Plasma Antibodies against *Trichomonas vaginalis* and Subsequent Risk of Prostate Cancer

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Abstract

Background: **Although several previous case-control studies have investigated associations between sexually transmitted infections (STI) and prostate cancer, most have focused on gonorrhea and syphilis, two well-recognized, symptomatic STIs. Another STI of interest for prostate carcinogenesis is trichomonosis, a less well recognized and frequently asymptomatic STI with known prostate involvement. We investigated this infection in relation to incident prostate cancer in a nested case-control study within the Health Professionals Follow-up Study.**

Methods: **Prostate cancer cases were men diagnosed with prostate cancer between the date of blood draw (1993-1995) and 2000 (n = 691). Controls were men who had had at least one prostate-specific antigen test and who were free of prostate cancer and alive at the time of case diagnosis. One control was individually matched to each case by age (n = 691). Serologic evidence of a history of trichomonosis was assessed by a recombinant *Trichomonas vaginalis* α-actinin IgG ELISA.**

Results: **Thirteen percent of cases and 9% of controls were seropositive for trichomonosis (adjusted odds ratio, 1.43; 95% confidence interval, 1.00-2.03). This association persisted after additional adjustment for such factors as a history of other STIs, and was strongest among men who used aspirin infrequently over the course of their lives (odds ratio, 2.05; 95% confidence interval, 1.05-4.02, $P_{interaction}$ = 0.11).**

Conclusions: **Serologic evidence of a history of trichomonosis was positively associated with incident prostate cancer in this large, nested case-control study of male health professionals. As this study is the first, to our knowledge, to investigate associations between *T. vaginalis* serology and prostate cancer, additional studies are necessary before conclusions can be made.** (Cancer Epidemiol Biomarkers Prev 2006;15(5):939–45)

Introduction

Previous case-control studies have observed positive associations between sexually transmitted infections (STI) and prostate cancer (1). However, many of these studies were limited by their small sample size, retrospective assessment of STI history, and narrow definition of STI exposure, typically only gonorrhea and syphilis. Another possible STI of interest is trichomonosis (*Trichomonas vaginalis* infection). This STI is much less well recognized and less symptomatic than gonorrhea or syphilis, which may allow it to persist for longer periods of time in the male genitourinary tract, and possibly to progress to the prostate, where it has been observed to infect prostatic epithelium and elicit an inflammatory immune response (2, 3).

Only one small case-control study has investigated potential associations between trichomonosis and prostate cancer, the results of which were largely inconclusive due to a lack of reported history of trichomonosis among both cases and controls (4). However, these self-reported rates of trichomonosis likely underestimated the true infection histories of participants because self-reported rates of more traditional, symptomatic STIs were higher than rates of trichomonosis in this study population and because trichomonosis is frequently asymptomatic in men, infrequently diagnosed in men seeking STI care, and generally not well-recognized as an STI.

To further explore associations between STIs and prostate cancer and to address some of the limitations of previous studies, we conducted a large nested case-control study of trichomonosis and incident prostate cancer in the Health Professionals Follow-up Study. A history of trichomonosis was assessed by antibody serostatus before prostate cancer diagnosis to establish a temporal relationship between trichomonal exposure and prostate cancer, to limit differential ascertainment biases, and to capture asymptomatic infections, symptomatic infections of possibly unrecognized origin, and infections that might otherwise not be captured by self-report. To our knowledge, this study is the first to investigate *T. vaginalis* serology and prostate cancer.

Materials and Methods

Study Population and Design. The Health Professionals Follow-up Study is an ongoing, prospective study of cancer and heart disease in men. It includes 51,529 American male health professionals ages 40 to 75 years at enrollment in 1986. At that time, participants completed a baseline epidemiologic questionnaire on demographics, lifestyle, and medical history,

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and a semiquantitative food frequency questionnaire. Since 1986, participants have completed questionnaires every 2 years to update exposure and disease information, and every 4 years to update dietary information. Between 1993 and 1995, 18,018 participants provided a blood sample for research purposes. These participants were similar to those who did not provide a blood sample on demographic and dietary factors. Blood samples were collected in tubes containing sodium EDTA and shipped to the Harvard School of Public Health by overnight courier in a chilled container. Upon arrival, specimens were centrifuged; aliquoted into plasma, buffy coat, and erythrocytes; and stored in liquid nitrogen.

