **p** | **RRR** | **p** | **RRR** | **p** |
| Age (per year) | 0.945 | 0.077 | 0.923 | **0.010** | 0.881 | **<0.001** | 0.954 | **0.021** |
| Current smoker at baseline | 1.385 | 0.774 | 2.939 | 0.308 | 1.839 | 0.574 | 0.626 | 0.339 |
| Regular partner at baseline3 | 1.178 | 0.516 | 1.547 | 0.073 | 1.509 | 0.094 | 0.975 | 0.872 |
| # sex partners 3 months prior to baseline4 | 1.877 | 0.113 | 1.773 | 0.131 | 1.919 | 0.090 | 1.082 | 0.752 |
| On hormonal contraception at baseline | 0.691 | 0.401 | 0.628 | 0.270 | 1.481 | 0.337 | 2.358 | **0.001** |
| Any hormonal contraception during study5 | 1.074 | 0.671 | 0.923 | 0.627 | 1.216 | 0.229 | 1.317 | **0.008** |
| Current condom use6 | 0.979 | 0.931 | 0.945 | 0.809 | 0.763 | 0.249 | 0.807 | 0.140 |
| Vaginal cleansing at least weekly | 0.997 | 0.987 | 0.820 | 0.295 | 0.878 | 0.493 | 1.070 | 0.580 |
| On ART at baseline | 1.877 | 0.138 | 1.179 | 0.674 | 0.800 | 0.568 | 0.678 | 0.114 |
| CD4 count (per cell/μl) | 1.000 | 0.946 | 0.999 | 0.348 | 0.998 | 0.053 | 0.999 | 0.128 |
| Log10 HIV plasma viral load | 0.830 | 0.257 | 1.026 | 0.869 | 1.231 | 0.175 | 1.200 | **0.050** |
| CT, NG or syphilis7 | 3.673 | 0.223 | 1.658 | 0.641 | 3.876 | 0.198 | 2.338 | 0.076 |
| Nugent score baseline8 | 1.049 | 0.444 | 1.126 | **0.050** | 1.087 | 0.170 | 0.965 | 0.367 |
| Yeasts on Gram stain baseline8 | 2.059 | 0.511 | 3.525 | 0.231 | 3.256 | 0.265 | 0.924 | 0.847 |
| Simpson index baseline | 1.460 | 0.518 | 2.066 | 0.198 | 1.717 | 0.344 | 0.831 | 0.619 |
| Simpson index endline | 2.395 | 0.132 | 2.165 | 0.162 | 5.981 | **0.003** | 2.763 | **0.012** |
| Lactobacilli RA baseline | 0.593 | 0.276 | 0.412 | 0.055 | 0.433 | 0.075 | 1.051 | 0.865 |
| Lactobacilli RA endline | 0.589 | 0.252 | 0.651 | 0.333 | 0.352 | **0.025** | 0.541 | **0.043** |
| BV-anaerobes RA baseline | 1.725 | 0.266 | 2.386 | 0.065 | 2.561 | **0.049** | 1.073 | 0.809 |
| BV-anaerobes RA endline | 1.453 | 0.426 | 1.287 | 0.575 | 2.412 | 0.060 | 1.874 | **0.039** |
| Pathobionts RA baseline | 0.594 | 0.764 | 1.391 | 0.832 | 0.375 | 0.576 | 0.269 | 0.275 |
| Pathobionts RA endline | 452.02 | 0.275 | 524.55 | 0.262 | 376.63 | 0.289 | 0.718 | 0.744 |
| **Multivariable models9** | All ≤CIN1; hrHPV at one visit vs no hrHPV at both visits | | All ≤CIN1; hrHPV at both visits vs no hrHPV at both visits | | CIN2+ at one or both visits vs ≤CIN1 and no hrHPV both visits | | CIN2+ at one or both visits vs ≤CIN1 and hrHPV at both visits | |
| **aRRR** | **p** | **aRRR** | **p** | **aRRR** | **p** | **aRRR** | **p** |
| Nugent score baseline8 | 1.042 | 0.518 | 1.114 | 0.081 | 1.073 | 0.262 | 0.963 | 0.348 |
| Simpson baseline | 1.381 | 0.585 | 1.941 | 0.246 | 1.653 | 0.391 | 0.852 | 0.671 |
| Simpson endline | 2.476 | 0.143 | 2.094 | 0.217 | 7.691 | **0.002** | 3.673 | **0.004** |
| Lactobacilli RA baseline | 0.587 | 0.267 | 0.404 | 0.051 | 0.417 | 0.067 | 1.034 | 0.911 |
| Lactobacilli RA endline | 0.534 | 0.207 | 0.631 | 0.339 | 0.325 | **0.027** | 0.515 | **0.050** |
| BV-anaerobes RA baseline | 1.729 | 0.264 | 2.414 | 0.063 | 2.738 | **0.039** | 1.134 | 0.677 |
| BV-anaerobes RA endline | 1.565 | 0.375 | 1.238 | 0.662 | 2.792 | **0.045** | 2.255 | **0.017** |

Abbreviations: *ART* antiretroviral therapy, *BV* bacterial vaginosis, *CIN* cervical intraepithelial neoplasia, *CT Chlamydia trachomatis*, *hrHPV* high-risk human papillomavirus, *NG Neisseria gonorrhoeae*, *RA* relative abundance, *aRRR* adjusted relative risk ratio. Statistically significant results (p<0.05) are shown in bold.

1. Each line represents one model. Each model includes the multi-category combined outcome and one independent variable.
2. With ‘hrHPV’ we mean detection of any type. With ‘no hrHPV’ we mean that not a single high risk type was present.
3. Comparing three categories: no, yes but not cohabiting, or cohabiting. The results are similar if analysed as an indicator variable.
4. Comparing three categories: none, one, or two or more. The results are similar if analysed as an indicator variable.
5. Comparing three categories: never, at one visit, and at both visits. The results are similar if analysed as an indicator variable. When four categories are analysed as an indicator variable (never, baseline only, endline only, or at both visits), hormonal contraceptive use at baseline and at both visits are both associated with CIN2+ compared to hrHPV twice, but hormonal contraceptive use at endline only is not.
6. Comparing four categories: never, sometimes, always, or no sex in last six months. The results are similar if analysed as an indicator variable.
7. *Trichomonas vaginalis* was only associated with hrHPV twice (RRR=2.544, p=0.142, compared to negative controls). *Mycoplasma genitalium* was not associated with any outcome.
8. Not assessed at endline.
9. Each line represents one multivariable model. All multivariable models included the multi-category combined outcome, one VMB independent variable, age, CD4+ count at baseline or endline, and current hormonal contraceptive use (baseline) or any hormonal contraception during the study (endline). See Table S5 in Supplement 1 for 95% confidence intervals.