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TRICHOMONOSIS AND SUBSEQUENT RISK OF PROSTATE CANCER IN THE PROSTATE CANCER PREVENTION TRIAL

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Abstract

We previously observed a positive association between a history of trichomonosis, a sexually transmitted infection caused by the protozoan, *Trichomonas vaginalis*, and prostate cancer risk in the Health Professionals Follow-up Study. To determine the reproducibility of this finding, we conducted a second, prospective investigation of trichomonosis and prostate cancer in the Prostate Cancer Prevention Trial. Participants were men ≥55 years of age with no evidence of prostate cancer at enrollment (n=18,882). Men were screened annually for prostate cancer, and if not diagnosed during the trial, were offered an end-of-study prostate biopsy. Cases were a sample of men diagnosed with prostate cancer on any biopsy after visit 2 or on their end-of-study biopsy (n=616). Controls were men not diagnosed with prostate cancer during the trial or on their end-of-study biopsy (n=616). Controls were frequency-matched to cases by age, treatment arm, and family history of prostate cancer. Serum from visit 2 was tested for anti-*T. vaginalis* IgG antibodies. No association was observed between *T. vaginalis* serostatus and prostate cancer. 21.5% of cases and 24.8% of controls had low seropositivity, and 15.2% and 15.0% had high seropositivity. Compared to seronegative men, the odds ratio of prostate cancer for men with low seropositivity was 0.83 (95% confidence interval (CI): 0.63–1.09), and that for men with high seropositivity was 0.97 (95% CI: 0.70–1.34). Given the original strong biologic rationale and

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Novelty and impact: We previously observed a positive association between the presence of plasma antibodies against *Trichomonas vaginalis*, a sexually transmitted protozoan, and prostate cancer risk in the Health Professionals Follow-up Study. This novel finding prompted our current, second investigation of trichomonosis (*T. vaginalis* infection) and prostate cancer in the Prostate Cancer Prevention Trial, in which we observed no association between *T. vaginalis* serostatus and risk of prostate cancer.

potential for prevention, additional studies are warranted to help resolve discrepancies between study findings, and further investigate this hypothesis from a variety of different approaches.

Keywords

trichomonosis; *Trichomonas vaginalis*; sexually transmitted infection; prostate cancer; epidemiology

INTRODUCTION

Trichomonas vaginalis is a sexually transmitted protozoan that causes vaginitis in approximately 20–50% of infected women, and non-gonococcal urethritis in a small percentage of infected men. Most other cases are asymptomatic.[1, 2] We previously hypothesized that this lack of specific symptoms might facilitate persistent and undetected trichomonosis (*T. vaginalis* infection) in men, thereby providing *T. vaginalis* with greater opportunity to ascend to the prostate than other more symptomatic sexually transmitted agents.[3] Indeed, early trichomonosis researchers believed the prostate to be the reservoir for *T. vaginalis* based on its frequent detection in prostate fluid from male partners of women with trichomonal vaginitis.[4–8] *T. vaginalis* has also been proposed as a cause of chronic prostatitis,[2] and has been observed in prostate tissue near areas of inflammation and epithelial hyperplasia, leading the authors to propose that *T. vaginalis* might be involved in prostate carcinogenesis.[9, 10] Other mechanisms by which we hypothesize that *T. vaginalis* may contribute to prostate carcinogenesis include urogenital epithelium damage,[11–13] inhibition of apoptosis,[14] and possible local perturbation of polyamine levels.[3] and references therein

In previous work on the relationship between trichomonosis and prostate cancer, we observed that men with plasma antibodies against *T. vaginalis* were significantly more likely to develop prostate cancer than men without anti-trichomonad antibodies in the Health Professionals Follow-up Study (HPFS, odds ratio (OR): 1.43, 95% confidence interval (CI): 1.00–2.03). Interestingly, this association was strongest among men who rarely used aspirin, and weakest among men who used aspirin regularly over the course of their lives and thus presumably at the time of infection. It was also stronger for high-grade than low-grade cancer. To determine the reproducibility of these findings, we have now conducted a second, prospective investigation of *T. vaginalis* serostatus and prostate cancer among participants in another large cohort of American men, the Prostate Cancer Prevention Trial (PCPT). This study has several design features appropriate for the study of prostate cancer etiology, including annual prostate cancer screening by digital rectal examination (DRE) and prostate specific antigen (PSA) testing, and end-of-study biopsies for all participants not diagnosed with prostate cancer during the trial to provide all participants with equal opportunity for prostate cancer detection.