On each biennial follow-up questionnaire, participants were asked to report prostate cancer diagnoses within the past 2 years. Information on death was obtained from the National Death Index, U.S. Postal Service, or next of kin in response to mailed follow-up questionnaires. We estimated that 96% of prostate cancer diagnoses and >98% of deaths were ascertained by these methods. Prostate cancer diagnoses were confirmed by medical record and pathology report review with permission from the participant or next of kin (>90% confirmation rate to date). Deaths from prostate cancer were confirmed by death certificate review. Disease stage (tumor-node-metastasis classification; ref. 5) and Gleason sum were abstracted from medical records by study investigators.

All participants who provided a blood sample in 1993 to 1995, who were free of reported cancer (except nonmelanoma skin cancer) at the time of blood draw, and who provided valid baseline food frequency information were eligible for inclusion in the nested case-control study. Cases were all men diagnosed with prostate cancer between the date of blood draw and January 31, 2000 (n = 691). Participants diagnosed with stage T_{1a} prostate cancers (n = 2) were not included as cases because their tumors comprise ≤5% of resected prostate tissue and are detected, by definition, at transurethral resection of the prostate for benign prostatic hyperplasia. Therefore, these tumors may be especially prone to detection bias due to varying rates of benign prostatic hyperplasia surgery by exposure. Controls were selected from among participants who had had at least one prostate-specific antigen (PSA) test between the date of blood draw and case diagnosis to allow for detection of possible occult prostate cancer. Controls were also required to be free of reported cancer (except nonmelanoma skin cancer) and alive at the time of case diagnosis. One control was matched to each case by age (±1 year), year (exact), time (midnight-9 a.m., 9 a.m.-noon, noon-4 p.m., and 4 p.m.-midnight) and season (January-March, April-June, July-September, and October-December) of blood draw in 1993 to 1995, and PSA testing history before 1993 to 1995 (yes/no). This study was approved by the Human Subjects Committee at the Harvard School of Public Health and the Committee on Human Research at the Johns Hopkins Bloomberg School of Public Health.

***T. vaginalis* Antibody Assessment.** *T. vaginalis* antibody serostatus was assessed by a novel ELISA in the laboratory of Dr. John F. Alderete. This assay detects IgG antibodies against purified, recombinant *T. vaginalis* α-actinin protein, one of the most immunogenic trichomonad proteins (6).

Recombinant *T. vaginalis* α-actinin antigen was prepared by inoculating a single *Escherichia coli* XL1-Blue (Stratagene, La Jolla, CA) colony containing a recombinant plasmid with the full-length *α-actinin* gene (7, 8) into 200 mL of Luria-Bertani medium. This colony was incubated for 5 hours at 37°C, after which it was induced with 1.0 mmol/L isopropyl-β-D-thiogalactopyranoside for 3 hours. The bacteria were then harvested by high-speed centrifugation, washed with 50 mmol/L Tris-HCl (pH 8.0), suspended in lysis buffer [62.5 mmol/L Tris-HCl (pH 6.8) containing 2% SDS, 10% glycerol, and 2% β-mercaptoethanol], and boiled for 5 minutes. The resulting lysates were centrifuged at 6,500 × g to remove insoluble debris and the α-actinin protein was purified by continuous elution electrophoresis (Model 491 Pre Cell, Bio-Rad, Hercules, CA). Fractions containing recombinant protein were detected by dot-blot immunoassays using a monoclonal antibody (ACT1) specific to α-actinin. Positive fractions were pooled and dialyzed against 10 mmol/L Tris-HCl (pH 8.0). Protein concentrations were measured by the Bradford assay (9) and adjusted to 20 μg/mL with 50 mmol/L sodium carbonate buffer [5.229 g Na_2CO_3, 4.2 g $NHCO_3$ in double-distilled water (pH 9.6)].

