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STUDY OF CYTOMEGALOVIRUS SEROSTATUS AND PROSTATE CANCER RISK IN THE PROSTATE CANCER PREVENTION TRIAL

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Abstract

Purpose—To investigate serologic evidence of infection by cytomegalovirus (CMV), a herpesvirus with known oncogenic potential that has been detected in malignant prostate tissue, in relation to prostate cancer (PCa) risk in a large case-control study nested in the Prostate Cancer Prevention Trial (PCPT).

Methods—Cases were men with a confirmed diagnosis of PCa after visit 2 (n=614) and controls were men not diagnosed with PCa during the trial who also had a negative end-of-study biopsy (n=616). Controls were frequency-matched to cases by age, treatment arm, and family history of PCa. Sera from visit 2 were tested for CMV IgG antibodies.

Results—No association was observed between CMV serostatus and PCa risk (adjusted CMV seroprevalence=67.9% for cases and 65.2% for controls, odds ratio=1.13, 95% confidence interval: 0.89–1.45).

Conclusions—Considering our null findings in the context of the full CMV literature, CMV infection, as measured by serostatus, does not appear to increase PCa risk.

Keywords

Cytomegalovirus; infection; prostate cancer; epidemiology

INTRODUCTION

Sexually transmitted infections, such as herpesviruses, have long been hypothesized to contribute to prostate cancer (PCa) risk (1). Cytomegalovirus (CMV), a common beta herpesvirus, has been detected in pre-malignant and malignant prostate tissue (2–4), and has been shown to transform cells *in vitro* (5) and to elicit prostate inflammation (6), a possible cause of PCa (7). At the time this study was conceived, a few small observational studies reported suggestive positive findings between CMV and PCa (4, 8, 9); however, since these studies were conducted, additional larger studies have observed generally null findings (10–14). To further inform the possible role of CMV infection in prostate carcinogenesis, we conducted a large case-control study of CMV serostatus and PCa risk nested within the Prostate Cancer Prevention Trial (PCPT). This study population is especially appropriate for investigations of infections and PCa risk because participants were screened annually for PCa and, if not diagnosed with PCa during the trial, were recommended for “end-of-study” prostate biopsy (15), both of which reduce possible detection bias caused by differential rates of screening by infection status, infection-mediated prostate specific antigen (PSA) elevation, or infection-mediated induction of any other prostate abnormalities that might trigger a prostate biopsy.

MATERIAL AND METHODS

Study design

The PCPT is a large randomized clinical trial designed to investigate whether the drug, finasteride, a 5 α -reductase type II inhibitor, prevents PCa. Generally healthy men ≥ 55 years of age who had no evidence of PCa (i.e., PSA concentration ≤ 3 ng/mL and a normal digital rectal examination (DRE)) or other clinically-significant chronic conditions, including severe benign prostatic hyperplasia (BPH, defined by an International Prostate Symptom Score (IPSS) ≥ 20), were recruited at 221 sites across the United States. Between 1994 and 1997, 18,882 of these men were enrolled into the trial and randomized to either the placebo or finasteride arm. At each annual study visit, participants were screened for PCa by DRE and PSA testing, and those found to have abnormal DRE results or elevated PSA (>4 ng/mL) were recommended for prostate biopsy (“for-cause” biopsy). To ensure that approximately equal proportions of men were recommended for biopsy in each arm, PSA concentrations were adjusted among men in the finasteride arm because finasteride is known to lower serum PSA. Serum remaining after PSA testing in both arms was stored for research purposes. After seven years of participation in the trial, men who had not been diagnosed with PCa were offered a prostate biopsy (“end-of-study” 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 that coincided with the “end-of-study” biopsy window were considered to have had a “for-cause” biopsy.