MATERIAL AND METHODS

Study population and design

The PCPT is a large randomized clinical trial designed to investigate whether finasteride, a 5α-reductase type II inhibitor, prevents prostate cancer.[15] Men eligible for the trial were those ≥55 years of age who were generally healthy and had no evidence of prostate cancer (i.e., PSA concentration ≤3 ng/mL and a normal DRE), or other clinically-significant chronic conditions, including severe benign prostatic hyperplasia (BPH) as defined by an American Urological Association symptom score ≥20. Between 1994 and 1997, 18,882 eligible men were randomized to either finasteride or placebo. Participants were screened

annually for prostate cancer by DRE and PSA testing, and those found to have abnormal DRE results or elevated PSA were recommended for prostate biopsy ("for-cause" biopsy). PSA levels in the finasteride arm of the trial were inflated to take into account the known lowering effects of finasteride on serum PSA. Serum remaining after PSA testing was stored for research purposes. After seven years of participation in the trial, men not diagnosed with prostate cancer were offered an "end-of-study" prostate biopsy as part of the trial protocol. This biopsy was included to ensure that biopsy referral patterns were not biased by use of finasteride. Men recommended for biopsy because of an abnormal PSA/DRE near the end of the trial were considered to have had a for-cause biopsy.

To investigate genetic and other serologic exposures in relation to prostate cancer, we nested a large case-control study in the PCPT. Only participants with an adequate baseline serum specimen and a definitive positive or negative diagnosis of prostate cancer, either by a confirmed prostate cancer diagnosis or a negative end-of-study biopsy, were eligible for inclusion (n=8,580). Cases were defined as men diagnosed with prostate cancer on their for-cause or end-of-study biopsy (n=1,809; 1,679 white, 83 black, and 47 Hispanic or other race/ethnicity). All biopsy material was reviewed by the PCPT central pathology laboratory. Prostate cancer diagnoses were established by agreement between pathologists at the central laboratory and study sites. Clinical and pathologic (if the participant underwent radical prostatectomy) stage was provided by the study sites, and Gleason patterns and sum were determined by central pathology review of biopsy or radical prostatectomy tissue. Eligible controls were defined as men not diagnosed with prostate cancer at any time during the trial or on their end-of-study biopsy. All non-white men who met this definition were selected as controls to enhance our ability to perform sub-group analyses based on race/ethnicity (n=372; 174 black, and 198 Hispanic or other race/ethnicity). The remaining controls were selected such that the entire distribution of controls was frequency-matched to cases by age (55–59, 60–64, 65–69, and ≥70 years), treatment arm, and family history of prostate cancer defined as at least one first degree relative with prostate cancer (n=1,437 white controls).

To conserve baseline serum specimens, we investigated associations between trichomonosis and prostate cancer in a subset of men from the parent nested case-control study with an adequate serum specimen from visit 2. In this subset, cases were defined as men diagnosed with prostate cancer after visit 2 (n=616; 557 white, 38 black, and 21 Hispanic or other race/ethnicity). Approximately equal numbers of cases diagnosed by for-cause and end-of-study biopsy, and approximately equal numbers of cases diagnosed with low-grade (Gleason sum <7) and high-grade (≥7) disease were selected to allow for more informative sub-analyses. Eligible controls were defined as men not diagnosed with prostate cancer at any time during the trial or on their end-of-study biopsy (n=616; 486 white, 63 black, and 67 Hispanic or other race/ethnicity). These men were frequency-matched to cases by age, treatment arm, and family history of prostate cancer. Although not specifically selected based on race/ethnicity, these men were enriched for non-white controls because of the original parent study control sampling scheme.

This study was approved by the Institutional Review Boards at the Johns Hopkins Bloomberg School of Public Health and the Fred Hutchinson Cancer Research Center. The PCPT was approved by the Institutional Review Boards of each of the institutions that randomized a participant.

