HHS Public Access

Author manuscript

Cancer Causes Control. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

Cancer Causes Control. 2017 August ; 28(8): 889–898. doi:10.1007/s10552-017-0919-6.

Trichomonas vaginalis infection and risk of prostate cancer: Associations by disease aggressiveness and race/ethnicity in the PLCO Trial

Miguelle Marous[1], **Wen-Yi Huang**[2], **Charles S. Rabkin**[3], **Richard B. Hayes**[4], **John F. Alderete**[5], **Bernard Rosner**[6], **Robert L. Grubb III**[7], **Anke C. Winter**[1], and **Siobhan Sutcliffe**[1]

[1]Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, St. Louis, MO

[2]Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[3]Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD

[4]Division of Epidemiology, Department of Population Health, New York University School of Medicine, New York, NY

[5]School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA

[6]Department of Biostatistics, Harvard T.H. Chan School of Public Health and the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

[7]Division of Urological Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, MO

Abstract

Purpose—Results from previous sero-epidemiologic studies of *Trichomonas vaginalis* infection and prostate cancer (PCa) support a positive association between this sexually transmitted infection and aggressive PCa. However, findings from previous studies are not entirely consistent, and only one has investigated the possible relation between *T. vaginalis* seropositivity and PCa in African-American men who are at highest risk of both infection and PCa. Therefore, we examined this possible relation in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, including separate analyses for aggressive PCa and African-American men.

Methods—We included a sample of participants from a previous nested case-control study of PCa, as well as all additional Caucasian, aggressive and African-American cases diagnosed since

Corresponding author: Siobhan Sutcliffe, Division of Public Health Sciences, Department of Surgery, Washington University School of Medicine, 600 S. Taylor Ave., Box 8100, Rm 208S, St. Louis, MO 63110, Tel: 314-362-3788, Fax: 314-747-3936, sutcliffes@wudosis.wustl.edu.

the previous study (total n=438 Gleason 7 Caucasian cases, 487 more advanced Caucasian cases (≥Gleason 8 or stage III/IV), 201 African-American cases, and 1,216 controls). We tested baseline sera for *T. vaginalis* antibodies.

Results—No associations were observed for risk of Gleason 7 (odds ratio (OR)=0.87, 95% confidence interval (CI)=0.55–1.37) or more advanced (OR=0.90, 95% CI=0.58–1.38) PCa in Caucasian men, or for risk of any PCa (OR=1.06, 95% CI=0.67–1.68) in African-American men.

Conclusions—Our findings do not support an association between *T. vaginalis* infection and PCa.

Keywords

Prostate cancer; Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; *Trichomonas vaginalis*; Sexually transmitted infections; Epidemiology

INTRODUCTION

Although accumulating evidence from several different disciplines supports an inflammatory contribution to prostate cancer (PCa) risk, responsible cause(s) of prostate inflammation have not yet been identified [1]. One possible, responsible cause is *Trichomonas vaginalis* infection, the most common non-viral sexually transmitted infection (STI) worldwide [2]. This STI has been proposed as a possible risk factor for PCa for several reasons, including its known prostatic tropism; its ability to elicit inflammation and damage prostate epithelium; its identification near foci of inflammation and hyperplastic prostate lesions, similar to those proposed as early PCa lesions; and its tendency to cause chronic, subclinical infections [3]. *T. vaginalis* has also been shown to alter polyamine levels, which have been linked to PCa in some studies [3]; and more recently, to upregulate expression of anti-apoptotic and other proto-oncogenes, and to increase the growth and invasiveness of benign and malignant prostate cells in some, but not all, *in vitro* studies [4–7].

Based on this rationale, *T. vaginalis* infection has been investigated in relation to PCa in a growing number of studies. The first of these studies, a nested case-control study in the Health Professionals Follow-up Study (HPFS), observed a modest positive association between *T. vaginalis* seropositivity and PCa risk (odds ratio (OR)=1.43, 95% confidence interval (CI)=1.00–2.03) and a slightly stronger association for high-grade disease (OR=1.76, 95% CI=0.97–3.18) [8]. Although the second study observed no association for risk of early-stage disease (OR=0.97, 95% CI=0.70–1.34 [9]), the third study, a nested case-control study in the Physicians' Health Study (PHS), observed a non-significant positive association for risk of any PCa (OR=1.23, 95% CI=0.94–1.61), and stronger significant associations for risks of extraprostatic and metastatic/lethal disease: OR=2.17 (95% CI=1.08–4.37) and 2.69 (95% CI=1.37–5.28), respectively [10]. Taken together, these results suggested that *T. vaginalis* might be associated with risk of aggressive, but not non-aggressive, PCa. Raising doubts about this hypothesis, however, are findings from a recent case-control study that observed a protective association for metastatic or fatal PCa (OR=0.51, 95% CI=0.28–0.93 [11]) in Washington State residents. These contradictory findings highlight the need for additional studies to resolve discrepancies by disease

aggressiveness. In addition, as only one study has examined African-American men (OR=1.12, 95% CI=0.76–1.66 [12]), who are at highest risk of both *T. vaginalis* infection [13] and PCa [14], additional studies are necessary in this high-risk group of men.