As part of a broader investigation of genetic and other serologic exposures in relation to PCa, we nested a large case-control study within the PCPT. Participants in either treatment arm who had an adequate baseline serum specimen and a definitive positive or negative diagnosis of PCa (i.e., a confirmed PCa diagnosis or a negative “end-of-study” biopsy) were

eligible for inclusion (n=8,580). We defined cases as men diagnosed with PCa on any “for-cause” or “end-of-study” biopsy (n=1,809). PCa diagnoses were established by agreement between pathologists at the central laboratory who reviewed all study biopsy material and pathologists at the study sites. Clinical stage was provided by the study sites, and Gleason patterns and sum were determined by central pathology review of biopsy tissue. We defined controls as men not diagnosed with PCa at any time during the trial who also had a negative “end-of-study” biopsy. All non-Caucasian men who met this definition were selected as controls to enhance our ability to perform stratified analyses by 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 PCa defined as at least one first degree relative with PCa (n=1,809).

We investigated CMV infection in relation to PCa risk in a subset of men from the parent nested case-control study (n=614 cases and 616 controls). To conserve valuable baseline specimens, we used visit 2 specimens for the CMV analysis and thus chose men with an adequate serum specimen from visit 2. We defined cases as men diagnosed with PCa after visit 2. To allow for investigations between CMV infection and different PCa outcomes with equal precision, we selected approximately equal numbers of cases diagnosed with low-grade (Gleason sum <7) and high-grade (≥7) disease, and approximately equal numbers of cases diagnosed by “for-cause” and “end-of-study” biopsy. We defined controls as men not diagnosed with PCa at any time during the trial who also had a negative “end-of-study” biopsy. We frequency-matched controls to cases by age, treatment arm, and family history of PCa. Although not specifically selected based on race/ethnicity, controls were also enriched for non-Caucasian men because of the original parent study control sampling scheme.

This study was approved by the Institutional Review Board at Johns Hopkins. The PCPT was approved by the Institutional Review Board at the Fred Hutchinson Cancer Research Center, as well as at each of the institutions that randomized a participant.

Assessment of CMV infection

We assessed the presence of CMV infection, a lifelong viral infection that reactivates periodically from latency throughout life, by measuring IgG antibodies against CMV. CMV IgG seropositivity is believed to be stable and lifelong based on data from longitudinal studies of CMV seropositive individuals (12, 16, 17), as well as the observed strong positive association between CMV seropositivity and age, with seroprevalence estimates as high as 91% by 80 years of age (18).

We performed CMV antibody testing on visit 2 serum using the Captia™ CMV IgG enzyme-linked immunosorbent assay (Trinity Biotech, Berkeley Heights, NJ). In a study conducted by the manufacturer, this assay had a sensitivity of 99.2% and specificity of 94.1% relative to another commercially available ELISA, and average intra-run, inter-day, and inter-lot coefficients of variation for the Immune Status Ratio (described below) of 5.9%, 5.4%, and 15.4%, respectively. We determined serostatus by dividing the optical density for each specimen by the product of the mean optical density of the two calibration specimens included in each run and the lot-specific correction factor to obtain an Immune Status Ratio. We considered specimens with ratios <0.90 to be seronegative, 0.91–1.09 to be equivocal, and ≥1.10 to be seropositive. We repeated testing for equivocal specimens until a non-equivocal result was obtained (27 tested twice, 1 tested three times, and 4 tested four times). We investigated assay reproducibility by testing 12 sets of approximately six blinded replicate samples from serum leftover after routine syphilis testing at the Baltimore Bureau of Disease Control laboratory. Samples were approved for use by the Baltimore City Health

Department public review process and anonymized prior to release. Nine of the replicate sets had 100% agreement and three had 83.3% agreement.

Assessment of covariates

At the baseline study visit, PCPT participants completed a detailed self-administered questionnaire on demographic and lifestyle characteristics, including race/ethnicity, education, occupation, 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 completed a 15-page diet and supplement questionnaire designed specifically for PCPT participants that 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 and supplements, and alcohol was derived from this questionnaire (19). Finally, information on treatment for BPH (surgical and medical) was obtained at the baseline, six month, and annual clinic visits, and at the three and nine month phone contacts between visits. Information on lower urinary tract symptoms (IPSS) was obtained at recruitment, baseline, and each clinic visit. This information was used to define a history of symptomatic BPH as a report of treatment for BPH or an IPSS >14 at baseline. Military experience was inferred from participants' form of payment (Veterans Affairs-sponsored payment for medical care) and type of study site (Veterans Affairs hospital, Army/Naval hospital, etc).