T. vaginalis antibody assessment

Serum collected at visit 2 was tested for anti-*T. vaginalis* antibodies in the laboratory of Dr. John F. Alderete. *T. vaginalis* serostatus was assessed by an in-house enzyme-linked immunosorbent assay (ELISA) that detects IgG antibodies against purified, recombinant *T. vaginalis* α-actinin protein.3 Case and control samples were tested in random order, with

blinding of laboratory personnel to the case-control status of each sample. All samples were tested in duplicate, and inferences were made based on the average of duplicate values. The coefficient of variation for the duplicate optical densities (ODs) was 9%. A control panel consisting of one seronegative specimen and five specimens of increasing seropositivity was included in each run. Four sets of control panels were used, such that four groups of four runs each (for a total of 16 runs of samples) had the same control panel. Cut-off points for seropositivity were determined by dividing the average duplicate OD value of the seropositive control specimens by the corresponding value for the seronegative control specimen to obtain a positive to negative ratio (P/N). The same was done for all prostate cancer case and control specimens. These values were then compared to the seropositive control panel values to obtain a score. Values less than the cut-off point were assigned the lower score (e.g., values less than the P/N for the lowest seropositive control were assigned a score of 0, and values greater or equal to the P/N for the lowest seropositive control and less than the P/N for the second lowest seropositive control were assigned a score of 1). Cut-off points of 1 and ≥2 were used to define low and high *T. vaginalis* seropositivity. Antibody scores of 2 through 5 were collapsed because of the small number of participants who fell into these higher categories. Scores of 1 and 2 were kept distinct because a combined category (i.e., ≥1) resulted in a much higher seroprevalence than observed in our previous study3 and in other studies of non-viral sexually transmitted infections (STIs).

To determine the reproducibility of *T. vaginalis* antibody testing, 14 sets of approximately five replicate blinded samples each were included in the testing sequence. These samples were obtained from the Baltimore Bureau of Disease Control laboratory from serum remaining after routine syphilis testing of STI clinic attendees. Before being released from the laboratory, specimens were anonymized and approved for use by the public review process of the Baltimore City Health Department. Clinic specimens were used as opposed to duplicate PCPT specimens to increase the likelihood that some reproducibility samples would have higher antibody scores. Using cut-off points of 1 and ≥2, 11 of the 14 sets of samples had 100% agreement, two had 80% agreement, and one had 60% agreement. Discrepancies tended to occur when replicate samples were tested in different runs, or when the specimen value fell close to the cut-off point for seropositivity.

Covariate assessment

At the baseline clinic visit, participants completed a detailed self-administered questionnaire on demographic and lifestyle characteristics, including race/ethnicity, education, occupation, military experience, marital status, cigarette smoking, physical activity, frequency of sexual activity in the past four weeks, histories of vasectomy and diabetes, and current and past regular aspirin use. Height and weight were also measured at the baseline visit. At the one year visit, participants additionally completed a 15-page diet and supplement questionnaire designed specifically for PCPT participants. This questionnaire included questions on usual consumption of 99 foods or food groups and nine beverages over the past year, 13 questions on food preparation and purchasing, and three questions on usual consumption of fruits, vegetables and fried foods. Information on usual intake of energy, protein, carbohydrate, fat, fruit, vegetables, tomato products, red meat, processed meat, fish, calcium and zinc from food plus supplements, and alcohol was derived from this questionnaire.16

Statistical analysis

To investigate the potential for confounding, standardized means and proportions of potential confounding variables were calculated by prostate cancer case-control status among all participants, and *T. vaginalis* serostatus among controls. Means and proportions were standardized by age, treatment arm and family history of prostate cancer to account for frequency-matching, and race to account for over-sampling of non-white controls in the

study design. Standardization was performed by linear regression. P-values were calculated by linear regression for continuous and binary variables, and by generalized logit models for categorical variables. Variables considered as potential confounders were those mentioned in the covariate section, and specimen storage time.

To explore potential associations between *T. vaginalis* serostatus and prostate cancer, standardized average OD values, P/N values and scores were calculated for prostate cancer cases and controls by linear regression. Values were compared by t-tests from linear regression models or by likelihood ratio tests from generalized logit regression models, as appropriate. Subsequent analyses used cut-off points of 1 and ≥2 to classify men as seronegative, low seropositive and high seropositive, and compared prostate cancer cases to controls by unconditional logistic regression, including terms for frequency-matched variables (age (continuous), treatment arm, and family history of prostate cancer) and race (white, non-white). Confounding was further investigated by adding each potential confounder individually to the regression model and comparing the point estimate to that obtained in a model including only age, treatment arm, family history of prostate cancer, and race.

