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# Patterns of repeated anal cytology results among HIV-positive and HIV-negative men who have sex with men

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## ARTICLE INFO

## ABSTRACT

*Background:* Men who have sex with men (MSM) are at increased risk for anal cancer. In cervical cancer screening, patterns of repeated cytology results are used to identify low- and high-risk women, but little is known about these patterns for anal cytology among MSM.

*Methods:* We analyzed Multicenter AIDS Cohort Study (MACS) data for MSM who were offered anal cytology testing annually (HIV-positive) or every 2 years (HIV-negative) for 4 years.

*Results:* Following an initial negative (normal) cytology, the frequency of a second negative cytology was lower among HIV-positive MSM with CD4 ≥ 500 (74%) or CD4 < 500 (68%) than HIV-negative MSM (83%) (p < 0.001). After an initial abnormal cytology, the frequency of a second abnormal cytology was highest among HIV-positive MSM with CD4 < 500 (70%) compared to CD4 ≥ 500 (53%) or HIV-negative MSM (46%) (p = 0.003). Among HIV-positive MSM with at least three results, 37% had 3 consecutive negative results; 3 consecutive abnormal results were more frequent among CD4 < 500 (22%) than CD4 ≥ 500 (10%) (p = 0.008).

*Conclusions:* More than one-third of HIV-positive MSM have consistently negative anal cytology over three years. Following abnormal anal cytology, a repeated cytology is commonly negative in HIV-negative or immunocompetent HIV-positive men, while persistent cytological abnormality is more likely among HIV-positive men with CD4 < 500.

## 1. Introduction

Anal cancer is rare in the United States general population (1.8 per 100,000) [1,2], though rates are increasing [3]. In contrast, incidence among HIV-seropositive men who have sex with men (HIV-positive MSM) is extremely high, estimated at 131 per 100,000 [4], due to increased human papillomavirus (HPV) prevalence and HIV-associated immunosuppression [5]. During 2001–2005, approximately 28% of U.S. anal cancers in males occurred in men living with HIV, the vast majority in HIV-positive MSM [6]. This burden is likely growing as the HIV-positive population size increases [7,8], though the trend in anal cancer incidence is unclear [9,10]. Anal cancer is also a concern for HIV-negative MSM, who have high prevalence of high-grade anal lesions [11] and 30-fold higher anal cancer incidence than the general population [12,13].

There is an urgent need for effective anal cancer screening methods among MSM. Though no national or international guidelines exist [14], the primary strategy is screening by anal cytology (collected with an anal swab) with referral to high-resolution anoscopy (HRA) for possible biopsy, diagnosis, and treatment of anal precancer/cancer [5,15,16]. This approach is analogous to cervical cancer screening by cytology with referral to colposcopy, but is not as well studied [5,17,18]. Using a threshold of ASC-US (atypical squamous cells of undetermined significance) and higher grades of cellular dysplasia on cytology as a

positive screen, the sensitivity of both anal and cervical cytology for biopsy-confirmed high-grade dysplasia are estimated at 90%; however, specificity appears lower for anal vs. cervical cytology (33% vs. 53%) [19]. There is some evidence that the sensitivity of anal cytology is higher in HIV-positive vs. HIV-negative MSM, while the specificity may be lower [20–23].

Due to the challenges and uncertainty associated with anal cytology, some have proposed that HIV-positive MSM be referred directly to HRA [24]. However, while anal cytology has high acceptability among MSM [25,26], there are a limited number of trained and experienced HRA providers, a higher cost for the procedure, and uncertain benefits of screening using this diagnostic tool. Thus, evaluating whether using cytology may be appropriate to identify men who do or do not need HRA is an important goal.

At the cervix, the predictive value of repeated cytology results (e.g., low risk after 3 consecutive negative results) is frequently utilized in screening guidelines [27,28]. For anal cytology, however, it is not known what proportion of HIV-positive MSM have consistently negative results. Further, different transition probabilities, such as the likelihood of a negative cytology if the previous cytology was abnormal, have not been described for anal cytology nor compared by HIV or immune status. Such data could inform decisions regarding when and whether to repeat anal cytology or refer MSM to HRA.