Ninety-six well ELISA plates (MAXISORP Immuno Module; Nunc, Roskilde, Denmark) were coated with 100 μL/well of diluted recombinant protein and incubated overnight at 4°C. The plates were then washed thrice with PBS-Tween 20 [0.05% Tween 20 in PBS (pH 7.0)] followed by the addition of 200 μL/well of blotting buffer (10% skim milk in PBS). After incubation at room temperature for 1 hour, the plates were washed once with PBS-Tween 20 and either used immediately or air-dried at room temperature and stored at 4°C.

Prostate cancer case and control plasma samples were diluted at 1:25 (v/v) in PBS-Tween 20 containing 10% skim milk and 100 μL of diluted plasma was added to each well. After incubation for 2 hours at 37°C, the plates were washed thrice with PBS-Tween 20, followed by the addition of 100 μL/well of secondary goat anti-human IgG (Fab fragment) conjugated to horseradish peroxidase at 1:1,500 dilution in PBS. Plates were incubated again for 2 hours at 37°C and then washed thrice with PBS-Tween 20. Color development was done by adding 100 μL/well of substrate solution (ABTS; phosphate-citrate buffer with 0.03% sodium perborate, Sigma Chemical Co., St. Louis, MO), and incubating the plates at room temperature for 15 minutes. Absorbance values of antibody were assessed by examining the supernatants spectrophotometrically at A_{405} using an ELISA reader (Bio-TEK Instruments, Inc., Winooski, VT).

All prostate cancer case and control samples were tested in duplicate (absorbance intra-assay coefficient of variation = 14%). Absorbance scores were assigned to each sample based on the mean duplicate absorbance value (0, absorbance < 0.150; 1+, 0.150 ≤ absorbance < 0.250; 2+, 0.250 ≤ absorbance < 0.500; 3+, 0.500 ≤ absorbance < 1.000; 4+, absorbance ≥ 1.000). All samples with scores 1+ through 4+ were retested in duplicate and observed to have the same absorbance score. Samples with *high* 2+ absorbance scores (0.400 ≤ absorbance < 0.500) were considered indicative of a history of trichomonosis, and those with 3+ or 4+ absorbance scores (absorbance ≥ 0.500) were considered highly indicative of a history of trichomonosis based on expert opinion (J.F. Alderete) without knowledge of prostate cancer status. Samples were tested in random case-control pair order, with case and matching control samples adjacent to one another, but in random within-pair order, and laboratory technicians blinded to the case-control status of each sample. Assay reliability was assessed by including two blinded seropositive and seronegative samples in the testing sequence for each plate (κ = 0.79). Samples of known serostatus were used as opposed to duplicate samples because of the low expected seroprevalence of trichomonosis in this population.

Statistical Analysis. To characterize participants and investigate confounding, means and proportions of known or suspected STI correlates or prostate cancer risk factors were compared by prostate cancer status for all participants and by *T. vaginalis* antibody serostatus for prostate cancer controls. Antibody absorbance means, medians, and proportions were calculated for prostate cancer cases and controls, and compared by paired t tests, Wilcoxon signed rank, McNemar's, and likelihood ratio tests. Conditional logistic regression was used to calculate matched multivariable-adjusted odds ratios (OR) and 95% confidence intervals (95% CI) for prostate cancer. Multivariable models included terms for known prostate cancer risk factors: race/ethnicity and family history of prostate cancer (cumulative through 1996). Additional models included

terms for height (inches), cigarette smoking in the past 10 years (pack-years), intakes of total energy (kcal/d), alcohol (g/d), tomato sauce (servings/d), red meat (servings/d), fish (servings/d), fructose (g/d), calcium (mg/d), and α-linolenic acid (g/d) in 1994, vitamin E supplementation, vigorous physical activity (metabolic equivalent-hours/wk), vasectomy, and diabetes mellitus type 2 in 1994, aspirin use since age 20 years, histories of other assessed STIs and clinical prostatitis, ejaculation frequency from ages 20 to 29 years (times/mo), alcohol consumption from ages 18 to 22 years (drinks/wk), cigarette smoking before age 30 years (pack-years), body mass index at age 21 years (kg/m^2), and vigorous physical activity in high school and college. As these variables did not alter the point estimate for *T. vaginalis* seropositivity, they were not retained in the primary model. The main outcome for each analysis was total prostate cancer. Additional outcomes considered were organ-confined (≤stage T_2 and N_0M_0), advanced (≥stage T_{3b}, or N_1 or M_1), low-grade (Gleason sum<7), and high-grade (Gleason sum ≥7) prostate cancer.