To address these remaining questions, we performed a nested case-control study of *T. vaginalis* infection and PCa risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), with enrichment for both aggressive PCa and African-American men. This study built upon findings from a previous study of STIs and PCa in PLCO, in which a suggestive positive association was observed for a history of any STIs, but not for any of the individual STIs studied. These non-specific findings suggested a possible association between another unmeasured STI and PCa risk in this group of men [15]. Given previous positive findings for *T. vaginalis* infection in other study populations, we sought to determine whether *T. vaginalis* might explain these suggestive positive findings for cumulative STI history in PLCO.

MATERIAL AND METHODS

Parent study population and design

The PLCO Trial was a large randomized controlled trial designed to investigate the effects of prostate, lung, colorectal, and ovarian cancer screening on cancer-specific mortality [16, 17]. Men 55–74 years of age with no reported histories of PCa or prostatectomy were eligible. Between 1993–2001, 76,705 men were enrolled at 10 screening centers across the U.S. Half of these men (n=38,350) were randomized to the intervention arm, which consisted of prostate-specific antigen (PSA) testing for five years and digital rectal examinations (DREs) for three years, and half (n=38,355) were randomized to the control arm, which consisted of usual medical care. Only men randomized to the intervention arm were included in the present study, as only these men provided a blood sample for study purposes.

At baseline, participants in the intervention arm completed a self-administered demographics and lifestyle questionnaire, and a food frequency questionnaire. Participants also underwent a DRE by a trained examiner and had their blood drawn for PSA testing as part of their baseline PCa screen. Blood samples were centrifuged, separated into serum and clot, aliquotted, and frozen at −70°C within 2–4 hours of collection. One aliquot was shipped to the central laboratory for PSA testing, and the remaining aliquots were shipped to the PLCO Biorepository for future research.

PCa diagnoses were ascertained in several different ways in PLCO. First, men with positive findings on their PCa screen (i.e., suspicious DRE characteristics or PSA values >4 ng/mL) were notified of their results and advised to visit their primary care providers for diagnostic follow-up. Relevant portions of participants' medical records were then obtained by PLCO staff, and information related to diagnostic and initial treatment procedures (within one year of diagnosis) was abstracted. Certified tumor registrars ascertained the stage (Tumor-Node-Metastasis system), Gleason grade, and type of all diagnosed cancers. These procedures were repeated for each PSA test and DRE. In addition to these screen-detected cancers, PCa diagnoses were also ascertained by: 1) self-report on study update questionnaires, which were mailed annually and asked about type and date of cancers diagnosed in the previous

year; and 2) periodic linkage to the National Death Index with subsequent death certificate request. Prostate cancers identified by these methods were investigated in the same way as for screen-detected PCa. Information on vital status was obtained by the annual study update questionnaires and linkage to the National Death Index.

Nested case-control study design

For the present nested case-control study, we included a large subset of participants from the previous nested case-control study of STIs and PCa in PLCO [15], as well as all additional aggressive Caucasian cases and African-American cases diagnosed since or not included in the previous study (diagnosed from 6/1994–9/2010). These additional cases were required to meet the same eligibility criteria as previous participants, including: 1) randomization to the intervention arm; 2) non-Hispanic African-American or Caucasian race/ethnicity; 3) having a valid baseline screen for PCa (DRE or PSA test); 4) completing the baseline questionnaire; 5) providing a baseline blood sample; and 6) reporting no history of PCa at baseline.

Cases for the previous study were defined as men diagnosed with pathologically-confirmed prostate adenocarcinoma ≥1 year after their first valid PCa screen for Caucasian men, and at enrollment or later for African-American men (i.e., without the restriction to one year after PCa screen to increase the number of African-American cases). Controls were selected by incidence-density sampling with replacement, and were frequency-matched to cases by age (5-year categories), race/ethnicity, and year of blood draw (1-year categories). From this original group of participants, we selected all Caucasian cases diagnosed with Gleason 7 cancer or worse, all African-American cases, and all original controls. We did not select Caucasian cases diagnosed with Gleason ≤6 cancer because of financial constraints and because these cases contributed less to our hypothesis. Instead, we enriched our study population with Gleason 7 Caucasian cases (n=255), more advanced Caucasian cases (Gleason ≥8 or stage III/IV; n=357), and African-American cases (n=83) diagnosed since the previous study to bring the total numbers of Caucasian Gleason 7 cases up to 438, more advanced Caucasian cases to 487, African-American cases to 201, and controls to 1,216. We then grouped newly selected and existing cases and controls into new incidence-density sampled clusters with individual-matching by age, race/ethnicity, and year of blood draw. This new study design resulted in the ability to make three main comparisons: 1) Gleason 7 Caucasian cases to matched Caucasian controls; 2) more advanced Caucasian cases to matched Caucasian controls; and 3) African-American cases (with any grade or stage PCa) to matched African-American controls. We did not perform our main African-American analyses separately by disease aggressiveness because of the smaller number of cases.

