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Prostate-specific antigen is unlikely to be a suitable biomarker of semen exposure from recent unprotected receptive anal intercourse in men who have sex with men

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Abstract

A biomarker of unprotected receptive anal intercourse (RAI) could improve validity of sexual behavior measurement. We quantified prostate-specific antigen (PSA) from rectal swabs from men who have sex with men (MSM). One swab was PSA-positive. Using current methods, PSA is an inadequate biomarker of recent unprotected RAI in MSM.

Keywords

MSM; Receptive anal intercourse; Prostate specific antigen (PSA); Measurement; Validity; biomarker

Men who have sex with men (MSM) in the United States are particularly vulnerable to and disproportionately affected by HIV.

The estimated probability of HIV transmission per act of unprotected receptive anal intercourse (RAI) ranges from 0.27% (1) to 3.38% (2). Valid measurement of sexual

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behaviors, including RAI, is critical for HIV prevention interventions. Yet, to date, nearly all sexual health research has relied on self-reported data, despite questionable validity (3, 4). Individuals may not accurately report whether, when, and how often they engage in risky behaviors, and research findings based on self-reported information may be biased. Because no other measures have been available, despite the recognized limitations of self-reported sexual behavior data, all large-scale HIV prevention clinical trials among MSM rely on self-reported information (5, 6). A biomarker that replaces self-reported sexual behavior data would be a meaningful methodological advancement.

Prostate-specific antigen (PSA) is a protein produced in the prostate and secreted into the urethra during ejaculation. The detection of PSA in vaginal fluid has been used in forensic medicine, and more recently in research, as a biomarker of recent exposure to semen (7, 8, 9). PSA is highly specific in confirming exposure to ≥1mL of semen in women, with excellent detection immediately after exposure and almost complete clearance by 48 hours after exposure (7, 10). PSA has been used in many studies to assess the reliability of self-reported sexual behavior and as a proxy measure of condom efficacy (7, 8, 11).

We evaluated PSA as a biomarker of recent unprotected RAI among MSM. We hypothesized that rectal specimens would be PSA-positive only following recent unprotected RAI or RAI marked by condom misuse or malfunction.

This investigation was part of a larger study of MSM recruited from a public sexual health clinic in the Midwestern United States. Participants were 18 or older, spoke and read English, and reported anal intercourse (receptive or insertive) with another man within the past year. The subset of men who reported receptive anal intercourse within the past two weeks was included in this analysis.

Per clinic protocol, any man reporting RAI in the past year had a rectal swab collected for assessment of gonococcal and chlamydial infection via nucleic acid amplification tests. Swabs were inserted 2–4 centimeters into the rectum and gently rotated clockwise for 2–3 seconds to ensure adequate specimen collection. To measure PSA, an additional rectal swab was collected from each participant and frozen at −80°C until testing.

Following their physical examination, participants completed a comprehensive behavioral questionnaire. Exposure to semen was assessed through questions about sexual practices in three time periods: the last 24 hours, 24 to 48 hours ago, and 48 to 72 hours ago. For each time period, participants were asked whether: they had RAI; a condom was used; any issues occurred with condom usage; the partner ejaculated; the partner withdrew prior to ejaculation; and lubricant was used. Men were also asked to provide the date and time of their last bowel movement and last anal douching. Men were compensated $10.

PSA was measured using methods previously developed for vaginal swab specimens (7, 9, 12). Swabs were hydrated for 10 minutes in 1 mL of phosphate-buffered saline and vortexed vigorously to elute the contents into the buffer. Eluates were then centrifuged for 10 minutes at 10,000 RPM in a microcentrifuge to remove particulate matter; 300μL of the resulting supernatant was tested with the Abbott Architect Total PSA assay (Abbott Diagnostics, Abbott Park, IL, USA). The PSA assay has excellent sensitivity (detectable range: 0.01–100

ng/mL) and specificity. In accordance with prior studies using vaginal swab specimens, a positive PSA result was defined as ≥1 ng PSA/mL rectal swab eluate.

Statistical analyses were conducted using SAS (Version 9.3, Cary NC). Using a Kruskal-Wallis test, we compared PSA concentrations by self-reported sexual behavior within the past 48 hours: no RAI, protected RAI only, and at least one unprotected RAI act. Among those who reported any RAI within 72 hours, we used Spearman's rank correlation coefficient to quantify the association between PSA concentration and hours since last reported RAI (13).

Fifty-four men met eligibility criteria, provided informed consent, and enrolled. Participants were 18–56 years of age (median: 26 years). Two-thirds had completed at least some college (n=36). Eighty-one percent (n=44) identified as gay. When asked about position preference, nine men (17%) classified themselves as "mostly top", 23 (43%) as "half top and half bottom", 20 (37%) as "mostly bottom", and two men (4%) as "exclusively bottom".