To investigate whether associations varied by surrogates of prostatic inflammation, stratified analyses were done by age at blood draw (<50, ≥50 years) as a marker for opportunity to have acquired trichomonosis before the introduction of metronidazole in 1959 (cited in ref. 10), and by reported aspirin use since age 20 years. Aspirin use was calculated by dividing the total number of years of reported aspirin use (of any frequency from age 20 years to age at blood draw) by the number of years of potential aspirin use. This percentage was then divided into infrequent (0-20% of the time), moderate (20-79%), and regular (80-100%) use over the course of participants' lives. Reasons for aspirin use were not collected in the full cohort. However, in a subset of 185 participants who reported aspirin use from 1986 to 1990, reasons for current aspirin use were cardiovascular disease

Table 1. Age-standardized characteristics of nested case-control study participants by prostate cancer and *T. vaginalis* plasma antibody status, Health Professionals Follow-up Study 1994

	Prostate cancer case and matched control status		*P**	*T. vaginalis* antibody serostatus (controls only)		*P*†
	Cases (*n* = 691)	Controls (*n* = 691)		Seropositive (*n* = 65)	Seronegative (*n* = 626)	
Mean age at blood draw (y)	65.8	65.7	Matching variable	65.8	65.7	0.98
Caucasian race (%)	94.6	94.2	0.56	92.4	94.4	0.52
Family history of prostate cancer (%)‡	21.8	16.8	0.02	9.3	17.6	0.08
Mean height in 1986 (in.)	70.1	70.1	0.76	70.2	70.1	0.86
Smoked cigarettes in the past 10 y (%)	16.5	17.7	0.61	14.4	18.0	0.46
Mean intakes of						
Total energy (kcal/d)	2,012	2,035	0.47	1,936	2,045	0.18
Alcohol (g/d)	11.8	11.5	0.70	13.0	11.4	0.40
Tomato sauce (servings/d)§	0.19	0.19	0.89	0.17	0.18	0.28
Red meat (servings/d)§	1.0	1.0	0.70	0.9	1.0	0.06
Fish (servings/d)§	0.31	0.33	0.04	0.36	0.33	0.40
Fructose in 1990 (g/d)‖	49.9	49.4	0.60	49.9	49.4	0.79
Calcium (mg/d)	950.1	951.5	0.95	899.1	956.9	0.32
α-Linolenic acid (g/d)‖	1.1	1.1	0.93	1.1	1.1	0.79
Vitamin E supplementation (%)	37.5	34.4	0.28	32.0	34.7	0.66
Any vigorous leisure-time physical activity (%)	58.0	57.4	0.87	61.3	57.0	0.51
Vasectomy (%)	25.9	26.5	0.85	32.3	25.9	0.26
Diabetes mellitus type 2 (%)	6.2	5.8	0.82	1.6	6.2	0.13
Aspirin use since age 20 y (%)¶						
Infrequent (0-19% of the time)	26.9	23.6		22.9	23.7	
Moderate (20-79% of the time)	29.5	31.8	0.40	29.8	32.0	0.68
Regular (80-100% of the time)	39.1	39.1		41.2	38.9	
PSA test after blood draw (%)**	99.6	99.7	1.00	100.0	99.4	0.52
Digital rectal examination after blood draw (%)**	98.1	96.8	0.16	97.1	96.8	0.90
History of (%)††						
Gonorrhea	2.6	2.6	1.00	1.4	2.7	0.54
Syphilis	0.14	0.00	1.00	0.0	0.0	–
Clinical prostatitis	22.0	18.0	0.07	21.8	17.6	0.39
Mean monthly ejaculation frequency, ages 20 to 29 y††	13.6	14.0	0.17	14.3	14.0	0.70
Consumed alcohol, ages 18 to 22 y (%)‡‡	69.6	68.2	0.61	75.4	67.4	0.18
Smoked cigarettes before age 30 y (%)§§	47.9	49.5	0.59	46.5	49.8	0.61
Mean body mass index at age 21 y (kg/m^2)§§	22.8	22.9	0.24	23.2	22.9	0.48
Any vigorous physical activity (%) in††						
High school	84.8	85.0	1.00	86.5	84.8	0.72
College	76.6	76.8	0.95	80.4	76.5	0.47