This study was approved by the Institutional Review Board at the National Institutes of Health. Specimens and data were de-identified before release from the National Cancer Institute.

Assessment of *T. vaginalis* serostatus

T. vaginalis antibody testing was performed using the same enzyme-linked immunosorbent assay (ELISA) and protocol as in all previous studies of *T. vaginalis* serostatus and PCa [8–12]. This ELISA, which detects IgG antibodies against recombinant *T. vaginalis* α-actinin

protein, had 85% sensitivity, 94% specificity [8], and 90–94% reproducibility [8–11] in previous studies.

Similar to the previous nested case-control study of STIs and PCa in PLCO [15], we tested baseline specimens for antibody seropositivity. Samples were tested in duplicate and inferences were made based on the mean duplicate absorbance (or optical density) value for each specimen. To standardize testing, we included a control panel of five specimens of increasing absorbance (0, 1+, 2+, 3+, and 4+) on each plate (approximately 42 specimens each). We obtained control specimens with scores ≤2+ from individuals without a history of *T. vaginalis* or other STIs, and specimens with scores ≥3+ from patients with known *T. vaginalis* infection [18]. Specimens with scores ≥2+ had no detectable reactivity to trichomonad proteins blotted onto nitrocellulose after SDS-PAGE of total *T. vaginalis* proteins and immunoblotting, whereas those with scores ≥3+ readily detected trichomonad proteins [19, 20]. Specimens with scores of 1+ and 2+ exhibited non-specific reactions above baseline by ELISA.

We included a separate control panel on each plate (n=58 plates). This panel was used to determine plate-specific cut-off points for seropositivity by dividing the mean absorbance for each control specimen (1+, 2+, 3+, and 4+) by the mean absorbance for the 0 score specimen to create a positive to negative (P/N) ratio. We then derived cut-off points by taking the mid-point between each P/N ratio – for instance, the cut-off point for the 3+ score was derived by taking the mid-point between the P/N ratios for the 2+ and 3+ control specimens. Scores were then assigned to each participant by comparing their P/N ratio (i.e., their mean absorbance divided by the plate-specific mean 0 score absorbance) to the plate-specific control panel cut-off values. Participants with scores ≥3+ were considered seropositive [10–12, 21].

Specimens were tested in random order with blinding of laboratory personnel to the case-control status of each specimen. To assess assay reproducibility, we distributed 89 blinded duplicate samples of unknown serostatus randomly across the testing sequence (% agreement=85.4%, 95% CI=78.1%–92.7%, Kappa=0.61, 95% CI=0.42–0.80). We also tested 47 blinded pairs of specimens from control participants collected one year apart to assess intra-individual variability over time. Of these men, 44 were seronegative at baseline, 42 (95.5%) of whom remained seronegative one year later and two (4.5%) of whom seroconverted. The three men who were seropositive at baseline remained seropositive one year later (100%; % agreement=95.7%, 95% CI=85.5%–99.5%, Kappa=0.73, 95% CI=0.37–1.00).

Assessment of other STIs

Self-reported histories of physician-diagnosed gonorrhea and syphilis were assessed on the baseline questionnaire, and serologic evidence of *Chlamydia trachomatis*, human papillomavirus (HPV) types 16 and 18, herpes simplex virus type 2 (HSV-2), human herpesvirus type 8 (HHV-8), and cytomegalovirus (CMV) infection were measured as part of the previous nested case-control study of STIs and PCa [15].

Statistical analysis

To inform confounding, we compared participants' baseline characteristics by case-control status within each stratum of race/ethnicity. Estimates were adjusted for age and year of blood draw by linear mixed models to take into account the matched design. We calculated global p-values (comparing controls, Gleason 7 cases, and more advanced cases) by competing risks Cox proportional hazards regression with data augmentation [22] in Caucasian men, and by conditional logistic regression in African-American men. We compared the distribution of baseline characteristics by *T. vaginalis* serostatus among controls using linear mixed models.

To explore the relation between *T. vaginalis* serostatus and PCa risk, we compared participants' *T. vaginalis* antibody absorbance values, P/N ratios, scores, and serostatus by case-control status within each stratum of race/ethnicity. Estimates were adjusted for age and year of blood draw by linear mixed models. Global p-values and p-values for heterogeneity (comparing the relation between controls and Gleason 7 cases to the relation between controls and more advanced cases) were calculated by competing risks Cox proportional hazards regression in Caucasian men, and by conditional logistic regression in African-American men. These same methods were used to calculate matched ORs and 95% CIs.

We explored potential confounding by including variables found to be associated with case-control status at an α-level of 0.25 in race/ethnicity-specific analyses, as well as more fine categories for age (1-year categories) and histories of gonorrhea and syphilis. Next, we investigated the potential for detection bias by including variables hypothesized to influence the likelihood of PCa investigation or detection (self-reported physician diagnosis or previous surgery for "an enlarged prostate or benign prostatic hypertrophy (BPH)" and nocturia). We also included self-reported physician diagnosis of "an inflamed prostate or prostatitis", as we hypothesized this variable might mediate the association between *T. vaginalis* serostatus and PCa. Inclusion of each of these sets of variables was performed in a sequential fashion.