## 2. Methods

### 2.1. Study population

We analyzed data from the Multicenter AIDS Cohort Study (MACS), a cohort study of HIV-positive and HIV-negative men who have sex with men (MSM). The MACS has 4 United States sites (Baltimore, Chicago, Pittsburgh, and Los Angeles) and has been ongoing since 1984. Visits occur every 6 months and include routine collection of biological and behavioral covariates of interest. For this sub-study, all MACS participants who attended any study visits between June 2010 and July 2011 were offered a free anal cytology test, with collection and testing done as previously described [18]. Men with unsatisfactory cytology results were offered another test at their next visit. By design, over the study period, HIV-positive men were offered annual cytology (up to 4 cytologies total), whereas HIV-negative men were offered a second cytology 2 years later (2 cytologies total). Thus, our analyses including both HIV-positive and HIV-negative MSM describe 2 cytology results typically collected 1 and 2 years apart, respectively. Analyses examining 3 or more cytology results could be performed among HIV-positive MSM only. Information about HRA and treatment of anal dysplasia occurring outside of regular MACS visits was collected using participant questionnaires and subsequent medical record review. This MACS sub-study was approved by the institutional review boards of each participating site.

### 2.2. Statistical analysis

A substantial proportion of cytology results were classified as being unsatisfactory for evaluation (18% overall, with no substantial changes over time). For the purposes of this analysis (excluding the generation of inverse probability weights described below) we omitted these results and only considered results deemed sufficient for interpretation. Adequate (valid) specimens were classified as negative (normal) or abnormal: ASC-US, low-grade squamous intraepithelial lesion (LSIL), atypical squamous cells cannot exclude HSIL (ASC-H), or high-grade squamous intraepithelial lesion (HSIL). As we stratify by HIV status, we excluded 1 man who acquired HIV after his first cytology. Men who had treatment of anal dysplasia (including imiquimod cream, trichloroacetic acid, cryotherapy, electrocautery, infrared coagulation, or surgery) between their first and second cytology (N = 38) were excluded from all analyses.

A total of 796 HIV-negative and 708 HIV-positive MSM had at least one anal swab collected and evaluated for anal cytology, including inadequate results (Table 1, top portion). In this study, HIV-negative men had up to 2 opportunities to have anal cytology collected while HIV-positive men had up to 4 opportunities. After excluding men with anal dysplasia treatment between their first and second cytology (6 HIV-negative and 32 HIV-positive men), 474/796 (60%) HIV-negative and 502/708 (71%) HIV-positive men had at least 2 valid results and were included in analysis. For analyses considering 3 consecutive cytologies (HIV-positive MSM only), 14 additional men with treatment between the second and third cytology were excluded.

Among men with at least 2 valid cytology tests, and considering only the first 2 valid (consecutive) results, we calculated frequencies of having a negative (vs. abnormal) cytology following a negative or abnormal cytology. We compared these frequencies across HIV-negative MSM and HIV-positive MSM with absolute CD4 + T cell counts (CD4) ≥ 500 cells/µL (immunocompetent) and < 500 cells/µL (potentially immunocompromised) at the first cytology; p-values were calculated using chi-square tests across all three groups. We also present this analysis after dividing abnormal results into more detailed categories (ASC-US, LSIL, ASC-H/HSIL). Among HIV-positive MSM with at least 3 valid results, we also calculated frequencies of having a negative (vs. abnormal) cytology at the third consecutive anal cytology following 2 consecutive negative or 2 consecutive abnormal cytologies, and compared these frequencies by CD4 count at the first cytology; p-values were calculated using chi-square tests across the two HIV-positive groups.

We recognized potential for selection bias in our analysis set of HIV-positive MSM with at least 3 valid results and no anal dysplasia treatment (N = 328), as this group represented less than half of the HIV-positive MSM who originally had at least one anal swab for cytology collected (N = 708). Therefore, we applied inverse probability weights in the analyses of cytology patterns conducted among this group [29]. We generated the weights using a logistic regression model including variables potentially related to consistent participation in cytology testing, including study center, wave of enrollment into cohort, age, race/ethnicity, educational level, first cytology result (including inadequate), HAART status, and number of sexual partners. Weights were stabilized by dividing the overall proportion with complete data by each individual's model-predicted probability of having complete data. We then applied these stabilized weights when calculating the prevalence of cytology patterns among HIV-positive MSM, and when fitting a logistic model comparing characteristics of men with consistently abnormal vs. consistently negative cytology (described below).