NOTE: Data are age-standardized by matching for comparisons between prostate cancer cases and controls, and by multivariable linear regression (including 2-year age indicator variables) for comparisons between *T. vaginalis* antibody seropositive and seronegative participants.

*Assessed by paired *t* test for continuous variables, McNemar's test for dichotomous variables, and the likelihood ratio test for polychotomous variables.

†Assessed by the Wald test.

‡Assessed in 1990 to 1996.

§Cumulative mean intake between 1986 and 1994.

‖Adjusted for total energy intake.

¶Percentages do not sum to 100% due to missing values.

**Assessed through 2000.

††Assessed in 1992.

‡‡Assessed in 1988.

§§Assessed in 1986.

Table 2. *T. vaginalis* α-actinin antibody levels in 691 prostate cancer cases and 691 matched controls in the Health Professionals Follow-up Study, 1993 to 2000

	Cases	Controls	P^*
Mean absorbance	0.22	0.21	0.24
Median absorbance	0.16	0.16	0.59
Score (n)			
0 (absorbance < 0.150)	312	302	
1+ (0.150 ≤ absorbance < 0.250)	167	193	
2+ (0.250 ≤ absorbance < 0.500)	158	158	0.21
3+ (0.500 ≤ absorbance < 1.000)	50	37	
4+ (absorbance ≥ 1.000)	4	1	
≥3+	54	38	0.11
Absorbance ≥ 0.400 (n)	87	65	0.07

*Assessed by paired *t* test for mean values, Wilcoxon signed rank test for median values, McNemar's test for dichotomous variables, and the likelihood ratio test for polychotomous variables.

(25.4%), cardiovascular disease prevention (58.4%), headaches (25.4%), joint or musculoskeletal pain (33.0%), and other reasons (7.0%; ref. 11). Additional stratified analyses were done by age at prostate cancer diagnosis and by prostate cancer testing (number of 2-year intervals with a digital rectal examination or PSA test) to investigate the possibility of detection bias. All stratified analyses, with the exception of those for age, were done by unconditional logistic regression, including exposure and matched variable terms. The statistical significance of any observed stratum-specific differences was assessed by including an additional cross-product term and evaluating its coefficient by the Wald test.

Results

The majority of prostate cancer cases were organ-confined (83% of 614 cases with stage information) with Gleason sums between 5 and 7 (79% of 647 cases with grade information at diagnosis). The mean age at prostate cancer diagnosis was 68.9 years, with a range of 47.7 to 84.3 years. The mean time from blood draw to diagnosis was 3.1 ± 1.7 years. In general, prostate cancer cases were similar to their matched controls, with the exception of differences by family history of prostate cancer, fish consumption, history of digital rectal examinations and clinical prostatitis, and ejaculation frequency from ages 20 to 29 years. Among controls, *T. vaginalis* antibody seropositive participants differed from seronegative participants by family history of prostate cancer, total energy and red meat consumption, history of diabetes, and alcohol consumption from ages 18 to 22 years (Table 1).