In preliminary analyses, we noted that the proportion of men with antibody scores of 1 and ≥2 increased across groups of runs defined by the same control panel (herein called run-groups), despite random specimen allocation to each run. Therefore, to investigate the potential influence of using four different control panels, we performed two additional analyses: 1) we included terms for individual run-groups in logistic regression models to account for any unintentional trends in case status across run-groups; and 2) we repeated the main analyses using cut-off points derived from the distribution of duplicate OD values among prostate cancer controls in each run-group (75^{th} and 90^{th} percentiles) rather than from the control panels, under the assumption that the proportion of men with antibody scores of 1 and ≥2 should be approximately equal across run-groups.

To investigate whether potential associations varied by clinical expression, grade or stage of prostate cancer, separate analyses were performed for prostate cancer diagnosed by for-cause and end-of-study biopsy, low- and high-grade prostate cancer (Gleason sum <7 versus ≥7, and <8 versus ≥8), organ-confined disease (≤stage T2 and N0M0), and organ-confined low- and high-grade disease. Too few men were diagnosed with advanced stage disease (>stage T2 or N1 or M1, n=14) to investigate its association with *T. vaginalis* serostatus. Stratified analyses were also performed by 1) finasteride to investigate whether it modified possible associations between trichomonosis and prostate cancer; 2) past regular aspirin use and zinc intake from food plus supplements to explore possible differences by use/intake of anti-inflammatory and anti-microbial substances; 3) age at prostate cancer diagnosis and family history of prostate cancer to investigate possible differences by underlying susceptibility to prostate cancer; and 4) race as a possible surrogate marker of unmeasured characteristics of trichomonosis (e.g., number of repeat infections, likelihood of co-infection, or duration of infection), responses to infection (e.g., strength and/or effectiveness of the immune response against *T. vaginalis*), and other variables that could potentially modify the association between trichomonosis and prostate cancer (e.g., general dietary profile).

RESULTS

Of the 616 cases of prostate cancer included in this analysis, 327 (53.1%) were diagnosed by for-cause and 289 (46.9%) by end-of-study biopsy. 312 (50.6%) cases were diagnosed with low-grade (Gleason sum <7) and 304 (49.4%) with high-grade (≥7) disease based on the study design. Almost all cases presented with organ-confined disease (n=566, 97.6% of 580

cases with stage information), as expected based on eligibility criteria for the trial (low PSA and normal DRE), and annual prostate cancer screening. When compared to controls, prostate cancer cases were more likely to be non-Hispanic white as per the design of the study, not to have had military experience, and to report a lesser number of pack-years of smoking. Otherwise, cases and controls were similar with respect to several potential confounders. Controls with the highest *T. vaginalis* antibody scores were less likely to have a family history of prostate cancer and to be non-Hispanic white than controls with lower *T. vaginalis* antibody scores. Controls with the highest *T. vaginalis* antibody scores were also more likely to have a professional occupation and to report lower intakes of most foods/food groups considered, and alcohol, and greater current and past regular aspirin use (Table 1). When the analyses were re-run using *T. vaginalis* antibody cut-off points derived from run-group-specific control distributions, differences for family history of prostate cancer and professional occupation attenuated (data not shown).

No differences were observed between cases and controls in the average OD, P/N and *T. vaginalis* antibody score. Using score cut-off points of 1 and ≥2 to define low and high seropositivity, 21.5% of cases and 24.8% of controls had low seropositivity, and 15.2% of cases and 15.0% of controls had high seropositivity (Table 2). The OR of prostate cancer for men with an antibody score of 1 was 0.83 (95% CI: 0.63–1.09), and that for men with a score of ≥2 was 0.97 (95% CI: 0.70–1.34, Table 3). The results were unchanged after adjustment for potential confounders, inclusion of individual terms for run-groups, and use of cut-off points derived from run-group-specific prostate cancer control distributions (data not shown). Null results were also obtained for prostate cancer diagnosed by for-cause and end-of-study biopsy, low- and high-grade prostate cancer (<7 and ≥7; and <8 and ≥8), organ-confined disease, and organ-confined low- and high-grade prostate cancer, with the exception of inverse associations between an antibody score of 1 and prostate cancer diagnosed by end-of-study biopsy and possibly organ-confined prostate cancer (Table 3 and data not shown).