Among HIV-positive MSM with at least 3 valid cytologies, we classified men as having different patterns of negative and abnormal results (e.g., negative-abnormal-negative) by considering the first 3 valid results. As a descriptive analysis, we further restricted to HIV-positive MSM with either consistently abnormal results (i.e., 3 consecutive abnormal cytologies) or consistently negative results (i.e., 3 consecutive negative cytologies) and fit a logistic regression model to compare demographic, behavioral, and biological characteristics between these two groups.

## 3. Results

Among MSM with at least two valid cytology results (Table 1, bottom portion), the median time interval between valid cytologies for HIV-negative MSM was 2.0 years (IQR 1.9–2.2) and for HIV-positive MSM was 1.0 years (IQR 0.96–1.3). The median age was 58 years for HIV-negative MSM and 54 years for HIV-positive MSM. Consistent with the MACS participants overall, most men in this sub-study were non-Hispanic White and had at least a college education. The first valid cytology was more commonly negative for HIV-negative MSM (75%) compared to HIV-positive MSM (64%). Among HIV-positive MSM, the median current CD4 cell count (at the first cytology) was 579 cells/µL,

**Table 1**

Description of HIV-negative and HIV-positive men who have sex with men (MSM) in the MACS study with anal cytology testing.

| Number of anal cytology tests | HIV-negative MSM N with valid results (N with any results) | HIV-positive MSM |
|---|---|---|
| 1 or more | 752 (796) | 665 (708) |
| 2 or more | 480 (625) | 534 (593) |
| 3 or more | NA | 369 (484) |

| Characteristics of MSM included in analyses (2 or more valid anal cytologies, no anal dysplasia treatment) | HIV-negative MSM N (%) or median (IQR) | HIV-positive MSM |
|---|---|---|
| Total number MSM | 474 | 502 |
| Age, years (at first cytology) | 58 (51–64) | 54 (50–59) |
| Race/ethnicity | | |
|   Non-Hispanic white | 410 (86) | 325 (65) |
|   Non-Hispanic black | 39 (8) | 129 (26) |
|   Hispanic | 19 (4) | 40 (8) |
|   Other | 6 (1) | 8 (2) |
| Education | | |
|   12th grade or less | 95 (20) | 194 (39) |
|   College graduate | 158 (33) | 173 (34) |
|   Post-graduate | 173 (36) | 107 (21) |
|   Unknown | 48 (10) | 28 (6) |
| Study site | | |
|   Baltimore | 130 (27) | 131 (26) |
|   Chicago | 37 (8) | 150 (30) |
|   Pittsburgh | 149 (31) | 102 (20) |
|   Los Angeles | 158 (33) | 119 (24) |
| First valid cytology result | | |
|   Negative | 355 (75) | 321 (64) |
|   ASC-US | 85 (18) | 101 (20) |
|   LSIL | 18 (4) | 68 (14) |
|   ASC-H/HSIL | 16 (3) | 12 (2) |
| CD4 count at first cytology, cells/µL | NA | 579 (429–749) |
| Nadir CD4 count (as of first cytology), cells/µL | NA | 252 (154–354) |
| Currently on HAART (at first cytology) | NA | 433 (88) |
| Time since first HAART (at first cytology), years | NA | 11.8 (7.4–13.8) |
| Mean number of condomless receptive anal sex partners reported at each visit during the previous 5 years | | |
|   0 | 307 (65) | 254 (52) |
|   0.1–0.9 | 107 (23) | 103 (21) |
|   1.0 or more | 58 (12) | 135 (27) |

MSM, men who have sex with men; HAART, highly active antiretroviral therapy; MACS, Multicenter AIDS Cohort Study. Small numbers of missing values are excluded and percentages may not sum exactly to 100 due to rounding. Men with treatment of anal dysplasia between the 1st and 2nd cytology (N = 38) are excluded in the lower portion of the table. For analyses involving 3 cytology results, HIV-positive men with treatment between the 2nd and 3rd cytology (N = 14) were additionally excluded.

while the median nadir CD4 count (prior to first cytology) was 252 cells/µL. When summarizing all MACS visits over the last 5 years, the mean number of condomless receptive anal sex partners reported at each visit was 1.0 or more for 12% of HIV-negative and 27% of HIV-positive men.

