Prospective Study of *Trichomonas vaginalis* Infection and Prostate Cancer Incidence and Mortality: Physicians' Health Study

Jennifer R. Stark, Gregory Judson, John F. Alderete, Vasanthakrishna Mundodi, Ashwini S. Kucknoor, Edward L. Giovannucci, Elizabeth A. Platz, Siobhan Sutcliffe, Katja Fall, Tobias Kurth, Jing Ma, Meir J. Stampfer, Lorelei A. Mucci

Background A recent nested case–control study found that the presence of antibodies against *Trichomonas vaginalis*, a common nonviral sexually transmitted infection, was positively associated with subsequent incidence of prostate cancer. We confirmed these findings in an independent population and related serostatus for antibodies against *T vaginalis* to prostate cancer incidence and mortality.

Methods We conducted a case–control study nested within the Physicians' Health Study that included 673 case subjects with prostate cancer and 673 individually matched control subjects who had available plasma samples. Plasma from blood samples collected at baseline was assayed for antibodies against *T vaginalis* with an enzyme-linked immunosorbent assay. We used conditional logistic regression to estimate the odds ratios (ORs) of incident prostate cancer, extraprostatic prostate cancer, and cancer that would ultimately progress to bony metastases or prostate cancer–specific death.

Results Although not statistically significant, the magnitude of the association between *T vaginalis*–seropositive status and overall prostate cancer risk (OR = 1.23, 95% confidence interval [CI] = 0.94 to 1.61) was similar to that reported previously. Furthermore, a seropositive status was associated with statistically significantly increased risks of extraprostatic prostate cancer (OR = 2.17, 95% CI = 1.08 to 4.37) and of cancer that would ultimately progress to bony metastases or prostate cancer–specific death (OR = 2.69, 95% CI = 1.37 to 5.28).

Conclusions This large prospective case–control study obtained further support for an association between a seropositive status for antibodies against *T vaginalis* and the risk of prostate cancer, with statistically significant associations identified for the risk of extraprostatic prostate cancer and for clinically relevant, potentially lethal prostate cancer.

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A number of inflammation-related factors have been implicated in prostate cancer risk and progression, but the origin of inflammation is unclear (1). Infections are one possible source. *Trichomonas vaginalis* is a common nonviral sexually transmitted infection, with an estimated 174 million annual infections globally (2). Prevalence in American men ranges from approximately 3% among young men in the general population (3) to 65% among military personnel with nongonococcal urethritis (4). Little is known about the prevalence of infection in older men; however, in contrast to other common sexually transmitted infections, the infection has been observed to be more prevalent among men aged 25–39 years than in men aged 18–20 years (3,5). Urethral symptoms associated with *T vaginalis* tend to be less severe than other common sexually transmitted infections, such as those due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae* (6). Furthermore, more recent studies have found that *T vaginalis* is associated with asymptomatic infections in 50%–75% of infected men (5,7). Consequently, many men are unaware that they are infected with the parasite.

Men infected with *T vaginalis* often experience spontaneous resolution, as shown by decreasing rates of infection with time

Affiliations of authors: Department of Epidemiology (JRS, GJ, ELG, KF, TK, MJS, LAM) and Department of Nutrition (ELG, MJS), Harvard School of Public Health, Boston, MA; Channing Laboratory (JRS, ELG, JM, MJS, LAM) and Division of Preventive Medicine (TK), Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; School of Molecular Biosciences, Washington State University, Pullman, WA (JFA, VM, ASK); Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD (EAP); Alvin J. Siteman Cancer Center and Department of Surgery, Washington University School of Medicine, St. Louis, MO (SS); Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden (KF); Institut National de la Santé et de la Recherche Médicale, Unit 708 Neuroepidemiology and Pierre et Marie Curie University, Paris, France (TK).

Correspondence to: Jennifer R. Stark, ScD, Department of Epidemiology, Harvard School of Public Health, 677 Huntington Ave, Boston, MA 02115 (e-mail: stark@hsph.harvard.edu).

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CONTEXT AND CAVEATS

Prior knowledge

The presence of antibodies against *Trichomonas vaginalis*, a common nonviral sexually transmitted infection, has been positively associated with subsequent incidence of prostate cancer.

Study design

Nested case–control study that included case subjects with prostate cancer and individually matched control subjects who had available plasma samples that were collected at baseline. Plasma was assayed for antibodies against *T vaginalis*. The relationship of incident prostate cancer, extraprostatic prostate cancer, and cancer known to progress to bony metastases or prostate cancer–specific death was investigated.

