

Challenges to implementing biomarkers of semen exposure in clinical trials

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Previous efforts in this area

- ◆ Meeting entitled “Evaluation of Markers of Intercourse in Trials of Vaginal Barriers” held in Washington on June 15, 2004, sponsored by CONRAD and IBIS.
- ◆ Discussed:
 - ▶ Role of biomarkers in HIV/STI prevention and contraceptive studies
 - ▶ Data on PSA, MHS-5 antigen, Y-chromosome DNA, and others.
- ◆ Concluded that the further development of biomarkers should be actively pursued by two smaller groups:
 - ▶ Preclinical
 - ▶ Clinical
- ◆ This meeting expands previous work to include biomarkers not just of semen exposure but also of cervicovaginal inflammation and HIV/STI infection

Settings in which semen biomarkers could be useful

1) Early safety trials in which intercourse is not permitted:

- ▶ If irritation is seen but a semen biomarker is also found, it means intercourse (not the gel) may have caused the irritation

2) In postcoital studies of barriers:

- ▶ Semen biomarker *should* be found in the cervix when barrier is not used and should *not* be found when the barrier is used, if it is successful

Settings in which semen biomarkers could be useful - continued

- 3) In microbicide effectiveness trials in which all participants are advised to use condoms:
- ▶ If semen biomarker is seen, can assume condoms not always used: greater risk of HIV
 - ▶ In study with gel+condoms arm and condom-only arm:
 - Women in gel+condom arm may feel more protected and use condoms less
 - Creates greater risk of HIV in that arm and may make gel look less effective
 - Biomarker may help in interpreting trial results.

Settings in which semen biomarkers could be useful - continued

4) Assessing compliance

- ▶ Assumes that biomarker is gold standard
 - In case of discordance:
 - “I did not have sex” + positive biomarker
 - Person being untruthful
 - False positive
 - “I had sex” + negative biomarker:
 - Person being untruthful
 - Biomarker no longer present/washed out
 - False negative
- ▶ Requires a biomarker that has low false positive & false negative rates

What's wrong with using sperm?

- ◆ Progressively motile sperm *are* the endpoint in postcoital studies of barriers
- ◆ Requires fresh specimen
- ◆ Counting sperm is subjective and dependent on examiner effort (prone to false negative results)
- ◆ Sperm are absent in vasectomized men and sperm counts vary among non-vasectomized men
- ◆ Sperm are larger than many STI pathogens – blocking them does not = blocking STIs

The ideal semen biomarker:

- ◆ *In the male reproductive tract:*
 - ▶ Always present when the “item of interest” (HIV/STI pathogen or sperm) is present
 - ▶ Is either part of the item of interest or of approximately the same size.
 - ▶ Concentration and absolute amount per ejaculate show low variability within and among men.

The ideal semen biomarker (continued):

◆ *In the female reproductive tract:*

- ▶ Never present in the female tract unless exposure to semen containing the item of interest has recently taken place.
- ▶ Distribution and residence time in the female tract is reproducible and the same as the item of interest
- ▶ Amount required to create a risk of pregnancy or HIV/STIs is known.
- ▶ Assays are not affected by the test item (e.g., microbicide) or anything normally present in the female tract.

The ideal semen biomarker (continued):

◆ *Once collected:*

- ▶ The survival time between sampling and assaying should be:
 - Known
 - Long enough to allow handling and batching.
- ▶ The assay should be:
 - Easy
 - Accurate
 - Reproducible
 - Affordable
 - Sensitive
 - Specific
- ▶ with high negative and positive predictive values. 😊

Semen biomarkers: two broad categories

1. Biomarkers of seminal plasma:

- ▶ Prostate-specific antigen (PSA)
- ▶ Semenogelins
- ▶ Acid phosphatase (AP)
- ▶ Others

2. Biomarkers of spermatozoa and other cells present in semen:

- ▶ Spermatozoa
- ▶ Y-chromosome DNA
- ▶ Others

PSA – example of challenges

◆ Types of clinical studies of PSA

- ▶ PSA values after unprotected intercourse or vaginal inoculation with semen
- ▶ Values after coitus during which a physical barrier was used:
 - Male and female condoms
 - Cervical barriers