We compared the frequency of a negative (vs. abnormal) cytology following an initial negative or abnormal cytology by HIV and CD4 status at the first cytology (Table 2). After an initial negative cytology, the frequency of a negative result on the second cytology (without accounting for differences in time interval) was 83%, 74%, and 68% among HIV-negative MSM, HIV-positive MSM with CD4 ≥ 500, and HIV-positive MSM with CD4 < 500, respectively (p < 0.001). After an initial abnormal cytology, corresponding frequencies of an abnormal result on the second cytology (without accounting for differences in time interval) were 46%, 53%, and 70% (p = 0.003). When the analyses were restricted to cytologies that were within 18–30 months of each other, so that HIV-negative and HIV-positive MSM had similar time intervals between tests (Supplementary Table 1), consecutive negative cytologies were still most frequent in HIV-negative MSM (83%), but there was no appreciable difference between the HIV-positive groups based on CD4 count (73–74%; overall p = 0.02). For consecutive abnormal cytologies, as in the primary analysis, the results showed comparable frequencies in HIV-negative MSM and HIV-positive MSM with CD4 ≥ 500 (43% and 40%, respectively), and higher

frequency in HIV-positive MSM with CD4 < 500 (65%, overall p = 0.02).

Further stratification of results from the first and second cytologies (Table 2) revealed that ASC-US results at the second cytology accounted for more than three-quarters of abnormal results following an initial negative cytology, with LSIL or higher grade results occurring in 6% of men or less, regardless of HIV or CD4 status. After an initial ASC-US cytology, more than one-quarter of men (27–31%) had ASC-US at their second cytology in all groups, while LSIL was more common in HIV-positive MSM with CD4 < 500 (27%) than CD4 ≥ 500 (14%) or HIV-negative MSM (6%) (overall p = 0.07). ASC-H and HSIL results were generally uncommon, but did represent 6–10% of results following an initial cytology of LSIL or higher grade.

Among HIV-positive MSM only, we also compared the frequency of a negative or abnormal cytology following 2 consecutive negative or abnormal cytologies (Table 2). After 2 consecutive negative cytologies, the frequency of the third cytology remaining negative was high (74–77%) regardless of CD4 count (p = 0.84). However, after 2 consecutive abnormal cytologies, the frequency of the third cytology remaining abnormal was higher among HIV-positive MSM with CD4 < 500 (79%) compared to CD4 ≥ 500 (60%), though the difference did not quite reach statistical significance (p = 0.08).

Among the 708 HIV-positive MSM who had at least one cytology collected, 328 (46%) had at least 3 valid results and no treatment.

**Table 2**

Frequencies of the next anal cytology result following 1 or 2 initial cytologies that were negative or abnormal among 976 MSM, by HIV and CD4 status at first cytology.

| | HIV-negative MSM | HIV-positive MSM, CD4 ≥ 500 | HIV-positive MSM, CD4 < 500 | p-value |
|---|---|---|---|---|
| After 1 negative cytology | 355 | 206 | 114 | < 0.001 |
|   Negative cytology | 295 (83) | 153 (74) | 77 (68) | |
|   Abnormal cytology | 60 (17) | 53 (26) | 37 (33) | |
| After 1 abnormal cytology | 119 | 99 | 81 | 0.003 |
|   Negative cytology | 64 (54) | 47 (48) | 24 (30) | |
|   Abnormal cytology | 55 (46) | 52 (53) | 57 (70) | |
| After 1 negative cytology | 355 | 206 | 114 | 0.004 |
|   Negative cytology | 295 (83) | 153 (74) | 77 (68) | |
|   ASC-US cytology | 46 (13) | 44 (21) | 30 (26) | |
|   LSIL cytology | 7 (2) | 6 (3) | 7 (6) | |
|   ASC-H/HSIL cytology | 7 (2) | 3 (1) | 0 (0) | |
| After 1 ASC-US cytology | 85 | 59 | 41 | 0.07 |
|   Negative cytology | 51 (60) | 34 (58) | 17 (41) | |
|   ASC-US cytology | 26 (31) | 16 (27) | 12 (29) | |
|   LSIL cytology | 5 (6) | 8 (14) | 11 (27) | |
|   ASC-H/HSIL cytology | 3 (4) | 1 (2) | 1 (2) | |
| After 1 LSIL/ASC-H/HSIL cytology | 34 | 40 | 40 | 0.49 |
|   Negative cytology | 13 (38) | 13 (33) | 7 (18) | |
|   ASC-US cytology | 11 (32) | 12 (30) | 14 (35) | |
|   LSIL cytology | 8 (24) | 11 (28) | 16 (40) | |
|   ASC-H/HSIL cytology | 2 (6) | 4 (10) | 3 (8) | |
| After 2 negative cytologies | – | 102 | 51 | 0.84 |
|   Negative cytology | – | 75 (74) | 39 (77) | |
|   Abnormal cytology | – | 27 (27) | 12 (24) | |
| After 2 abnormal cytologies | – | 35 | 38 | 0.08 |
|   Negative cytology | – | 14 (40) | 8 (21) | |
|   Abnormal cytology | – | 21 (60) | 30 (79) | |