Statistical analysis

To investigate the potential for confounding, we calculated adjusted means and proportions of potential confounding variables by PCa case-control status among all participants and by CMV serostatus among controls. We adjusted means and proportions by age, treatment arm, and family history of PCa to account for frequency-matching, and by race to account for over-sampling of non-white Caucasian controls in the study design. We considered as potential confounders all variables mentioned in the covariate section, as well as specimen storage time. To begin to investigate the relation between CMV serostatus and PCa risk, we examined age, treatment arm-, family history-, and race-adjusted distributions of CMV antibodies by PCa case-control status. Next, we calculated odds ratios (ORs) of PCa by CMV serostatus, using unconditional logistic regression and adjusting for age, treatment arm, family history, and race. We further investigated confounding by adding each potential confounder described above individually to the regression model and comparing the point estimate to that obtained from a model including only age, treatment arm, family history of PCa, and race. No covariates were found to shift the point estimate for CMV serostatus appreciably. To investigate the relation between CMV infection and different PCa outcomes, we performed separate analyses for PCa diagnosed by low- and high-grade cancer, and organ-confined disease. To investigate the potential for detection bias by infection-mediated PSA elevation or -induction of prostate abnormalities, we performed additional separate analyses for cases diagnosed by "for-cause" and "end-of-study" biopsy, and we stratified analyses by follow-up time (within one year of blood draw at visit 2 versus later in follow-up, and 2.5 versus >2.5 years of follow-up from visit 2) for men diagnosed by "for-cause" biopsy. We did not perform similar analyses for men diagnosed by "end-of-study" biopsy because these cases were diagnosed, by definition, at the end of the study. Finally, to investigate effect modification by age at PCa diagnosis, treatment arm, family history of PCa, race, and past regular aspirin use, we performed further stratified analyses by these covariates. We calculated p-values for interaction by the likelihood ratio test.

A priori, we had 80% power to detect an OR 1.48 for an expected control seroprevalence of approximately 75% (18). Statistical analysis was performed using SAS version 9.2 (SAS, Cary, NC).

RESULTS

We selected 616 PCa cases and 616 controls for analysis. Two cases did not have sufficient serum for testing, leaving 614 cases and 616 controls. By study design, 50.7% of cases were diagnosed with low-grade PCa (Gleason sum <7) and 49.3% were diagnosed with high-grade (Gleason sum ≥7) disease. 53.3% of cases were diagnosed by “for-cause” biopsy and 46.7% by “end-of-study” biopsy. Almost all cases presented with organ-confined disease (97.6% of 578 cases with stage information), as expected based on eligibility criteria for the trial (low PSA and normal DRE), and annual PCa screening. The median time from visit 2 to diagnosis was 4.02 years (range: 0.02–5.46 years) for cases diagnosed by “for-cause” biopsy and 5.02 years (range: 4.69–5.44 years) for cases diagnosed by “end-of-study” biopsy. Compared to controls, cases were more likely to be Caucasian, as per the study design; not to have military experience; and to report a lesser number of pack-years of smoking. Otherwise, cases and controls were similar with respect to covariates considered. Among controls, CMV seropositive men were more likely to be older and in the finasteride arm; less likely to have a family history of PCa, to be Caucasian, and to have 16 years of education; and more likely to have military experience. Seropositive men also had a higher average body mass index; consumed less overall protein, fish, and calcium; and had smoked a greater average number of pack-years of cigarettes (Table 1).