To examine the influence of re-grouping participants into incidence-density sampled, matched clusters, we repeated the re-grouping process nine additional times and compared the results to our main findings. We also examined the influence of our case definitions by: 1) repeating the analyses redefining Caucasian cases by clinical variables only rather than by pathologic variables when these were available and by clinical variables otherwise, as is typically done in PLCO; 2) defining Caucasian cases by grade (Gleason ≥8) or stage only (III or IV); and 3) examining African-American Gleason 7 and more advanced cases separately.

Finally, as suggestive positive findings were observed for serologic evidence of ≥1 STI in Caucasian men and for ≥2 STIs in African-American men in the previous nested case-control study, but not for any of the individual STIs tested [15], we investigated whether these findings might be explained by *T. vaginalis* serostatus. First, we re-examined these findings in our different sub-sample of participants (excluding Gleason 6 participants not sent for *T. vaginalis* testing) to confirm that we could replicate the original suggestive positive findings, after which we: 1) adjusted them for *T. vaginalis* serostatus; and 2) added

T. vaginalis serostatus to the previous cumulative STI history variables to examine the combined influence of all eight STIs on PCa.

Analyses were performed using SAS® software v.9.4 (SAS Institute, Cary, NC) and a two-sided α-level of 0.05.

RESULTS

We included 2,342 participants (or participant observations) in our analysis, 1,786 of which were from Caucasian men and 556 were from African-American men. Of the Caucasian men, 861 were controls, 438 were Gleason 7 cases, and 487 were more advanced cases (Gleason ≥8 or stage III/IV). Of the African-American men, 355 were controls and 201 were cases, 92 of whom had Gleason 7 grade disease or worse. Compared to Caucasian controls, cases were more likely to have a family history of PCa, to have never smoked cigarettes, and to have higher PSA at baseline. Compared to their respective controls, African-American cases consumed non-significantly less alcohol, had higher baseline PSA, and were more likely to report a physician diagnosis of an enlarged prostate/BPH, nocturia, and a previous biopsy at baseline. Otherwise, cases and controls were similar (Table 1). With respect to *T. vaginalis* serostatus, Caucasian controls were considerably less likely to be seropositive than African-American controls (9.5% versus 21.0%, $p<0.0001$). In analyses stratified by race/ethnicity, *T. vaginalis* seropositive Caucasian controls were non-significantly older, more likely to be married, less likely to consume alcohol or smoke cigarettes, had higher baseline PSA (although these findings attenuated after adjustment for BPH), were more likely to report a prior prostate biopsy, and were less likely to report a vasectomy or gonorrhea than seronegative controls. African-American seropositive controls were less likely to be married or to report a family history of PCa, more likely to report nocturia and a history of syphilis, and less likely to report a vasectomy than seronegative controls (Supplemental Table 1).

Considering *T. vaginalis* antibody levels, although Caucasian cases had lower geometric mean absorbance values, P/N ratios, and antibody scores than controls, no differences were observed by serostatus. For African-American participants, similar distributions of absorbance values, P/N ratios, antibody scores, and serostatus were observed for cases and controls (Table 2). In crude analyses, no associations were observed for *T. vaginalis* seropositivity and Gleason 7 or more advanced disease in Caucasian participants, or for total PCa in African-American participants. Similar null findings were observed after adjustment for variables identified as potential race/ethnicity-specific confounders, variables that might influence the likelihood of PCa detection, and a physician diagnosis of prostatitis (Table 3); as well as in all sensitivity analyses (data not shown). Combining results for Caucasian and African-American participants yielded an OR of 1.02 (95% CI=0.73–1.42) for Gleason 7 disease, 0.88 (95% CI=0.59–1.30) for more advanced disease, and 0.97 (95% CI=0.73–1.27) for any PCa.

When we limited the sample to participants from the previous nested case-control study of STIs and PCa, we confirmed that suggestive positive associations were still observed for ≥1 STI and risk of more advanced PCa in Caucasian men and for ≥2 (versus ≤1) STIs and PCa risk in African-American men. These findings were largely unaltered by adjustment for *T.*

vaginalis serostatus or by addition of *T. vaginalis* serostatus to the cumulative STI history variable, with the exception of a slight strengthening of the findings for African-American men when *T. vaginalis* serostatus was added to STI history (OR=1.83, 95% CI=0.79–4.28 versus OR=1.54, 95% CI=0.71–3.31, Supplemental Table 2).

DISCUSSION

In our large nested case-control study of *T. vaginalis* infection and PCa, no associations were observed for *T. vaginalis* seropositivity and risk of aggressive PCa in Caucasian men, or for *T. vaginalis* seropositivity and risk of any PCa in African-American men. Additionally, adjustment for *T. vaginalis* did not influence previous estimates for other STIs, suggesting that this infection did not confound or explain previous findings for cumulative STI history in PLCO. Finally, addition of *T. vaginalis* to our cumulative STI history variable did not influence study findings, with the exception of a slight strengthening of the findings for African-American men.