N or N (%). MSM, men who have sex with men; ASC-US, atypical squamous cells of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; ASC-H, atypical squamous cells cannot exclude HSIL; HSIL, high-grade squamous intraepithelial lesion. Men with treatment of anal dysplasia during the cytologies being considered were excluded for each analysis (see Methods), as were 2 HIV-positive men with missing CD4 cell counts. Data are unweighted and p-values were calculated using chi-square tests. Due to differences in study design, the median length of time between cytologies was longer for HIV-negative MSM (2.0 [IQR 1.9–2.2] than for HIV-positive MSM (1.0 years [IQR 0.96–1.3]). Percentages may not sum exactly to 100 due to rounding.

**Table 3**

Patterns of the first 3 consecutive anal cytology results among 328 HIV-positive MSM with at least 3 valid cytology results.

| Pattern | HIV-positive MSM, CD4 ≥ 500 | HIV-positive MSM, CD4 < 500 |
|---|---|---|
| Consistently negative | 37% | 38% |
|   Negative – Negative – Negative | 37% | 38% |
| 2 negative, 1 abnormal | 35% | 25% |
|   Negative – Negative – Abnormal | 11% | 7% |
|   Negative – Abnormal – Negative | 9% | 8% |
|   Abnormal – Negative – Negative | 15% | 9% |
| 1 negative, 2 abnormal | 17% | 16% |
|   Abnormal – Abnormal – Negative | 8% | 5% |
|   Abnormal – Negative – Abnormal | 4% | 4% |
|   Negative – Abnormal – Abnormal | 7% | 7% |
| Consistently abnormal | 10% | 22% |
|   Abnormal – Abnormal – Abnormal | 10% | 22% |

MSM, men who have sex with men. Percentages are weighted to correct for missing cytology-pattern data among 708 eligible HIV-positive MSM (i.e., 708 HIV-positive MSM with at least one anal cytology specimen collected). Men with treatment for anal dysplasia between the first and third cytology were excluded, as were 2 men with missing CD4 cell counts. Numbers may not sum exactly due to rounding.

Among these 328 HIV-positive MSM, we explored frequencies of different patterns of results observed over the first 3 cytologies, using inverse-probability weighting to approximate what would have been observed in the original 708 MSM (Table 3). Across categories of CD4 count, 37–38% of HIV-positive MSM had consistently (3 out of 3) negative cytology; thus, a high proportion (62–63%) had abnormal results for at least 1 of the 3 cytologies. However, most men did not have consistently abnormal cytology, and the proportion of men with consistently abnormal cytology was higher among HIV-positive MSM with CD4 < 500 than CD4 ≥ 500 (22% vs. 10%, p = 0.008). Conversely, the proportion of men with 2 negative and 1 abnormal cytology was lower among HIV-positive MSM with CD4 < 500 vs. CD4 ≥ 500 (25% vs. 35%). A pattern of 1 negative and 2 abnormal cytologies was also

common in both groups (16–17%). A detailed description of all patterns across the first 3 consecutive cytologies in HIV-positive MSM is provided in Supplementary Table 2.

Finally, we explored demographic, behavioral, and biological risk factors for a consistently (over 3 consecutive results) abnormal cytology pattern, compared to a consistently negative pattern, using inverse-probability weighted logistic regression (Table 4). The odds of consistently abnormal cytology increased by 28% with each 100 cells/μL decrease in CD4 cell count at the first cytology (95%CI 4–59%). Additionally, a nadir CD4 cell count less than 100 cells/μL (threshold chosen based on exploratory analysis) indicated 4.4-fold higher odds of consistently abnormal cytology (95%CI 1.18–16.5). Men who reported a mean of 1 or more condomless receptive anal sex partners at each visit

**Table 4**
Logistic regression identifying risk factors for a pattern of first 3 consecutive abnormal anal cytologies (N = 51) compared to first 3 consecutive negative cytologies (N = 113) among HIV-positive MSM.