Of 54 participants, 41 reported no RAI in the past 48 hours, 3 reported only protected (condom) RAI in the past 48 hours, and 10 reported unprotected RAI in the past 48 hours (Table 1). PSA concentrations for the 54 specimens ranged from 0.000 ng/mL to 1.512 ng/mL, with a median of 0.017 ng/mL (IQR: 0.003 to 0.040). One specimen with 1.512 ng/mL tested positive for PSA according to the 1 ng/mL threshold for positivity (Table 1). The single positive specimen was collected from a man who reported *protected* RAI 14 hours prior to swab collection, and no *unprotected* RAI in the 72 hours preceding swab collection.

PSA concentrations for men who reported no RAI in the past 48 hours (median, 0.017 ng/mL); protected RAI only (median, 0.051 ng/mL); or at least one unprotected act (median, 0.018 ng/mL) did not differ significantly (Kruskal-Wallis test, p=0.49) (Table 1). Among those who reported any RAI in the last 72 hours (n=21), PSA concentration was not significantly correlated with hours since last RAI (p=0.41). Figure 1 depicts the relationship among MSM who report RAI in the previous 72 hours between PSA concentration and hours since last RAI by self-report of RAI behavior (unprotected, protected, or none) in the previous 48 hours (Figure 1).

HIV/STI prevention research in women has been meaningfully strengthened by the availability of biomarkers of recent unprotected sex, including PSA. The validation of a biological marker of unprotected RAI would help to assess exposure to HIV or STIs and substantially improve the validity and reliability of prevention research in MSM.

Of 54 men, five reported RAI in the previous 24 hours (3 unprotected), 13 in the previous 48 hours (10 unprotected), and 21 in the previous 72 hours (14 unprotected). Given these self-reports, we detected PSA in substantially fewer men than expected, especially compared to similar work in women (3). Low PSA levels prevented us from performing more sophisticated analyses of discordance (positive PSA despite report of no unprotected sex). The low number of men reporting recent RAI may be related to our STD clinic sample. Men often present at an STD clinic because of symptoms or suspicion of infection, which may limit their sexual activity in the days immediately preceding the visit. However, Anderson et

al, recently detected PSA in 8% of symptomatic women presenting to an STD clinic, which provides support for our study design (14).

In women, PSA sensitivity is highest immediately after exposure (96%) and decreases over time, with 65% sensitivity six hours after sex, 21–29% after 24 hours, and 3–7% after 48 hours (7,10). Specificity ranges from 91–97% (7). Given the high specificity of the PSA assay in women, a positive test for PSA is unlikely to occur without exposure to semen. However, whether PSA persists similarly in the rectum and in men is unknown. The only previous study on PSA testing on rectal swabs from men was completed on cadavers, and found PSA in 64% of male rectal swabs (15). Sexual orientation of men in that study was unknown (15), and whether PSA migrated to the rectum post-mortem is unclear. Notably, the single individual in whom PSA was detected in our study reported *protected* RAI 14 hours prior to collection of the rectal swab, highlighting the potential weaknesses of self-report.

PSA concentration was not significantly correlated with time since last RAI among men who reported RAI in the past 72 hours. While Figure 1 suggests a correlation between PSA and time since last RAI among men with the most recent sexual encounters, with one exception, detected PSA levels in this small study were all below the accepted threshold for positivity and orders of magnitude below levels reported in post-coitus vaginal samples (3). Even if the positive result detected in this analysis represents a true positive, a biomarker with such limited range would be of limited utility, as it would be difficult to separate true positives from background noise. In contrast, PSA used for identifying vaginal exposures results in much higher concentrations. Gallo *et al* found PSA levels of 100 ng/mL or higher in more than 20% of women (8), while median PSA levels in our study were well below 1 ng/mL, even after stratification by timing of last RAI (Table 1).

We hypothesized that PSA clearance from the rectum could be affected by anal douching, bowel movements, or lubricant use (16). However, of the 13 men reporting sex in the last 48 hours, only one did not report having a bowel movement in the time between last RAI and his examination. Similarly, 12 of 13 reported lubricant use at last RAI. Thus, we could not assess the effect of these behaviors on PSA detection because of lack of variability in participant reports. Similarly, PSA detection may be affected by whether ejaculation occurred. Nine of the 13 men reporting RAI in the last 48 hours report ejaculation as part of the sex act.

Our data suggest that PSA is not a suitable biomarker of recent unprotected RAI in MSM recruited from a STD clinic. However, our data do not rule out a use for PSA in a highly controlled setting. Future studies may assess whether PSA can be reliably measured immediately after a known rectal exposure – in the absence of lubricant use, anal hygiene, or bowel movements – in order to use the biomarker in future studies to evaluate the effectiveness of barrier methods for RAI (17).

To our knowledge, the lack of association between PSA and recent RAI presented here is the first evidence that PSA, as currently measured, is not a suitable biomarker of unprotected RAI among MSM. The need for a biomarker in this population remains high.