PSA – values after unprotected intercourse or vaginal inoculation with semen

- ◆ Some studies have found measurable PSA in all women tested immediately after unprotected intercourse
- ◆ Other studies have shown mixed results, however.
 - ▶ May be due to errors in self-reporting
- ◆ Alternative: inoculate the vagina with a known volume of semen in the clinic and then take vaginal samples at various time-points afterward.
 - ▶ Two studies done:
 - In one, all swabs taken immediately post exposure had PSA.
 - In the other, 98% sensitivity & 97% specificity - Details in Maurizio's talk

PSA – condom studies

- ◆ Studies have used less sensitive rocket electrophoresis assay
- ◆ Intact male condom without breakage and slippage: up to 19% of vaginal specimens had PSA
 - ▶ Condom may not have been used for the entire act, samples may have been contaminated, or there may have been an undetected break.
- ◆ Broken male condoms: 81% of vaginal samples had PSA
 - ▶ Unlikely that a break would be reported when there was none: results most likely reflect the low sensitivity of the rocket assay.
- ◆ Deliberately punctured male condoms: 35-60% of vaginal samples had PSA , depending on whether slippage or breakage was noted by the user.
 - ▶ Again, these results may reflect low sensitivity of the rocket assay.
- ◆ Despite these shortcomings, PSA was better than sperm counts at predicting pregnancy rates.

PSA – cervical barrier studies

- ◆ **CONRAD studies show that small amounts of PSA can always be found in the cervix after use of a barrier not thought to have been breached.**
 - ▶ **A cervical barrier alone, without the use of a chemical adjunct, is apparently not an absolute barrier**
 - ▶ **Cervical barriers have been associated with a lower incidence of STIs: it is unknown whether an absolute barrier is required.**

PSA - summary

- ◆ PSA is a useful, but not perfect, semen biomarker.
- ◆ Studies are limited by:
 - ▶ Differences in assay sensitivities
 - ▶ The need to rely on self-reports of intercourse
 - ▶ The lack of information on actual intactness of condoms
 - ▶ Possible interference with assays by the use of vaginal gels
 - ▶ The lack of knowledge of how much of a biomarker is needed to indicate increased risk of infection or pregnancy.

PSA – summary (continued)

- ◆ **Studies which use inoculation should carry much more weight than studies that rely on self-reporting.**
 - ▶ If self-reporting were accurate, there would be no need for a biomarker of semen exposure.
 - ▶ More studies of this type are needed and several are underway.
- ◆ **PSA may not provide truly quantitative information comparing individual women.**
 - ▶ The amount of PSA is too minute
 - ▶ Variability among women and in sampling scenarios is too large
 - ▶ For the same reasons, it is unlikely that a threshold level of PSA can be found, above which the risk of pregnancy or HIV/STIs can be assumed to be present.

Other biomarkers of seminal plasma

◆ Semenogelins

- ▶ Robin and Michael to cover

◆ Acid phosphatase (AP):

- ▶ Low specificity and low sensitivity - no longer considered an accurate biomarker of semen exposure.

◆ Others:

- ▶ Isozymes of lactate dehydrogenase (LDH-C4) and creatine phosphokinase (CPK-BB)
- ▶ Glyceryl phosphocholine
- ▶ Gamma-glutamyltransferase
- ▶ Prostaglandin E2 (PGE2).
- ▶ Specific assays exist for these, but none has been used as a biomarker of semen exposure in clinical trials

Biomarkers of spermatozoa and other cells present in semen

◆ Sperm

◆ Y-chromosome DNA:

- ▶ In one study Yc-DNA was detectable for up to 15 postcoital days, with a half-life clearance of 4 days.
- ▶ In another, mean Yc-DNA was significantly lower among consistent condom users.
- ▶ In a recent study, poor agreement was seen between Yc-DNA and PSA with poor repeatability for Yc-DNA identification, however.
- ▶ A new study comparing PSA and Y-c DNA is being conducted by the CDC and Johns Hopkins University

Biomarkers of spermatozoa and other cells present in semen (continued)

◆ Others:

- ▶ Gender differentiating alleles
- ▶ Gamete-specific nuclear proteins
- ▶ Sperm-specific antigens

Steps to an improved semen biomarker:

1) *Standardization of sampling procedures*

◆ Considerations include:

- ▶ Whether specimen is collected in the clinic or at home.
- ▶ Whether multiple samples are taken after a single coital act.
- ▶ How sampling is done: swabbing vs. cervicovaginal lavage (CVL) vs. tampon:
 - Women prefer swabs and self-sampling
 - Swab measures biomarker concentration
 - Lavage or tampons measure the absolute amount of biomarker in the vagina
 - Tampons may cause increase in vaginal fluid production and/or discomfort and mucosal damage

Steps to an improved semen biomarker: *Standardization of sampling procedures (cont)*

◆ Considerations also include:

- ▶ Where samples are taken, e.g., the cervical os vs. cervical face vs. inside cervical barrier.
- ▶ Presence of spermicide.
- ▶ Post-collection handling.