| Characteristic at first cytology | Unadjusted odds ratio (95% CI) | Adjusted odds ratio (95% CI) |
| --- | --- | --- |
| Absolute CD4 cell count, per 100 cells/μL decrease | 1.30 (1.08–1.57) | 1.28 (1.04–1.59) |
| Nadir CD4 count as of first cytology | | |
| ≥100 cells/μL | Reference | Reference |
| < 100 cells/μL | 3.5 (1.07–11.2) | 4.4 (1.18–16.5) |
| Mean number of condomless receptive anal sex partners reported at each visit during the previous 5 years | | |
| 0–0.9 | Reference | Reference |
| 1.0 or more | 2.1 (0.84–5.1) | 5.7 (2.1–15.2) |
| Age, years | | |
| < 50 | Reference | Reference |
| 50–59 | 1.10 (0.38–3.2) | 1.20 (0.32–4.5) |
| ≥60 | 1.88 (0.60–5.9) | 6.1 (1.33–27.6) |
| Race/ethnicity | | |
| Non-Hispanic white | Reference | Reference |
| Any other | 1.99 (0.89–4.5) | 6.2 (1.92–20.0) |

Higher odds ratios indicate a higher likelihood of having 3 consistently abnormal cytologies, as compared with having 3 consistently negative cytologies. Cytology was tested approximately annually. Odds ratios are weighted to correct for missing cytology-pattern data among 708 eligible HIV-positive MSM (i.e., 708 HIV-positive MSM with at least one anal cytology specimen collected). Men with treatment for anal dysplasia between the first and third cytology were excluded. Of 166 MSM, two observations were excluded due to missing data, leaving 164 in the analysis set.

over the past 5 years had 5.7-fold higher odds (95%CI 2.1–15.2) compared to men who reported fewer or none of these partners. Odds of consistently abnormal cytology were increased in men aged 60 and older (OR 6.1, 95%CI 1.33–27.6) and of non-white race/ethnicity (OR 6.2, 95%CI 1.92–20.0).

## 4. Discussion

Identifying MSM who might (or might not) have risk for anal dysplasia is an important goal, particularly for HIV-positive MSM who are at high risk of anal cancer. Anal cytology is one potentially useful tool for this process. In one of few studies to repeatedly collect and evaluate anal swabs for cytological abnormalities, our data show that more than one-third of our population of HIV-positive MSM have consistently negative (normal) anal cytology when tested three times over the course of approximately three years. Since negative anal cytology may indicate lower risk of ultimate development of anal cancer, it is possible that repeatedly negative cytology might define a subset of HIV-positive MSM who are at lower anal cancer risk and thus less likely to benefit from an invasive procedure such as HRA. Conversely, consistently abnormal anal cytology in HIV-positive MSM over a three-year period may identify men at higher risk of anal dysplasia. A fluctuating pattern of negative and abnormal cytology was very common, but additional research is necessary to understand how such a pattern should be interpreted.

Even among MSM with the same recent cytology result, we found that the likelihood of the next cytology being abnormal was related to HIV status or to the level of HIV-associated immunosuppression. After a negative cytology result, a second negative cytology was seen in the majority of men regardless of HIV or CD4 status, but the frequency was lower in HIV-positive MSM. When the first cytology was abnormal, the likelihood that the next result would remain abnormal was highest among HIV-positive MSM with CD4 < 500 cells/μL, with much lower frequencies of a second abnormal Pap in HIV-positive MSM with CD4 > 500 cells/μL and HIV-negative MSM. One of multiple possible explanations for this finding is that anal dysplasia was less likely to regress in more immunosuppressed men [30,31]. This topic is poorly studied, though some data do suggest that HIV reduces clearance of anal HPV [11,32,33].

Our findings motivate further study of the utility of repeated cytology for managing an initial abnormal cytology. For example, MSM with an initial ASC-US cytology (the least severe of the abnormal cytology results) were likely to have a negative second cytology if HIV-

negative (60%) or HIV-positive with CD4 > 500 cells/μL (58%). If this accurately represents a low-risk status (which could only be determined by performing HRA on all men), then repeating cytology after an ASC-US result may provide one way to distinguish between men who are not at high risk of anal dysplasia and men who might benefit from prompt referral to HRA. Repeated cytology was less likely to revert to negative among HIV-positive MSM with lower CD4 counts, particularly when the initial result was LSIL or worse.