Although prostate cancer cases and controls had similar mean and median *T. vaginalis* antibody levels, a borderline significantly greater proportion of cases had high absorbance scores (absorbance ≥ 0.400, 12.6%) than controls (9.4%, P = 0.07; Table 2). This difference was strengthened slightly after adjustment for race/ethnicity and family history of prostate cancer (OR, 1.43; 95% CI, 1.00-2.03; Table 3). Similar associations were observed after further adjustment for additional covariates, including a history of other assessed STIs (OR, 1.42; 95% CI, 1.00-2.02) and clinical prostatitis (OR, 1.42; 95% CI, 1.00-2.02), and for organ-confined, and low- and high-grade prostate cancer (Table 3). When the data were stratified by aspirin use since age 20 years, a significant positive association was observed between *T. vaginalis* seropositivity and prostate cancer among men who used aspirin infrequently over the course of their lives, a weaker association was observed among moderate users, and no association was observed among regular users (Table 4). No effect modification was suggested for age at prostate cancer diagnosis or digital rectal examination/PSA testing history (all $P > 0.20$).

Discussion

In this large nested case-control study of male health professionals, a positive association was observed between *T. vaginalis* antibody seropositivity and prostate cancer. This association persisted across early stage, and low and high grades of prostate cancer, and after adjustment for a history of other STIs, STI correlates, and prostate cancer risk factors. It was particularly strong among men who used aspirin infrequently over the course of their lives.

We originally investigated *T. vaginalis* seropositivity in relation to prostate cancer because trichomonads have been observed to infect and elicit a strong inflammatory immune response within the prostate (2, 3) and because intraprostatic inflammation is believed to influence later risk of prostate cancer (12). However, beyond this observation, relatively little is known about trichomonosis in men. Although a small proportion of infections are known or suspected to cause nongonococcal urethritis, clinical prostatitis, and other genitourinary conditions in men between the ages of 15 to 40 years, the majority of trichomonal infections are believed to be asymptomatic. However, the duration and site of these asymptomatic infections is unclear (13). Very little is also known about the serologic response to *T. vaginalis* infection. In studies conducted to date, serum antitrichomonad antibodies have been observed in male and female patients with current trichomonosis (14-27), and in those with a documented history of trichomonosis months to years previously (16, 21). Although not specifically investigated for *T. vaginalis*, the response to other genital, mucosal infections (e.g., *Chlamydia trachomatis* infection) is frequently stronger in those with chronic rather than acute infections (28). Based on this information, the typically lower age of *T. vaginalis* infection and the high antibody titers observed in our study population, we inferred that a large proportion of detected infections were chronic, asymptomatic infections, which, without treatment, may have progressed to the prostate. Indeed, early trichomonosis researchers believed the prostate to be the reservoir for *T. vaginalis* based on its frequent detection in prostate specimens from husbands/partners of women with trichomonosis (29-33).

Table 3. OR and 95% CI values of prostate cancer for *T. vaginalis* antibody serostatus in 691 matched pairs nested in the Health Professionals Follow-up Study, 1993 to 2000

	Seronegative		Seropositive		
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)*	Multivariable-adjusted OR (95% CI)*,†
Total prostate cancer	604/626	1.00	87/65	1.41 (0.99-2.00)	1.43 (1.00-2.03)
Organ-confined ($\leq T_2$ and N_0M_0)	440/455	1.00	67/52	1.35 (0.91-2.00)	1.36 (0.92-2.02)
Low-grade (Gleason sum <7)	333/342	1.00	47/38	1.30 (0.81-2.09)	1.27 (0.79-2.06)
High-grade (Gleason sum ≥7)	210/222	1.00	33/21	1.67 (0.93-2.99)	1.76 (0.97-3.18)

NOTE: Too few participants were diagnosed with advanced-stage prostate cancer (n = 62) to investigate its association with *T. vaginalis* antibody serostatus.
*Estimated by conditional logistic regression.
†Adjusted for race (Caucasian, non-Caucasian) and cumulative family history of prostate cancer through 1996 (yes/no).

Table 4. OR and 95% CI values of total prostate cancer stratified by aspirin use since age 20 years in 691 matched pairs nested in the Health Professionals Follow-up Study, 1993 to 2000

	T. vaginalis antibody seronegative		*T. vaginalis* antibody seropositive		
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)*	Multivariable-adjusted OR (95% CI)*,†
Aspirin use since age 20 y ‡					
Infrequent use (0-19% of the time)	154/148	1.00	32/15	2.00 (1.02-3.89)	2.05 (1.05-4.02)
Moderate use (20-79% of the time)	181/201	1.00	23/19	1.33 (0.70-2.55)	1.37 (0.71-2.62)
Regular use (80-100% of the time)	241/243	1.00	29/27	1.03 (0.59-1.80)	1.05 (0.60-1.85)§

*Estimated by unconditional logistic regression, including terms for *T. vaginalis* antibody serostatus, age, time of day, season and year of blood draw, and PSA screening history before blood draw.
†Adjusted for race (Caucasian, non-Caucasian) and cumulative family history of prostate cancer through 1996.
‡Percentage of years that aspirin was used with any frequency.
§*P* values for interaction with aspirin use = 0.11.