Steps to an improved semen biomarker:

2) *Understanding differences among women*

◆ Volume of cervical and vaginal secretions:

▶ In one study of 22 women:

- 1.55 gm produced in 8 hours, on average
- Highest on day 14: 1.96 gm/8 hr
- Lowest on day 7: 1.3 gm/ 8 hr
- All values had large ranges

▶ Affected by:

- Infection
- Menses
- Vaginal products (medications, lubricants, douches, tampons, etc.)

▶ These factors should be considered in clinical trials of biomarkers

Issues that deserve further research in the field of semen biomarkers

1) *More studies of existing biomarkers (e.g. PSA, Y-chromosome DNA)*

- ▶ More studies of sensitivity/specificity and vaginal residence time (“decay curve”), using vaginal inoculation
- ▶ Correlation of levels of biomarker with risk of pregnancy and HIV/STI infection
- ▶ Determine consistent methodology for collecting specimens and carrying out the assay.

Issues that deserve further research in the field of semen biomarkers (cont.)

1) *Studies of existing biomarkers (e.g. PSA, Y-chromosome DNA) - continued*

- ▶ Determine interference by various vaginal products and cervicovaginal secretions.
- ▶ Compare assays with differing levels of sensitivity (e.g., rocket immunoelectrophoresis vs. ELISA in the case of PSA); determine the best use for each assay.
- ▶ Evaluate air drying vs. use of desiccant vs. freezing as a post-collection method of storage.
- ▶ All 3 of these are being addressed by members of the clinical working group

Issues that deserve further research in the field of semen biomarkers (cont.)

2) *Studies of new biomarkers:*

- ▶ Use of proteomic data on sperm and seminal plasma to select new biomarkers of semen.
 - Ongoing study at the University of Alabama Birmingham of PSA and SELDI-TOFMS - Details in Andrzej's talk
- ▶ Set up analytical assays to quantify those biomarkers.
- ▶ Additional studies as already described for existing markers

Issues that deserve further research in the field of semen biomarkers (cont.)

3) *Studies of factors affecting the amount of biomarker present:*

- ▶ Additional studies on the volume of cervicovaginal fluid present in healthy women in the follicular and luteal phases
- ▶ Assessment of the utility of epithelial cells and mucin to verify vaginal self-sampling – stay tuned for Robin's talk.

Issues that deserve further research in the field of semen biomarkers (cont.)

4) *Studies of protocol compliance - example*

- ▶ Phase III study of the diaphragm used with and without Replens gel is being done in Zimbabwe (the MIRA trial)
- ▶ Audio computer-assisted self interview (ACASI) is used to collect data on sexual behavior and product use.
 - ACASI may improve self-report compared to face to face (FTF) interviewing.
- ▶ After completing the trial, subset of women will be randomized either to one additional ACASI session or to FTF interview about recent sexual activity.
- ▶ Proportion reporting no sex in past 48 hours but testing positive for PSA will be compared by interviewing technique.
- ▶ Will help with interpretation of the main study results by giving information on whether ACASI data collected during the main trial is more valid than what would have been collected with FTF interviewing alone.

Issues that deserve further research in the field of semen biomarkers (cont.)

5) As a first step in finding a biomarker for HIV/STI infection:

- ▶ Correlation of size and cellular uptake of seminal biomarkers and STI pathogens of bacterial and viral origin.

In conclusion

- ◆ Sensitive, specific, easy to assay, reliable and validated biomarkers of semen are critical for efficient testing of new vaginal contraceptives & microbicides.
- ◆ It is likely that more than one biomarker of semen exposure will be developed for use in different settings.
 - ▶ Surrogate biomarkers of HIV infection may be combined with semen biomarkers to assess chemical/physical barrier efficacy and protocol compliance.

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