To investigate whether finasteride influenced the results, stratified analyses were performed by treatment arm. No statistically-significant difference was observed in the magnitude of association between *T. vaginalis* seropositivity and prostate cancer among men in the finasteride (1: OR=1.03, 95% CI: 0.69–1.53; ≥2: OR=1.01, 95% CI: 0.63–1.61) and placebo arms of the trial (1: OR=0.68, 95% CI: 0.46–1.00; ≥2: OR=0.95, 95% CI: 0.60–1.51, p-interaction=0.35). No differences were also observed when the data were stratified by past regular aspirin use, zinc intake from food plus supplements, age at prostate cancer diagnosis, family history of prostate cancer, and race (all p-interaction≥0.20).

DISCUSSION

In this large study of older American men, no association was observed between *T. vaginalis* seropositivity and prostate cancer risk. This null association persisted after adjustment for potential confounders, investigation of several different prostate cancer endpoints, and stratification by finasteride, factors hypothesized to influence prostatic infection/inflammation, markers of underlying susceptibility for prostate cancer, and race. Although inverse associations were observed in some sub- and stratified analyses, these associations were limited to men with lower seropositivity. No associations were observed for men with higher seropositivity, among whom a relationship between trichomonosis and prostate cancer might be more expected based on the lesser degree of misclassification and greater likelihood of capturing infections hypothesized to involve the prostate, such as prolonged or multiple infections.17

Our predominantly null finding differs from our previous observation of a modest, positive association between *T. vaginalis* seropositivity and prostate cancer risk in the HPFS, which was stronger among men who used aspirin infrequently over the course of their lives, and for men diagnosed with high-grade prostate cancer.3 One likely reason for these differences in study findings is chance, as these are the only two studies to have investigated *T. vaginalis* seropositivity and prostate cancer to date.

Another possible reason for disparate study results is differences in the spectrum of prostate cancer cases between the two study populations. In the PCPT, almost half of prostate cancer cases were diagnosed by end-of-study biopsy, indicating that their prostate cancer was not detectable by prostate cancer screening or symptoms. In contrast, nearly all prostate cancer cases in the HPFS nested case-control study were diagnosed by for-cause biopsy because of an elevated PSA concentration or abnormal DRE. Therefore, PCPT cases likely had smaller foci of cancer with possibly lesser potential for progression than cases in the HPFS. Even when only considering prostate cancer cases diagnosed by for-cause biopsy, PCPT cases likely still had smaller foci of cancer with potentially lesser potential for progression than HPFS cases because PCPT cases were selected to have had low baseline PSA concentration (<3 ng/mL), were screened annually for prostate cancer, and were diagnosed with prostate cancer after visit 2 and thus after typically at least three prostate cancer screenings, whereas participants in the HPFS nested case-control study were only required to have had at least one PSA test. Therefore, if, for instance, trichomonosis is only associated with larger foci of prostate cancer or foci with greater potential for progression, then this may explain differences in study findings.

Although the PCPT study design likely resulted in detection of smaller foci of prostate cancer with potentially lesser potential for progression, it also provided the following benefits. First, by screening participants annually for prostate cancer and requesting that all participants undergo a prostate biopsy, it allowed us to address two potential methodologic concerns: 1) differential prostate cancer screening by a history of trichomonosis, and b) differential, incidental detection of prostate cancer by a history of trichomonosis due to possible prostatic *T. vaginalis* infection- or residual prostatic inflammation-mediated PSA elevation.3 A second possible benefit of investigating trichomonosis and prostate cancer in the PCPT is that it allowed us to investigate the specificity of the association for larger foci of cancer or foci with potentially greater potential for progression. For instance, if both the PCPT and HPFS studies had observed positive findings, one hypothesis might have been that a history of trichomonosis is associated with all foci of prostate cancer, perhaps at an early stage of carcinogenesis common to all tumors. However, given that null findings were observed in the PCPT, another more relevant hypothesis might be that trichomonosis is preferentially associated with early development of potentially more aggressive tumors, or possibly later progression of small, less aggressive tumors to larger or more aggressive tumors.