Overall, PCa cases and controls had a similar distribution of CMV antibody levels, with the possible exception of a slight, non-significantly higher geometric mean Immune Status Ratio for cases than controls. CMV seroprevalence was similar for PCa cases and controls (67.9 versus 65.2%, $p=0.31$, Table 2). No association was observed between CMV serostatus and risk of overall PCa, low- and high-grade PCa, organ-confined disease, and PCa diagnosed by “for-cause” and “end-of-study” biopsy (Table 3). In analyses stratified by follow-up time, a non-significant positive association was observed for men diagnosed by “for-cause” biopsy within one year of visit 2 (OR=1.61, 95% confidence interval (CI): 0.75–3.43, $n=38$ cases) and a positive association was observed for men diagnosed in the first half of follow-up (OR=1.67, 95% CI: 1.01–2.76, $n=96$ cases). No association was observed for men diagnosed by “for-cause” biopsy in the second half of follow-up (OR=1.03, 95% CI: 0.74–1.42, $n=231$ cases). When we excluded cases diagnosed early in follow-up from the entire analysis, no association was observed between CMV serostatus and PCa risk (OR=1.11, 95% CI: 0.87–1.42 excluding cases diagnosed in the first year of follow-up, and OR=1.06, 95% CI: 0.83–1.37 excluding cases diagnosed in the first half of follow-up). The slight, non-statistically significant difference in geometric mean Immune Status Ratio between cases and controls also diminished (data not shown). No effect modification was observed by age at PCa diagnosis, treatment arm, family history of PCa, race, or past regular aspirin use (all p -interaction>0.20). Finally, null results were observed in sensitivity analyses defining CMV seropositivity based on the first test result (for originally equivocal results), adjusting for each run, and excluding the one run that yielded two sets of discordant replicate quality control results.

DISCUSSION

In general, no association was observed between CMV serostatus and PCa risk in this large, prospective study. The sole possible exception to this statement was the suggestive positive finding for men diagnosed by “for-cause” biopsy early but not later in follow-up. In addition to chance, another possible explanation for this positive finding may be detection bias.

Although CMV seropositivity is generally believed to be stable over time, it is possible that, in men with borderline CMV seropositivity, recent (i.e., close in time to visit 2) reinfection with a different CMV strain or reactivation may lead to both a rise in CMV antibody levels at visit 2 and either infection-mediated PSA elevation or infection-mediated prostate abnormalities at visit 2 or subsequent visits, as PSA has been found to remain elevated for at least six months following sexually transmitted infectious episodes (20). Either of these two possibilities might then trigger a “for-cause” prostate biopsy and detection of occult PCa, creating a positive association between CMV antibody seropositivity and PCa among men diagnosed early in follow-up. Thus, in this scenario, results for men diagnosed by “for-cause” biopsy early in follow-up would be falsely positive, whereas those for men diagnosed by “for-cause” biopsy later in follow-up and those diagnosed by “end-of-study” biopsy would be more likely to reflect the true association between CMV serostatus and PCa risk in our study population. As men diagnosed by “for-cause” biopsy early in follow-up accounted for only a small proportion of the study population, our overall findings and even those for men diagnosed by “for-cause” biopsy were largely un-influenced by the results for men diagnosed early in follow-up.

Our generally null findings for CMV serostatus and PCa risk are consistent with those from most previous, larger studies, three of which observed no association between CMV serostatus and PCa (10, 12, 21) and one of which observed lower CMV antibody levels in PCa cases than controls with BPH (14). A further two studies detected no CMV DNA in prostate tissue from PCa cases and controls (11) or in only a few participants (13). Thus, taken together, these findings suggest that CMV infection is not associated with PCa risk.

Although CMV serology is sensitive and specific for CMV infection, and CMV antibodies are believed to be stable over time, one possible drawback to measurement of CMV antibodies in studies of CMV infection and prostate carcinogenesis is its inability to indicate when primary infection occurred. In the United States, approximately 30–40% of children are seropositive for CMV infection by 6–11 years of age, a further 5% seroconvert during adolescence, and another 45% go on to acquire CMV infection later in adulthood (18).

Therefore, if, for instance, primary infection during a critical window of susceptibility (e.g., by sexual transmission during adolescence when the prostate is growing and developing) is necessary for prostate carcinogenesis, then this type of time-sensitive association might not be detectable by serology, as infections acquired during this critical window would not be distinguishable from infections acquired outside of this window. The same concern would also be true for tissue-based studies of CMV DNA or RNA. Another possible limitation to both of these types of assessments is their inability to determine when and how often CMV reinfection or reactivation occurred. Therefore, if, for instance, reinfection or reactivation during a critical window of susceptibility is important or the cumulative lifetime frequency of reinfection or reactivation is important, these types of hypothetical associations might also be missed.