In men with chronic, prostatic infection, trichomonosis may potentially contribute to the development of prostate cancer by several different, but synergistic, mechanisms. First, *T. vaginalis* adhesion has been observed to up-regulate expression of numerous host epithelial cell genes, including proinflammatory genes, such as *interleukin-8* and *monocyte chemoattractant protein-1* (34), involved in the recruitment of large numbers of neutrophils and monocytes/macrophages to the site of infection. These inflammatory cells secrete oxygen- and nitrogen-based reactive molecules, which may inadvertently damage cellular DNA and nearby cells. Second, *T. vaginalis* may directly lyse host epithelial cells (35). Cellular lysis necessitates cellular renewal, which may be facilitated by the controlled secretion of growth factors from inflammatory cells. However, if uncontrolled, this secretion may potentially lead to hyperproliferation of prostatic epithelial cells. Additionally, proliferating cells may be more vulnerable to a potentially genotoxic, inflammatory environment than non-proliferating cells. Third, *T. vaginalis* adhesion has also been observed to up-regulate expression of antiapoptotic genes, such as *defender against cell death* and *cycloxygenese-2* (34), which may prevent apoptosis of damaged cells. In response to inflammation and cell injury/death due to infection or other possible sources, proliferative inflammatory atrophic lesions, regenerative prostatic lesions characterized by highly proliferative atrophic epithelial cells and decreased levels of apoptosis are believed to form near areas of chronic and, at times acute, inflammation. These lesions have been postulated to serve as prostate cancer precursor lesions or as markers of a cellular environment conducive to the development of prostate cancer (36). Although not specifically described as proliferative inflammatory atrophic lesions, focal areas of epithelial hyperplasia have been observed near *T. vaginalis* organisms and associated inflammatory infiltrates in older histologic studies of prostate tissue (37, 38). Independent of proliferative inflammatory atrophic lesions or subsequent to their formation, trichomonosis may also contribute to the development of or progression toward prostate cancer by the mechanisms just described.

To explore the potential role of inflammation in the observed association between *T. vaginalis* seropositivity and prostate cancer, we adjusted the analyses for a history of clinical prostatitis (which encompasses symptomatic prostatic infection/inflammation) and stratified the analyses by crude surrogates of prostatic inflammation. Adjustment for clinical prostatitis did not affect the point estimate for *T. vaginalis* seropositivity, suggesting that the observed association between *T. vaginalis* seropositivity and prostate cancer was unlikely to have been mediated by symptomatic prostatic inflammation. The slightly higher prevalence of clinical prostatitis among cases was contrary to our previous findings in this cohort and may be explained by sampling variation.[7] Although not conclusive, the stronger observed association among men who used aspirin infrequently over the course of their lives than among regular aspirin users is consistent with a potential role for symptomatic or asymptomatic inflammation.

An additional, possibly more speculative mechanism by which trichomonosis may contribute to the development of prostate cancer is by altering luminal concentrations of spermine and putrescine, polyamines involved in cellular growth, differentiation, and death (39). As trichomonads cannot synthesize spermine, they must import exogenous spermine in exchange for putrescine through an antiporter system (40). This exchange may lead to increased extracellular levels of putrescine, as observed in genital secretions from patients with trichomonosis (41), and possibly to decreased levels of extracellular spermine. Interestingly, spermine has been observed to inhibit prostatic carcinoma growth *in vitro* and *in vivo* (42), and lower concentrations of spermine have been observed in malignant prostate tissue than in normal or hyperplastic tissue (43), suggesting that spermine may be involved in prostate carcinogenesis. Finally, the fact that putrescine secretion by trichomonads contributes to a complex interplay between parasite and host cells is evidence of the intricate interrelationship that exists during infection (44).