Beyond the potentially different types of prostate cancer detected in each study, a further possible reason for differences in study results is differing exclusion criteria. Although both studies were composed of predominantly white, older American men, PCPT participants were required to have had a PSA concentration ≤3 ng/mL and a normal DRE at enrollment, whereas no PSA or DRE restrictions were placed on HPFS participants. Therefore, if prostatic *T. vaginalis* infections/residual inflammation cause PSA concentrations to rise (i.e., >3 ng/mL), then this difference in exclusion criteria may have potentially resulted in a lower proportion of participants with prostatic *T. vaginalis* infection/residual inflammation relative to participants with purely urethral or resolved prostatic infections at enrollment in the PCPT than in the HPFS. These infections are not currently distinguishable by the serum anti-trichomonad IgG ELISA. If we further hypothesize that persistent, prostatic infections/

residual inflammation are associated with prostate cancer, but not purely urethral or resolved short-term prostatic infections, then exclusion of men more likely to have had prostatic infections/residual inflammation at enrollment may have potentially reduced our ability to detect associations between *T. vaginalis* seropositivity and prostate cancer in the PCPT. Even though the seroprevalence of trichomonosis appeared to be higher in the PCPT (15.0% high seropositivity among controls) than in the HPFS (9.4% among controls), these seroprevalences are not directly comparable because the two studies used different methods to define seropositivity: in the PCPT, cut-off points were derived from seronegative and seropositive quality control panel values, whereas in the HPFS, they were based on expert opinion (Alderete) without knowledge of prostate cancer status.3 Therefore, these estimates are more useful for relative comparisons, such as by case-control status, than for absolute estimates of the lifetime prevalence of trichomonosis. It is also conceivable that, even though the observed PCPT seroprevalence was higher than in the HPFS, the actual PCPT seroprevalence may have been even higher had men with a PSA concentration >3 ng/mL or an abnormal DRE not been excluded at enrollment.

Another difference in exclusion criteria between the PCPT and HPFS was the presence of comorbidities. In the PCPT, men with comorbid conditions, such as BPH, were not eligible for enrollment, whereas HPFS nested case-control participants were only required to be free of a diagnosis of cancer at the time of blood draw. Therefore, if, for instance, persistent, prostatic *T. vaginalis* infection/residual inflammation is associated with both severe BPH and prostate cancer, then exclusion of men with BPH at enrollment in the PCPT may have depleted the study population of men with prostatic infection/residual inflammation who might have gone on to develop prostate cancer, thus potentially limiting our ability to detect associations between *T. vaginalis* seropositivity and prostate cancer.

Reasons unlikely to explain differences in study findings include 1) differences in statistical power because both studies were similarly-sized; 2) differences in study design because both studies were nested within a cohort; and 3) differences in serologic testing because both studies used the same in-house assay to measure *T. vaginalis* IgG antibodies. The two studies did, however, use different methods to define seropositivity, yielding seemingly different study-specific seroprevalences as discussed above. To investigate whether these differences led to differing associations between *T. vaginalis* seropositivity and prostate cancer, we compared the entire distribution of OD values between cases and controls in the present study, we included terms for run-groups in regression models, and we re-ran the analyses using cut-off points derived from the run-group-specific distribution of prostate cancer controls rather than the control panels, all of which yielded null results.

In summary, contrary to our previous positive findings, we observed no association between *T. vaginalis* seropositivity and subsequent risk of prostate cancer among participants in the PCPT. Given the strong biologic plausibility of this hypothesis and its potential for prostate cancer prevention, we believe that additional studies are warranted to help resolve discrepancies between our two study findings using a variety of different epidemiologic and molecular approaches, and in study populations with varying trichomonosis history and prostate cancer presentation.

Abbreviations

BPH	benign prostatic hyperplasia
CI	confidence interval
DRE	digital rectal examination

ELISA	enzyme-linked immunosorbent assay
HPFS	Health Professionals Follow-up Study
OD	optical density
OR	odds ratio
P/N	positive to negative ratio
PCPT	Prostate Cancer Prevention Trial
PSA	prostate specific antigen
STI	sexually transmitted infection

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Table 1

Baseline characteristics[1] of participants by prostate cancer status and *T. vaginalis* antibody score in the Prostate Cancer Prevention Trial