Compared to HIV-positive MSM with 3 consecutive negative anal cytologies, we found that HIV-positive MSM with 3 consecutive abnormal cytologies were more likely to be older, of race/ethnicity other than non-Hispanic white, to have lower CD4 counts at first cytology as well as lower nadir CD4 counts, and to have more condomless receptive anal sex partners. These characteristics are largely consistent with known risk factors for having anal lesions/cancer or for acquiring anal HPV [12,34–36]. One possible explanation for the higher likelihood of persistently abnormal cytology among non-white HIV-positive MSM is that, in our data, the likelihood of treatment for anal dysplasia after a first abnormal cytology was 30% among white non-Hispanic men compared to only 12% among other men (p = 0.002). Thus, white non-Hispanic men with a first abnormal cytology were more likely to be excluded from the cytology-patterns analysis, and the racial difference that we observed might be due to other factors related to treatment access or treatment-seeking behavior. We recommend further study of this difference in the likelihood of referral and potential treatment, as an analogous disparity in follow-up after abnormal cervical cytology has produced a substantial racial disparity in cervical cancer incidence among older U.S. women [37].

Our results show that using repeated anal cytology over time identifies patterns of negative and abnormal anal cytology. These patterns may have potential to identify men at low and high risk of anal lesions, but further research linking cytology patterns to anal dysplasia outcomes is needed. We emphasize that cytology screening is not diagnostic, and cannot prevent anal cancer without the possibility of referral to HRA and ultimately to treatment of anal precancer among those identified to be at risk. Digital anorectal examination should always be included in the evaluation of individuals at risk for anal dysplasia, and can be used to identify some anal cancers at earlier stages if HRA is not readily available [38,39]. In our study, the proportion of inadequate cytology specimens was higher than recommended [40], but previous investigations by our group did not suggest that clinician training or experience was the source of this problem [18].

A major weakness of our study is that we could not relate anal

cytology patterns (from anal swabs) to histologically verified anal dysplasia (from anal biopsies). The utility of repeated anal cytology can only be confirmed through unbiased follow-up by HRA (i.e., HRA in MSM with both negative and abnormal anal cytology results) and biopsy when indicated. It is possible that, as in cervical cancer screening, molecular testing of anal swabs for the presence of oncogenic HPV subtypes, or co-testing for HPV subtype and cytology, will help overcome the current suboptimal diagnostic accuracy of anal cytology testing alone [41,42]. The prevalence of anal HPV is very high among HIV-positive MSM, though testing specifically for HPV16 (which confers higher risk) may have utility in screening, including for triage of lower-risk abnormal cytology results [11,43].

Our analysis did not relate cytology patterns to histologically verified anal precancer, and thus we cannot say (for example) that risk of biopsy-confirmed anal HSIL is truly lower among those with consistently negative cytology. While this is likely to be true, it must be studied directly. In addition, treatment of anal HSIL has not yet been conclusively shown to prevent anal cancer; this is the topic of an ongoing randomized trial [44]. We did not have complete data on serial anal cytology for all participants, but we attempted to correct for potential selection bias using inverse probability weighting, and results were similar when weights were disregarded. We also did not attempt to describe or account for differences in demographic, biological, or behavioral characteristics by HIV status or CD4 count when calculating the prevalence of different transition probabilities and cytology patterns. Thus, factors other than HIV status (for example, differences in sexual behavior) may contribute to the differences in cytology patterns between HIV-positive and HIV-negative MSM. Cytology samples were collected more frequently for HIV-positive MSM, which could have increased the likelihood of concordant results over time as compared to HIV-negative MSM. However, in sensitivity analyses, this did not explain the differences in transition probabilities that we observed. Finally, while the MACS study is a large and rich data source for studying HIV among MSM, it may not be representative of all HIV-positive MSM in the U.S [45]. Despite these limitations, we hope that our comprehensive description of anal cytology patterns by HIV and CD4 status may inform management of anal cytology results and suggest new avenues for future research.

In conclusion, for HIV-negative and HIV-positive MSM, patterns of repeated anal cytology may prove useful as an indicator of low or high risk of anal disease. More than one-third of HIV-positive MSM have consistently negative annual anal cytology over 3 years, and lower CD4 counts are associated with consistently abnormal anal cytology and with a transition to abnormal cytology after a negative cytology. Further study of the cytology patterns we described, including direct relation of cytology patterns to biopsy-confirmed anal precancer, will be important to enable more effective anal cancer prevention for MSM.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.pvr.2018.04.001.

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