In our study population, 13% of cases and 9% of controls were seropositive for trichomonosis. In the only other epidemiologic study of trichomonosis and prostate cancer to date, none of the 40 prostate cancer cases nor 64 benign prostatic hyperplasia controls reported a history of trichomonosis (4). However, as stated previously, these rates may have underestimated the participants' true infection histories because rates of more symptomatic STIs (gonorrhea and syphilis) were much higher in this study population than rates of trichomonosis, and because trichomonosis is frequently asymptomatic in men, presumptively treated rather than specifically diagnosed in symptomatic men, and generally not well-recognized as an STI. In other studies unrelated to prostate cancer, estimates of the seroprevalence of trichomonosis have ranged from 1% in male Scottish STI clinic patients (45) to 65% in American military personnel with nongonococcal urethritis (17). The seroprevalence of trichomonosis in men unselected for STIs and using a higher sensitivity assay, as was used in our study, is unknown. However, irrespective of the generalizability of seroprevalence estimates in this cohort, we expect that our observed positive association between *T. vaginalis* seropositivity and prostate cancer is likely applicable to other populations.

Alternate noncausal explanations for our findings include increased diagnostic work-up for prostate cancer (e.g., prostate biopsy) among men with chronic, latent trichomonosis due to inflammation-mediated increased PSA. The likelihood of this possibility depends on the type of infection detected by antibody seropositivity, which we suspect to be chronic asymptomatic infection, and the propensity of trichomonosis-mediated inflammation to increase circulating levels of PSA

[7] S. Sutcliffe, et al. Gonorrhea, syphilis, clinical prostatitis, and the risk of prostate cancer, submitted for publication.

to clinically elevated concentrations. A second possibility is residual confounding by other sexually transmitted agents causally associated with prostate cancer and more frequently transmitted with *T. vaginalis* than *Neisseria gonorrhoeae*, *Treponema pallidum*, human papillomavirus, or *C. trachomatis*, as no associations were observed between these agents and prostate cancer in other analyses conducted in this cohort.[7,8] Residual confounding by other unmeasured risk factors or the strength of participants' general humoral immune response is also unlikely, again because no associations were observed between histories of other STIs and prostate cancer, which should be subject to similar confounders as associations between *T. vaginalis* seropositivity and prostate cancer. A third possibility is chance, particularly given our relatively low estimated magnitudes of association and marginal statistical significance. On the other hand, potentially stronger associations between *T. vaginalis* seropositivity and prostate cancer may have been attenuated by nondifferential detection of infections of possibly lesser relevance to prostate carcinogenesis, including recently acquired infections, treated infections of shorter duration, and infections without prostate involvement.

Several notable strengths distinguish this study from previous studies. First, it is the largest study of trichomonosis and prostate cancer to date. Second, it assessed trichomonal exposure before prostate cancer diagnosis to establish a temporal relationship between trichomonosis and prostate cancer, and to limit ascertainment biases. Third, it assessed trichomonal exposure by antibody seropositivity against a highly immunogenic trichomonad protein to improve the sensitivity of exposure ascertainment by potentially capturing asymptomatic infections, presumptively treated undiagnosed infections, and infections that might otherwise not be reported. Finally, it was conducted within an extremely well characterized cohort to allow for adjustment for STI correlates and prostate cancer risk factors.

In conclusion, serologic evidence of a history of trichomonosis was positively associated with incident prostate cancer in this large, nested case-control study. As this study is the first, to our knowledge, to investigate associations between *T. vaginalis* serology and prostate cancer, additional studies are necessary before conclusions can be made. However, if confirmed, our findings may offer the potential for innovative new approaches to prostate cancer prevention by reducing *T. vaginalis* acquisition and treating existing infections.

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[8] S. Sutcliffe et al. Plasma antibodies against *Chlamydia trachomatis*, human papillomavirus and human herpesvirus type 8 in relation to risk of prostate cancer, in preparation.

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