	Case-control status			*T. vaginalis* antibody score[2] (controls only)			
	Case (n=616)	Control (n=616)	P-value[3]	0 (n=368)	1 (n=154)	≥2 (n=94)	P-value[3]
Mean age (years)	64.0	64.0	Matched	63.7	64.0	64.7	0.31
Finasteride (%)	48.9	48.9	Matched	48.6	47.4	52.1	0.76
First-degree relative with prostate cancer (%)	20.6	20.6	Matched	21.7	22.1	13.8	0.21
Race (%):							
White	90.4	78.9		80.4	79.2	72.3	
Black	6.2	10.2	N/A[4]	10.3	9.1	11.7	0.16
Other race/ethnicity	3.4	10.9		9.2	11.7	16.0	
Education (%):							
Some high school or less	5.3	4.3		4.6	4.6	4.3	
High school graduate	14.8	14.9	0.72	12.8	19.5	17.0	0.29
Some post-secondary education or more	79.9	80.8		82.6	76.0	78.7	
Occupation (%):							
Professional	65.4	63.8		61.1	65.6	68.1	
Craftsman	12.9	12.9		15.2	9.7	11.7	
Sales	9.5	8.8	0.73	10.6	5.2	6.4	0.15
Farming	1.5	2.3		1.4	4.6	2.1	
Other	10.6	12.3		11.7	14.9	11.7	
Military experience (%)[5]	25.9	28.3	0.14	30.1	30.0	29.3	0.98
Marital status (%):							
Currently married or in marriage-like relationship	88.9	90.3		89.7	92.9	86.2	
Divorced/widowed	8.8	7.3	0.63	7.9	5.2	10.6	0.90
Never married	2.1	2.4		2.4	2.0	3.2	
Mean height (inches)	69.9	69.7	0.39	69.7	69.8	69.6	0.80
Mean body mass index (kg/m^2)	27.6	27.6	0.87	27.4	27.8	27.8	0.55
Mean intakes of:							
Energy (kcal/day)	2171.3	2140.7	0.53	2148.8	2148.4	2005.2	0.32

	Case-control status			*T. vaginalis* antibody score[2] (controls only)			
	Case (n=616)	Control (n=616)	P-value[3]	0 (n=368)	1 (n=154)	≥2 (n=94)	P-value[3]
Protein (g/day)	92.3	92.1	0.93	92.2	93.0	86.8	0.46
Carbohydrate (g/day)	264.7	259.4	0.37	257.8	265.6	244.7	0.30
Fat (g/day)	79.8	78.6	0.59	79.5	77.5	73.5	0.43
Fruit consumption (fruit/day)	2.2	2.0	0.03	2.0	2.1	2.0	0.58
Vegetable consumption (vegetables/day)	2.3	2.3	0.59	2.3	2.4	2.1	0.17
Tomato products (1 cup servings/day)	0.48	0.47	0.81	0.46	0.46	0.40	0.06
Red meat (6 oz servings/day)	0.60	0.63	0.27	0.62	0.67	0.57	0.18
Processed meat (6 oz servings/day)	0.35	0.38	0.23	0.36	0.43	0.36	0.07
Fish (6 oz servings/day)	0.23	0.21	0.19	0.20	0.23	0.20	0.42
Calcium from food plus supplements (mg/day)	1073	1063	0.76	1067	1050	991	0.49
Zinc from food plus supplements (mg/day)	25.5	25.4	0.93	26.0	23.7	25.1	0.52
Total alcohol (g/day)	9.3	8.9	0.64	9.5	7.5	7.7	0.25
Cigarette smoking:							
Smoked regularly before 25 years of age (%)	61.7	63.8	0.44	62.0	68.2	66.0	0.37
Currently smoke (%)	6.1	6.6	0.70	7.9	4.6	7.4	0.39
Mean pack-years smoked	22.0	24.1	0.05	24.1	22.7	25.7	0.45
Physical activity (%):							
Sedentary	14.7	15.8		19.6	9.9	11.1	
Light activity	44.6	40.2	0.25	39.2	35.8	51.1	0.28
Moderate activity	33.2	32.0		29.6	39.5	28.9	
Very active	7.5	12.0		11.6	14.8	8.9	
Frequency of sexual activity in the past 4 weeks (%):							
Not at all	14.0	12.0		11.7	10.4	14.9	
Once	11.7	12.5		11.4	13.6	13.8	
2–3 times	23.4	24.3	0.46	22.8	28.6	22.3	0.84
Once/week	30.9	27.7		29.1	28.6	21.3	
2–3 times/week	16.7	19.5		20.1	16.2	23.4	
≥4 times/week	2.1	3.3		3.8	2.6	3.2	
Vasectomy (%)	29.4	31.2	0.49	30.7	29.9	28.7	0.93

	Case-control status			*T. vaginalis* antibody score[2] (controls only)			
	Case (n=616)	Control (n=616)	P-value[3]	0 (n=368)	1 (n=154)	≥2 (n=94)	P-value[3]
Mean age at vasectomy	38.7	37.5	0.11	37.4	38.0	38.6	0.71
History of diabetes mellitus type 2 (%)	6.2	7.1	0.54	9.2	3.2	8.5	0.06
Aspirin use on a regular basis (%):							
Current use	42.5	40.8	0.53	36.1	46.8	43.6	0.06
Past use	8.1	8.6	0.76	7.9	8.4	13.8	0.19

[1] Case and control values collected at baseline in 1994–7 or at the first annual visit were standardized by age, treatment arm, family history of prostate cancer and race (non-white versus white) using linear regression.