Contribution

The size of the association between *T vaginalis*–seropositive status and overall prostate cancer risk, although not statistically significant, was similar to that reported previously. A seropositive status was associated with statistically significantly increased risks of extraprostatic prostate cancer, cancer that is known to progress to bony metastases, or prostate cancer–specific death.

Implications

Further investigation is warranted to determine whether local prostatic inflammation could lead to downstream events that influence prostate cancer risk and to confirm the association between *T vaginalis* serostatus and aggressive prostate cancer.

Limitations

The time between *T vaginalis* infection and blood collection was not known. Men with *T vaginalis* infection might visit their physicians more frequently than those without such infection and so increase the possibility of prostate cancer diagnosis. Because other sexually transmitted infections occur concurrently with *T vaginalis* infections, the possibility that *T vaginalis* is acting as a marker for another pathogen cannot be ruled out.

From the Editors

since last sexual contact with an infected partner (8) and natural history studies (9), in which as many as one-third of men cleared the infection within 2 weeks without treatment (7). Nevertheless, a smaller proportion of men experience long-term asymptomatic infection (7,9). *T vaginalis* can ascend the urethra to the prostate and infect the prostate epithelium (10,11), and in that epithelium, it is associated with evidence of acute and chronic inflammation (10). As such, chronic prostatic infection with *T vaginalis* may initiate an inflammatory response that could increase the risk of developing prostate cancer (10) and increase the risk of disease progression.

A recent case–control study (12) nested in the Health Professionals Follow-up Study found that seroprevalence of *T vaginalis* infection was positively associated with subsequent prostate cancer risk, with a suggestion of the greatest risk for more aggressive disease that was defined as high Gleason grade disease. As a follow-up on the positive finding between *T vaginalis* serostatus and prostate cancer risk, we conducted a large nested case–control study within the Physicians' Health Study to further investigate a potential association between *T vaginalis* serostatus and prostate cancer incidence. We also investigated potential associations between *T vaginalis* serostatus and subgroups of prostate cancer defined by tumor stage, tumor grade, age at diagnosis, and cancer that ultimately progressed to bony metastases or prostate cancer–specific death.

Study Subjects and Methods

Study Population

The Physicians' Health Study (13,14) was initiated in 1982 as a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene for the primary prevention of cardiovascular disease and cancer. The study included 22 071 healthy US male physicians aged 40–84 years at baseline. Before being randomly assigned to a treatment group, 14 916 (68%) of the 22 071 men provided a blood sample (15). These participants constitute the study base for the nested case–control study.

We included 673 case subjects who were diagnosed with prostate cancer up to 18 years after blood collection (1982–2000) and who had available plasma samples. We selected 673 control subjects from the population at risk at the time of the case subject's diagnosis (ie, those who had provided blood, had not had a prostatectomy, and had not reported a diagnosis of prostate cancer at the time the case subject was diagnosed with prostate cancer). For statistical efficiency, control subjects were individually matched to case subjects by age (within 1 year), smoking status (never, former, or current), and follow-up time.

Laboratory Assessment

Plasma from prospectively collected blood samples from each case subject and his matched control subject (stored at −80°C) was thawed and assayed for antibodies against *T vaginalis* with an assay that detects IgG antibodies against purified, recombinant α-actinin protein from *T vaginalis*. Enzyme-linked immunosorbent assays were optimized with known negative and positive pooled plasma of uninfected individuals and patients with trichomonosis, respectively, that gave reproducible readings after incubation with microtiter wells containing immobilized α-actinin. In this study, paired plasma samples from case and control subjects were diluted at 1:10 (vol/vol) in phosphate-buffered saline–Tween-20 containing 5% skim milk, and 100 μL of the diluted plasma was added to each well of a 96-well plate (Nunc, Rochester, NY). After incubation for 3 hours at 37°C, the plates were washed three times with phosphate-buffered saline–Tween-20 followed by the addition of 100 μL of secondary goat anti–human IgG (Fc-specific) conjugated to horseradish peroxidase at a 1:1500 dilution in phosphate-buffered saline–Tween-20 containing 5% skim milk to each well. Plates were incubated again for 1 hour at 37°C and then washed three times with phosphate-buffered saline–Tween-20. Color was allowed to develop by adding 100 μL of substrate solution per well (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); phosphate–citrate buffer with 0.03% sodium perborate, Sigma Chemical Co, St. Louis, MO) according to the manufacturer's recommendations, and plates were incubated at room temperature for 10 minutes. Absorbance values at a wavelength of 405 nm were then obtained by examining the supernatants spectrophotometrically with an enzyme-linked immunosorbent assay plate reader (Bio-Tek instruments, Inc, Winooski, VT).