In summary, findings from our large prospective study of CMV serostatus and PCa risk do not support a role for CMV infection in prostate carcinogenesis. However, we cannot rule out the less likely possibility of a time-sensitive association between CMV infection and PCa risk or an association between reinfection/reactivation history and PCa risk.

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Baseline characteristics¹ of participants by prostate cancer status and cytomegalovirus serostatus in the Prostate Cancer Prevention Trial

Table 1

	Case-control status			Cytomegalovirus serostatus(controls only)		
	Case (n=614)	Control (n=616)	P-value ²	Positive (n=409)	Negative (n=207)	P-value ²
Mean age (years)	64.1	64.0	Matched	64.4	63.1	0.01
Finasteride (%)	48.9	48.9	Matched	51.3	44.0	0.08
First-degree relative with prostate cancer (%)	20.5	20.6	Matched	18.3	25.1	0.05
Race (%):						
Caucasian	90.4	78.9		73.8	88.9	
African-American	6.5	10.7	N/A ³	13.7	4.8	<0.001
Other race/ethnicity	3.1	10.4		12.5	6.3	
Education (%):						
12 years	20.2	19.2		22.2	14.5	
13–15 years	26.9	30.4	0.74	33.0	26.6	<0.001
16 years	53.0	50.5		44.7	58.9	
Occupation (%):						
Professional	65.5	63.8		61.9	66.2	
Craftsman	13.0	12.9		15.2	9.7	
Sales	9.6	8.8	0.27	8.1	9.7	0.08
Farming	1.5	2.3		1.5	3.9	
Other	10.5	12.3		13.4	10.6	
Military experience (%) ⁴	24.8	29.5	0.06	34.2	25.1	0.02
Marital status (%):						
Currently married/marriage-like relationship	88.9	90.3		89.2	91.3	
Divorced/widowed	8.8	7.3	0.66	8.1	6.8	0.71
Never married	2.1	2.4		2.7	1.9	
Mean height (inches)	69.9	69.7	0.37	69.6	69.9	0.09
Mean body mass index (kg/m ²)	27.6	27.6	0.93	27.8	27.1	0.02
Mean intakes/day of:						
Energy (kcal)	2168.7	2140.7	0.56	2090.4	2195.8	0.14

	Case-control status			Cytomegalovirus serostatus (controls only)		
	Case (n=614)	Control (n=616)	P-value ²	Positive (n=409)	Negative (n=207)	P-value ²
Protein (g)	92.0	92.1	0.98	88.6	97.2	0.01
Carbohydrate (g)	264.7	259.4	0.37	253.0	266.9	0.11
Fat (g)	79.7	78.6	0.62	78.1	77.8	0.93
Fruit consumption (fruit) ⁵	1.6	1.5	0.18	1.5	1.5	0.89
Vegetable consumption (vegetables)	2.2	2.3	0.29	2.2	2.3	0.49
Tomato products (1 cup servings)	0.48	0.47	0.84	0.47	0.47	0.88
Red meat (6 oz servings)	0.60	0.63	0.21	0.61	0.65	0.30
Processed meat (6 oz servings)	0.35	0.38	0.19	0.37	0.38	0.75
Fish (6 oz servings)	0.23	0.21	0.18	0.20	0.24	0.03
Calcium from food and supplements (mg)	1072	1063	0.78	1008	1132	0.01
Zinc from food and supplements (mg)	25.5	25.4	0.94	25.2	25.4	0.90
Total alcohol (g)	9.2	8.9	0.66	8.6	8.9	0.82
Cigarette smoking:						
Smoked regularly before 25 years of age (%)	61.7	63.8	0.45	65.0	62.3	0.51
Currently smoke (%)	6.1	6.6	0.71	7.3	6.3	0.63
Mean pack-years smoked	13.8	16.0	0.02	17.0	13.9	0.03
Physical activity (%):						
Sedentary	16.9	15.5		15.4	16.9	
Light activity	41.8	41.6	0.18	39.1	44.9	0.16
Moderate activity	33.4	31.8		33.7	28.5	
Very active	7.5	10.9		11.5	9.7	
Frequency of sexual activity in the past 4 weeks (%):						
Not at all	13.7	12.0		12.7	10.1	
Once	11.7	12.5		12.5	12.1	
2-3 times	23.5	24.3	0.25	22.5	27.5	0.63
Once/week	31.0	27.7		26.7	30.0	
2-3 times/week	16.7	19.5		21.5	15.9	
4 times/week	2.1	3.3		3.7	2.9	
Vasectomy (%)	29.3	31.2	0.48	28.9	32.9	0.31