T. vaginalis infection and PCa risk

Our null findings for *T. vaginalis* seropositivity and PCa are generally consistent with findings from two previous studies [9, 11], but differ from those from two others [8, 10]. Our findings are consistent with those from the Prostate Cancer Prevention Trial (PCPT), a large study of predominantly Caucasian men that observed no association between *T. vaginalis* seropositivity and risk of generally earlier-stage PCa [9], and with those from a recent, small case-control study that observed a protective association between *T. vaginalis* seropositivity and metastatic and fatal PCa in predominantly Caucasian men [11]. Our findings differ, however, from those from two additional studies. These two studies, which were nested in cohorts of predominantly Caucasian health professionals, observed positive associations for *T. vaginalis* seropositivity and risk of aggressive PCa, including risks of high-grade [8], extra-prostatic, metastatic, and lethal disease [10]. These positive findings motivated our current analysis of aggressive PCa in Caucasian men in PLCO. With respect to our results for African-American men, our null findings are consistent with those from the only published study to date to include a sizeable number of African-American men. This nested case-control study observed no association between *T. vaginalis* seropositivity and PCa risk in African-American men in the Southern Community Cohort Study [12].

Reasons for differences between our findings and those from previous studies of Caucasian men are not immediately apparent. Our study used a similar prospective study design as three of the four previous studies (i.e., a nested case-control study design); it was at least as large or larger than all previous studies, giving it similar or greater power to detect associations; and it measured *T. vaginalis* exposure by the same assay as in all previous studies. In addition, although our assay reproducibility was lower than in previous studies that observed positive findings (85% versus 90–91%), we do not believe this difference is sufficient to explain our null versus significantly positive findings. Finally, differences in participant characteristics are unlikely to explain our null findings because our study population had a similar demographic composition as most previous populations studied to

date – i.e., it had a higher educational and socio-economic status than the general population.

Despite use of common methodology and similar study populations, one place where our findings differed from previous studies of Caucasian men is in our estimate of *T. vaginalis* exposure. We estimated a prevalence of 9.5% in Caucasian controls (similar to the HPFS study: 9.4% [8]), whereas the PCPT, PHS, and King County studies estimated prevalences of 15.0–23.2% in their controls [10, 11]. However, these types of differences are not unusual for serologic testing [23, 24] and should not have affected our relative comparisons between cases and controls. We were also able to detect an expected association between *T. vaginalis* seropositivity and African-American race/ethnicity in our study, supporting the criterion validity of our serologic results. Therefore, we do not believe that differences in seroprevalence across studies likely explain our findings. Finally, a further possible reason for differences between our study findings and those from previous studies is chance.

Cumulative STI history and PCa risk

Another one of the goals of our nested case-control study was to examine the contribution of *T. vaginalis* infection to previous positive findings for cumulative STI history in PLCO. When we adjusted these findings for *T. vaginalis* seropositivity, we observed no change in the estimates for STI history, suggesting that *T. vaginalis* infection did not explain previous positive findings. Additionally, incorporation of *T. vaginalis* seropositivity into our STI history variable resulted in no change to the findings, except for a slight strengthening of the results for African-American men. These findings suggest that either another unmeasured, but correlated, STI may be responsible for the suggestive positive associations for STI history and PCa risk, or that the cumulative burden of these infections is important.

Considering our findings for all eight measured STIs in the context of the broader STI history literature, our suggestive positive findings for Caucasian men are consistent with those from two previous prospective studies of predominantly Caucasian men, one of which observed a non-significant, positive finding for seropositivity against *C. trachomatis*, HPV, HSV-2, and HHV-8 infection in Scandinavian men [25], and the other observed a suggestive positive association for seropositivity against *C. trachomatis*, HPV, and HSV-2 infection in predominantly Caucasian-American men [26]. Our suggestive positive findings are also consistent with those from a cohort study of Taiwanese men that observed a positive association for medical-record documented, later-life STIs (gonorrhea, syphilis, *C. trachomatis*, genital warts, genital herpes, and epididymitis/orchitis) and PCa risk [27], and with those from several previous retrospective case-control studies that observed positive associations for self-reported cumulative STI history in men of various race/ethnicities [28, 29]. In contrast, our findings differ from those from a recent cohort study that observed no association for self-reported STIs (gonorrhea, syphilis, *C. trachomatis*, genital warts, and genital herpes) in older Caucasian- and African-American participants from the California Men's Health Study [30], as well as from those from several other case-control studies that observed null associations for cumulative STI history in Caucasian or African-American men [28]. However, given the methodologic differences across these studies, it is unclear whether their differing findings are explained by recall bias in the case of retrospective case-

control studies of self-reported STIs; differences in the cumulative burden of STIs across study populations (e.g., differing number and type of STIs, repeat or untreated infections, or infections of longer duration); or chance. Additional large prospective studies with more detailed exposure information will be necessary to resolve these discrepancies.