Case–control sample pairs were assayed in adjoining wells, with blinding of laboratory personnel as to the case–control status of the samples. All samples were tested in duplicate and inferences were based on the mean of duplicate values. To create absorbance scores, we used a control plasma panel consisting of pooled plasma from known seronegative patients and four plasma samples with increasing seropositivity. We divided the mean duplicate absorbance value for each seropositive sample in the control panel by the mean duplicate absorbance value of the seronegative control plasma to obtain a minimum positive to negative (P/N) ratio for each absorbance score (0 = 1 to <1.81; 1 = 1.81 to <2.78; 2 = 2.78 to <3.31; 3 = 3.31 to <4.07; or 4 = ≥4.07). The positive to negative ratio was computed for all case subjects with prostate cancer and all control subjects, and the resulting values were then compared with the specified cut points determined from the control panel to assign an absorbance score (ie, 0, 1, 2, 3, or 4). Samples from the control panel were included with each plate to monitor reproducibility; values for these samples always fell within the previously determined range. Samples with absorbance scores of 3 or 4 were considered positive for history of trichomonosis. We also included 29 quality-control duplicate or triplicate samples that were randomly distributed across plates. Concordance in serostatus was achieved for 26 of 29 (90%) of the quality-control samples; 17 of 26 of the concordant replicate samples were seropositive.

Statistical Analysis

We used conditional logistic regression to analyze prostate cancer risk according to serostatus adjusting for matching factors. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by comparing men who were *T vaginalis* seropositive at baseline with men who were *T vaginalis* seronegative. We additionally controlled for randomization to aspirin assignment and body mass index (continuous) and evaluated risk within subgroups of stage and grade at diagnosis. All *P* values were from two-sided statistical tests, with α of .05 considered to be statistically significant.

Analyses were undertaken with the SAS Statistical Analysis version 9.1.3 (SAS Institute, Cary, NC). The research protocol was approved by the institutional review board at Partners Healthcare. Questionnaire data were collected with implied consent, and biomarker data were collected with written authorization.

Results

On average, case subjects were aged 68.7 years (SD = ±7.4 years) at diagnosis. Most case subjects were diagnosed with well-differentiated tumors (54% with a Gleason score of 2–6) at a localized stage (83% with a stage of T1 or T2). Mean time between blood collection and prostate cancer diagnosis was 9.3 years (range = 0.3–17.9 years). The seroprevalence of *T vaginalis* infection was 21% in control subjects and 25% in case subjects (Table 1). *T vaginalis* absorbance scores were not associated with age or baseline prostate-specific antigen (PSA) levels in case subjects or control subjects.

T vaginalis seropositivity was not statistically significantly associated with total prostate cancer risk (OR = 1.23, 95% CI = 0.94 to 1.61) or high-grade disease (OR for Gleason 7–10 scores = 1.10, 95% CI = 0.72 to 1.68). However, serological evidence of *T vaginalis* infection was associated with a statistically significant increase in the risk of diagnosis of advanced-stage prostate cancer (OR = 2.17, 95% CI = 1.08 to 4.37) and in the risk of cancer that would ultimately progress to distant metastases or cancer-specific death (OR = 2.69, 95% CI = 1.37 to 5.28) (Table 1). We also found that the association between *T vaginalis* and prostate cancer was stronger for men who were diagnosed more closely to blood collection (Table 1). Compared with case subjects overall (n = 673), the 94 case subjects who were diagnosed within 5 years of blood collection tended to be somewhat older at diagnosis (eg, those who were aged >65 years = 72 [77%] vs 452 [67%]) and more advanced (eg, those who were at stage T3 or T4, N1, or M1 = 23 [25%] vs 105 [16%]). However, cross-classifying men on these characteristics suggested that the time scale that most influenced effect estimates was the duration between blood collection and diagnosis. Given the observed increased risk for cancer soon after blood collection, we explored the association between *T vaginalis* serostatus and lethal prostate cancer, according to years from blood collection to diagnosis. Among the 39 men diagnosed with lethal cancer within 5 years of blood collection and their matched control subjects, men positive for history of trichomonosis (n = 15) were statistically significantly more likely to develop lethal prostate cancer than seronegative men (OR = 6.4, 95% CI = 1.5 to 27.9).