[2] Scores were derived by first calculating the ratio of the average duplicate optical density value for each participant's specimen to the average duplicate optical density value for the seronegative control included in each run, and then comparing this value to the corresponding values for the five seropositive controls with increasing positivity (1–5) also included in each run.

[3] P-values were calculated by linear regression for continuous and binary variables, and by generalized logit models for categorical variables.

[4] Black controls and controls of other race/ethnicity were over-sampled in the design of the study. All analyses are adjusted for race to take this feature of the design into account.

[5] May underestimate the true prevalence of military experience in this population.

Table 2

Trichomonas vaginalis antibody distribution[1] for 616 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

	Case[2]	Control	P-value[3]
OD (mean)	0.39	0.40	0.32
P/N[4] (mean)	2.28	2.33	0.40
Score[5] (%):			
0	63.3	60.2	
1	21.5	24.8	
2	13.1	13.8	0.33
3	2.1	1.2	
4	0.0	0.0	
5	0.0	0.0	
≥2	15.2	15.0	0.39[6]

OD=optical density, P/N=positive to negative ratio

[1] Standardized by age, treatment arm, family history of prostate cancer and race (non-white versus white) using linear regression.

[2] Cases were a sample of men diagnosed with prostate cancer on any biopsy after their second visit or on their end-of-study biopsy (1996–2003).

[3] P-values were calculated by linear regression for continuous variables, and by generalized logit models for categorical variables.

[4] P/N values were calculated by dividing the average duplicate OD value for each specimen by the average duplicate OD value for the seronegative control included in each run.

[5] Scores were derived by comparing the P/N for each specimen to the P/N for the five seropositive controls with increasing positivity (1–5) included in each run.

[6] P-value for the comparison of 0, 1, and ≥2 scores.

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer for *Trichomonas vaginalis* serostatus in 616 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

***T. vaginalis* antibody score**[1]	**Cases (n)**[2]	**Controls (n)**	**OR (95% CI)**[3]
Total prostate cancer:			
0	393	368	1.00
1	131	154	0.83 (0.63–1.09)
≥2	92	94	0.97 (0.70–1.34)
Prostate cancer diagnosed by for-cause biopsy[4]:			
0	198	368	1.00
1	76	154	0.94 (0.68–1.30)
≥2	53	94	1.09 (0.74–1.61)
Prostate cancer diagnosed by end-of-study biopsy[5]:			
0	195	368	1.00
1	55	154	0.69 (0.48–0.99)
≥2	38	94	0.82 (0.54–1.25)
Low-grade (Gleason sum <7) prostate cancer:			
0	200	368	1.00
1	61	154	1.21 (0.92–1.60)
≥2	51	94	1.03 (0.75–1.44)
High-grade (Gleason sum ≥7) prostate cancer:			
0	193	368	1.00
1	70	154	0.87 (0.62–1.22)
≥2	41	94	0.82 (0.54–1.24)
Organ-confined (≤T2 and N0M0) prostate cancer:			
0	364	368	1.00
1	115	154	0.78 (0.59–1.04)
≥2	87	94	1.00 (0.71–1.39)

[1] Scores were derived by first calculating the ratio of the average duplicate optical density value for each participant's specimen to the average duplicate optical density value for the seronegative control included in each run, and then comparing this value to the corresponding values for the five seropositive controls with increasing positivity (1–5) also included in each run.

[2] Cases were a sample of men diagnosed with prostate cancer between their second visit and the end of the trial (1996–2003).

[3] Calculated by unconditional logistic regression, including terms for age (continuous), treatment arm, family history of prostate cancer and non-white race.

[4] For-cause biopsy refers to a biopsy performed because of an elevated prostate specific antigen concentration or an abnormal digital rectal examination.

[5] End-of-study biopsy refers to a biopsy performed without indication after seven years of participation in the study as part of the study protocol.