In summary, findings from our large nested case-control study of *T. vaginalis* seropositivity and PCa risk provide no evidence to support a role for *T. vaginalis* infection in PCa development in either Caucasian or African-American men. However, findings for all eight measured STIs combined continue to support a link between another unmeasured STI or possibly the cumulative burden of these infections and PCa. These possibilities should be explored further in future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the National Cancer Institute (NCI) for providing the human material collected by PLCO; Karen Pettit, Sally Larson, and Amy Hutchinson for performing or coordinating serum specimen retrieval and aliquoting; Mike Furr, Craig Williams, and Ryan Noble for managing study data; Calvin Neace and Patrick A. Joyce for performing *T. vaginalis* antibody testing; Drs. Raphael P. Viscidi, Francis K. Lee, Yun F. Wang, and Denise Whitby and their staff for performing or supervising STI testing in the previous nested case-control study in PLCO; and Dr. Graham A. Colditz for helpful review of our NCI grant.

PLCO is supported by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, and by contracts from the Division of Cancer Prevention, NCI, National Institutes of Health, Department of Health and Human Services. This study was funded by NCI grant R03 CA143949; the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, NCI, National Institutes of Health, Department of Health and Human Services; the Barnes-Jewish Hospital Foundation; and the Alvin J. Siteman Cancer Center.

References

1. De Marzo AM, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7(4):256–69. [PubMed: 17384581]
2. Holmes, KK., et al., editors. Sexually transmitted diseases. 4th. McGraw-Hill; New York: 2008.
3. Sutcliffe S. Sexually transmitted infections and risk of prostate cancer: review of historical and emerging hypotheses. Future Oncol. 2010; 6(8):1289–311. [PubMed: 20799875]
4. Twu O, et al. Trichomonas vaginalis homolog of macrophage migration inhibitory factor induces prostate cell growth, invasiveness, and inflammatory responses. Proc Natl Acad Sci U S A. 2014; 111(22):8179–84. [PubMed: 24843155]
5. Zhu Z, et al. Trichomonas vaginalis: a possible foe to prostate cancer. Med Oncol. 2016; 33(10):115. [PubMed: 27613161]
6. Han IH, et al. Signalling pathways associated with IL-6 production and epithelial-mesenchymal transition induction in prostate epithelial cells stimulated with Trichomonas vaginalis. Parasite Immunol. 2016; 38(11):678–687. [PubMed: 27543848]
7. Sutcliffe S, et al. Trichomonosis, a common curable STI, and prostate carcinogenesis–a proposed molecular mechanism. PLoS Pathog. 2012; 8(8):e1002801. [PubMed: 22912571]
8. Sutcliffe S, et al. Plasma antibodies against Trichomonas vaginalis and subsequent risk of prostate cancer. Cancer Epidemiol Biomarkers Prev. 2006; 15(5):939–45. [PubMed: 16702374]
9. Sutcliffe S, et al. Trichomonosis and subsequent risk of prostate cancer in the Prostate Cancer Prevention Trial. Int J Cancer. 2009; 124(9):2082–7. [PubMed: 19117055]

10. Stark JR, et al. Prospective study of Trichomonas vaginalis infection and prostate cancer incidence and mortality: Physicians' Health Study. J Natl Cancer Inst. 2009; 101(20):1406–11. [PubMed: 19741211]
11. Shui IM, et al. Trichomonas vaginalis infection and risk of advanced prostate cancer. Prostate. 2016; 76(7):620–3. [PubMed: 26818005]
12. Fowke JH, et al. A prospective study of Trichomonas vaginalis and prostate cancer risk among African American men. BMC Res Notes. 2016; 9(1):224. [PubMed: 27091219]
13. Miller WC, et al. The prevalence of trichomoniasis in young adults in the United States. Sex Transm Dis. 2005; 32(10):593–8. [PubMed: 16205299]
14. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65(1):5–29. [PubMed: 25559415]
15. Huang WY, et al. Sexually transmissible infections and prostate cancer risk. Cancer Epidemiol Biomarkers Prev. 2008; 17(9):2374–81. [PubMed: 18768506]
16. Prorok PC, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials. 2000; 21(6 Suppl):273S–309S. [PubMed: 11189684]
17. Andriole GL, et al. Prostate Cancer Screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial: findings from the initial screening round of a randomized trial. J Natl Cancer Inst. 2005; 97(6):433–8. [PubMed: 15770007]
18. Alderete JF. Enzyme linked immunosorbent assay for detecting antibody to Trichomonas vaginalis: use of whole cells and aqueous extract as antigen. Br J Vener Dis. 1984; 60(3):164–70. [PubMed: 6610453]
19. Neace CJ, Alderete JF. Epitopes of the highly immunogenic Trichomonas vaginalis alpha-actinin are serodiagnostic targets for both women and men. J Clin Microbiol. 2013; 51(8):2483–90. [PubMed: 23616456]
20. Alderete JF, Neace CJ. Identification, characterization, and synthesis of peptide epitopes and a recombinant six-epitope protein for Trichomonas vaginalis serodiagnosis. ImmunoTargets & Therapy. 2013; 2:91–103. [PubMed: 27471691]
21. Sutcliffe S, et al. Persistence of Trichomonas vaginalis serostatus in men over time. Cancer Causes Control. 2015; 26(10):1461–6. [PubMed: 26223890]
22. Lunn M, McNeil D. Applying Cox regression to competing risks. Biometrics. 1995; 51(2):524–32. [PubMed: 7662841]
23. Sutcliffe S, et al. Prospective study of human herpesvirus type 8 serostatus and prostate cancer risk in the placebo arm of the Prostate Cancer Prevention Trial. Cancer Causes Control. 2015; 26(1): 35–44. [PubMed: 25359302]
24. Cannon MJ, Schmid DS, Hyde TB. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. Rev Med Virol. 2010; 20(4):202–13. [PubMed: 20564615]
25. Korodi Z, et al. No serological evidence of association between prostate cancer and infection with herpes simplex virus type 2 or human herpesvirus type 8: a nested case-control study. J Infect Dis. 2005; 191(12):2008–11. [PubMed: 15897985]
26. Dennis LK, et al. Sexually transmitted infections and prostate cancer among men in the U.S. military. Cancer Epidemiol Biomarkers Prev. 2009; 18(10):2665–71. [PubMed: 19755645]
27. Chung SD, et al. Increased risk of prostate cancer following sexually transmitted infection in an Asian population. Epidemiol Infect. 2013; 141(12):2663–70. [PubMed: 23461984]
28. Caini S, et al. Sexually transmitted infections and prostate cancer risk: a systematic review and meta-analysis. Cancer Epidemiol. 2014; 38(4):329–38. [PubMed: 24986642]
29. Vazquez-Salas RA, et al. History of gonorrhea and prostate cancer in a population-based case-control study in Mexico. Cancer Epidemiol. 2016; 40:95–101. [PubMed: 26706364]
30. Cheng I, et al. Prostatitis, sexually transmitted diseases, and prostate cancer: the California Men's Health Study. PLoS One. 2010; 5(1):e8736. [PubMed: 20090948]