	Case-control status		Cytomegalovirus serostatus(controls only)			
	Case (n=614)	Control (n=616)	P-value ²	Positive (n=409)	Negative (n=207)	P-value ²
Mean age at vasectomy	38.8	37.5	0.10	37.4	38.2	0.50
History of diabetes mellitus type 2 (%)	6.3	7.7	0.33	8.8	7.2	0.51
History of benign prostatic hyperplasia (%) ⁶	10.7	12.2	0.42	13.0	11.1	0.51
Aspirin use on a regular basis (%):						
Current use	42.5	40.8	0.54	39.6	40.6	0.82
Past use	8.0	8.6	0.70	10.0	6.8	0.18

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

² P-values were calculated by linear regression for continuous and binary variables, and by generalized logit models for categorical variables.

³ African-American 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. African-American Hispanics are included in the African-American category. Previous analyses in this case-control study population included African-American Hispanics in the “other race/ethnicity” category.

⁴ Defined as use of Veterans Affairs-sponsored payment for medical care or medical care at a military site. Previous analyses in this case-control study population defined military experience solely by use of Veterans Affairs-sponsored payment for medical care.

⁵ Based on the “5-a-day” definition. Previous analyses in this case-control study population defined fruit consumption by the “summation method”.

⁶ Defined as surgical or medical treatment for benign prostatic hyperplasia or an International Prostate Symptom Score>14.

Table 2

Cytomegalovirus antibody distributions¹ for 614 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

	Case ²	Control	P-value ³
Mean ISR	1.73	1.69	0.58
Geometric mean ISR	0.94	0.84	0.22
Seropositive (%)	67.9	65.2	0.31

ISR=Immune Status Ratio (see Methods section for details).

¹ Adjusted for age, treatment arm, family history of prostate cancer, and race (non-Caucasian versus Caucasian) 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.

Table 3

Odds ratios (ORs) and 95% confidence intervals (CIs) of prostate cancer for cytomegalovirus serostatus in 614 prostate cancer cases and 616 frequency-matched controls in the Prostate Cancer Prevention Trial

	Cases (n) ¹	Controls (n)	OR (95% CI) ²
Total prostate cancer			
Seronegative	204	207	1.00
Seropositive	410	409	1.13 (0.89–1.45)
Low-grade (Gleason sum <7) prostate cancer			
Seronegative	101	207	1.00
Seropositive	210	409	1.24 (0.92–1.67)
High-grade (Gleason sum ≥ 7) prostate cancer			
Seronegative	103	207	1.00
Seropositive	200	409	1.04 (0.77–1.40)
Organ-confined (T2 and N0M0) prostate cancer			
Seronegative	188	207	1.00
Seropositive	376	409	1.13 (0.88–1.45)
Prostate cancer diagnosed by “for-cause” biopsy³			
Seronegative	105	207	1.00
Seropositive	222	409	1.17 (0.87–1.56)
Prostate cancer diagnosed by “end-of-study” biopsy⁴			
Seronegative	99	207	1.00
Seropositive	188	409	1.10 (0.81–1.50)

¹Cases 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).

²Calculated by logistic regression, including terms for age (continuous), treatment arm, family history of prostate cancer, and non-white race.

³“For-cause” biopsy refers to a biopsy performed because of an elevated prostate-specific antigen concentration or an abnormal digital rectal examination.

⁴“End-of-study” biopsy refers to a biopsy performed without indication after seven years of participation in the study as per trial protocol.