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SHORT SUMMARY

Prostate-specific antigen (PSA), collected via rectal swab, is an inadequate biomarker of recent unprotected receptive anal intercourse among men who have sex with men (MSM).

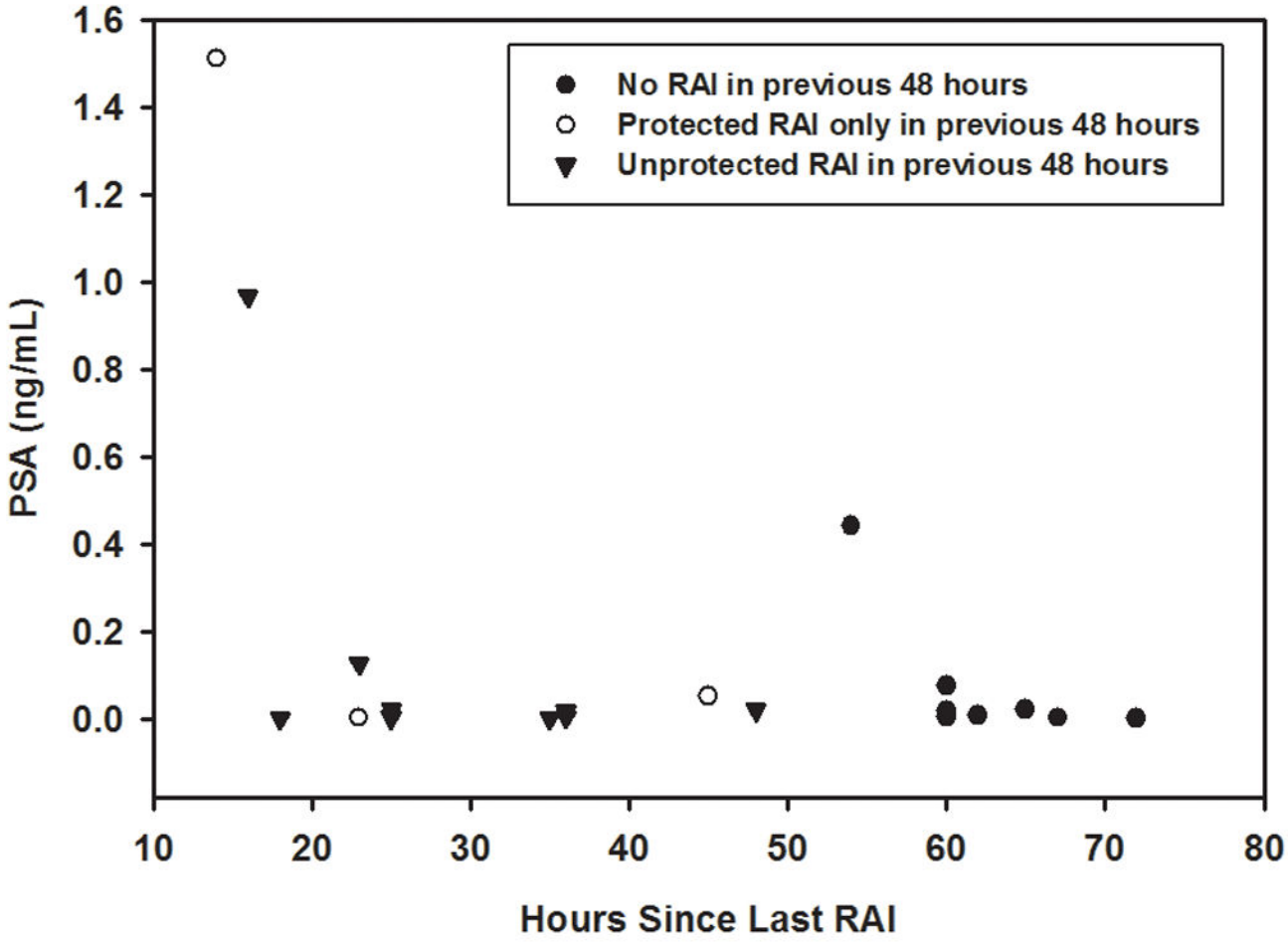

Figure 1.

Among those who reported RAI in the previous 72 hours (n=21), PSA concentration (ng/mL) by hours since last RAI

Table 1

PSA Level by Self-reported Sexual Behavior (n=54)

Self-Reports	Median (ng PSA/mL)	IQR (ng PSA/mL)	PSA Positive		PSA Negative	
			N	(%)	N	(%)
Past 24 hours						
>=1 Unprotected RAI	0.127	0.000–0.966	0	(0)	3	(6)
Protected RAI only	0.758	0.003–1.512	1	(2)	1	(2)
No RAI	0.017	0.003–0.034	0	(0)	49	(91)
Past 48 hours						
>=1 Unprotected RAI	0.018	0.000–0.020	0	(0)	10	(19)
Protected RAI only	0.051	0.003–1.512	1	(2)	2	(4)
No RAI	0.017	0.002–0.037	0	(0)	41	(76)