Table 1

Baseline characteristics[1] of Caucasian and African-American prostate cancer cases and controls in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, 1993–2001

	Caucasian				African-American		
	Controls (n=861)	Gleason 7 cases (n=438)	Gleason ≥8 or stage III/IV cases (n=487)	Global p-value[2]	Controls (n=355)	Cases (n=201)	Global p-value[3]
Age (mean, yrs)[4]	64.9	64.1	63.4	-----	63.8	63.1	-----
Married or living as married (%)	86.7	88.6	89.0	0.29	71.9	71.0	0.70
College graduate or higher (%)	41.7	43.4	43.6	0.62	30.8	29.7	0.61
First-degree family history of prostate cancer (%)	5.5	12.4	12.5	<0.0001	6.7	8.6	0.32
Body mass index (kg/m^2, %)							
<25	26.0	27.2	24.1		26.4	25.3	
25–29	51.1	52.7	57.1	0.37	44.5	49.2	0.56
≥30	22.9	20.1	18.8		29.1	25.5	
Moderate to vigorous physical activity (hrs/wk)[5]							
None	11.1	12.0	11.9		23.0	19.4	
<1	17.6	14.6	14.4		14.0	13.0	
1	12.6	12.3	10.8	0.32	6.5	9.1	0.61
2–3	26.8	30.7	28.7		17.8	22.5	
≥4	29.2	26.6	29.5		17.2	17.7	
Energy intake (kcal/day, %)[5]							
Quintile 1	19.0	20.4	16.9		14.9	18.1	
Quintile 2	19.4	16.4	16.4		15.4	15.8	
Quintile 3	19.3	18.3	23.7	0.41	14.6	9.6	0.52
Quintile 4	18.5	18.3	17.8		15.7	18.8	
Quintile 5	19.2	19.6	19.4		13.9	13.2	
Alcohol intake (g/day, %)[5]							
0–4.9	51.5	53.2	50.9		48.2	53.9	
5.0–14.9	15.4	13.5	14.4		7.7	10.6	
				0.31			0.17

	Caucasian				African-American		
	Controls (n=861)	Gleason 7 cases (n=438)	Gleason ≥8 or stage III/IV cases (n=487)	Global p-value[2]	Controls (n=355)	Cases (n=201)	Global p-value[3]
15.0–29.9	12.7	15.3	13.1		12.3	11.5	
≥30.0	17.8	14.4	17.1		11.1	6.4	
Cigarette smoking (%)							
Never	39.4	43.1	44.9		33.7	34.9	
Current smoker	9.3	8.8	4.8	0.047	17.1	14.7	0.69
Former smoker	51.4	48.1	50.3		49.2	50.4	
Regular NSAID (aspirin or ibuprofen) use (≥1/week, %)	54.1	52.8	54.2	0.86	37.3	37.2	0.97
PSA at entry (ng/mL, geometric mean)	1.1	2.6	2.2	<0.0001	1.1	3.5	<0.0001
Self-reported medical history:							
Physician diagnosis of an inflamed prostate/ prostatitis	8.3	8.1	7.8	0.72	6.1	5.9	0.85
Physician diagnosis of an enlarged prostate or BPH	24.2	26.1	23.6	0.98	21.4	25.8	0.14
Nocturia (>1 time/night) in the past year	32.1	28.2	32.0	0.37	39.2	47.8	0.035
BPH surgery	4.5	4.5	3.8	0.76	2.8	2.5	0.93
Prostate biopsy	5.9	7.9	7.1	0.44	5.3	11.4	0.0009
Vasectomy	28.1	24.5	28.7	0.50	7.3	6.3	0.69
Syphilis	0.8	0.0	0.2	NE	5.2	5.2	0.82
Gonorrhea	2.6	3.7	3.7	0.36	29.1	24.6	0.43

BPH=benign prostatic hypertrophy; DRE=digital rectal examination; NE=not estimated; NSAID=non-steroidal anti-inflammatory drug; PSA=prostate-specific antigen

[1] All values were calculated by linear mixed models, adjusting for age and year of blood draw.

[2] P-values were calculated by competing risks Cox proportional hazards regression with data augmentation. Controls were selected by incidence-density sampling, and were individually-matched to cases by age, race/ethnicity, and year of blood draw.

[3] P-values were calculated by conditional logistic regression.

[4] Crude value because age was one of the matching variables.

[5] Percentages do not sum to 100% because of missing information.

Table 2

Trichomonas vaginalis antibody levels[1] among Caucasian and African-American prostate cancer cases and controls in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, 1993–2001

	Caucasian							African-American		
	Controls (n=861)	**Gleason 7 cases (n=438)**	**P-value[2]**	**Gleason ≥8 or stage III/IV cases (n=487)**	**P-value[2]**	**Global P-value[2]**	**P-value for heterogeneity[2]**	**Controls (n=355)**	**Cases (n=201)**	**Global p-value[3]**
Absorbance (geometric mean)	0.15	0.14	0.0003	0.14	0.0035	<0.0001	0.62	0.18	0.17	0.65
P/N ratio (geometric mean)	1.20	1.10	0.0003	1.13	0.0018	<0.0001	0.72	1.54	1.52	0.72
Score (%)										
0	43.7	51.3		50.1				19.4	20.3	
1	22.1	21.3		21.3				17.1	27.8	
2	24.7	19.8	0.042[4]	19.3	0.053[4]	0.020[4]	0.88[4]	42.5	30.0	0.33[4]
3	7.8	5.9		8.2				16.2	16.8	
4	1.7	1.6		1.2				4.8	5.0	
≥3	9.5	7.5	0.42	9.3	0.62	0.64	0.80	21.0	21.8	0.61

P/N=positive to negative

[1] All values were adjusted for age and year of blood draw.

[2] Calculated by competing risks Cox proportional hazards regression with data augmentation. Controls were selected by incidence-density sampling, and were individually-matched to cases by age, race/ethnicity, and year of blood draw.

[3] Calculated by conditional logistic regression.

[4] P-trend.

Table 3

Multivariable-adjusted matched odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer by *Trichomonas vaginalis* serostatus in a nested case-control study of Caucasian and African-American men in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, 1993–2001

	Caucasian							African-American			
	Controls	Gleason 7 PCa		Gleason ≥8 or stage III/IV PCa		Global p-value[1]	P-value for heterogeneity[1]	Controls	Total PCa		Global p-value[2]
	N	N	OR (95% CI)[1]	N	OR (95% CI)[1]			N	N	OR (95% CI)[2]	
Seronegative	777	405	1.00	443	1.00	-----	-----	280	158	1.00	-----
Seropositive	84	33	0.83 (0.53–1.30)	44	0.90 (0.59–1.37)	0.64	0.80	75	43	1.12 (0.72–1.73)	0.61
Seropositive[3]	84	33	0.87 (0.55–1.37)	44	0.89 (0.58–1.37)	0.73	0.93	75	43	1.12 (0.72–1.75)	0.61
Seropositive[3,4]	84	33	0.87 (0.55–1.37)	44	0.89 (0.58–1.38)	0.74	0.93	75	43	1.05 (0.67–1.65)	0.55
Seropositive[3,4,5]	84	33	0.87 (0.55–1.37)	44	0.90 (0.58–1.38)	0.74	0.92	75	43	1.06 (0.67–1.68)	0.79

PSA=prostate-specific antigen; STI=sexually transmitted infection.

[1] Calculated by competing risks Cox proportional hazards regression with data augmentation.

[2] Calculated by conditional logistic regression.

[3] Adjusted for age (in 1-year categories), first degree family history of PCa, cigarette smoking status, and a history of gonorrhea in Caucasian men, and for age (in 1-year categories), alcohol intake, and histories of gonorrhea and syphilis in African-American men.

[4] Additionally adjusted for self-reported physician diagnosis or prior surgery for an enlarged prostate or benign prostatic hypertrophy, and for self-reported nocturia.

[5] Additionally adjusted for self-reported physician diagnosis of an inflamed prostate or prostatitis.