Discussion

In this large nested case–control study, we provide further evidence to support the previously reported association between a *T vaginalis*–seropositive status and prostate cancer risk (12). The magnitude of the overall association of *T vaginalis*–seropositive status with incidence in our study, although not statistically significant, was similar to that observed in the previous case–control study nested in the Health Professionals Follow-up Study (OR = 1.43, 95% CI = 1.00 to 2.03). The Health Professionals Follow-up Study found a suggestion that infection was primarily associated with more aggressive disease, as shown by the higher Gleason scores at diagnosis, but small numbers prohibited a subgroup analysis among men with advanced disease. In this analysis with more than two decades of follow-up for case subjects with prostate cancer, we found that *T vaginalis*–seropositive status was primarily associated with clinically relevant prostate cancer. That is, compared with a seronegative status, a seropositive status before cancer diagnosis was associated with a statistically significant risk of developing prostate cancer that was diagnosed at an advanced stage. Moreover, *T vaginalis* infection appears to be associated with cancer that will ultimately progress to bony metastases and prostate cancer death, independent of body mass index, smoking status, aspirin randomization group, age at diagnosis, and tumor stage and grade. We found no evidence of a stronger association with higher Gleason grade but the subjectivity of Gleason grading and the shift in scores over time (16–18) could explain this discrepancy, because Gleason scores in the Health Professionals Follow-up Study tended to be assigned more recently and, thus, may be better predictors of lethal disease (18).

Our study had several limitations. Because all men provided blood samples in 1982 and all *T vaginalis* assays of plasma samples were completed in 2008, the performance of the assay should not

Table 1. Association between *Trichomonas vaginalis* antibody serostatus and prostate cancer risk among 673 matched pairs nested in the Physicians' Health Study (1982–2000)*

	T vaginalis serostatus	
	Negative	**Positive**
Control subjects, No. (%)	529 (78.6)	144 (21.4)
All prostate cancer		
Case subjects, No. (%)	508 (75.5)	165 (24.5)
OR (95% CI)	1.00 (Ref)	1.23 (0.94 to 1.61)
Tumor grade: Gleason 2–6		
Case subjects, No. (%)	238 (76.3)	74 (23.7)
OR (95% CI)	1.00 (Ref)	1.16 (0.77 to 1.74)
Tumor grade: Gleason 7–10		
Case subjects, No. (%)	204 (76.7)	62 (23.3)
OR (95% CI)	1.00 (Ref)	1.10 (0.72 to 1.68)
Tumor stage: localized (T1 or T2)		
Case subjects, No. (%)	406 (76.6)	124 (23.4)
OR (95% CI)	1.00 (Ref)	1.10 (0.81 to 1.49)
Tumor stage: extraprostatic (T3 or T4, N1, and M1)		
Case subjects, No. (%)	70 (66.7)	35 (33.3)
OR (95% CI)	1.00 (Ref)	2.17 (1.08 to 4.37)
Nonlethal cancer		
Case subjects, No. (%)	416 (76.7)	126 (23.3)
OR (95% CI)	1.00 (Ref)	1.01 (0.75 to 1.37)
Lethal cancer or development of bony metastases		
Case subjects, No. (%)	92 (70.2)	39 (29.8)
OR (95% CI)	1.00 (Ref)	2.69 (1.37 to 5.28)
Age at diagnosis: <65 y		
Case subjects, No. (%)	169 (76.5)	52 (23.5)
OR (95% CI)	1.00 (Ref)	1.41 (0.86 to 2.31)
Age at diagnosis: ≥65 y		
Case subjects, No. (%)	339 (75.0)	113 (25.0)
OR (95% CI)	1.00 (Ref)	1.12 (0.81 to 1.56)
Time from blood draw to diagnosis: ≤5 y		
Case subjects, No. (%)	64 (68.1)	30 (31.9)
OR (95% CI)	1.00 (Ref)	2.86 (1.27 to 6.47)
Time from blood draw to diagnosis: >5 y		
Case subjects, No. (%)	444 (76.7)	135 (23.3)
OR (95% CI)	1.00 (Ref)	1.09 (0.81 to 1.46)

* From logistic regression conditioned on age and smoking and additionally adjusted for randomized aspirin assignment and body mass index. CI = confidence interval; OR = odds ratio; Ref = referent.

be differentially influenced by specimen quality according to date of cancer diagnosis. The unknown period of time between infection and blood collection, however, could influence assay sensitivity. Presumably, men who were infected with *T vaginalis* closer to the time of blood collection in 1982 would be more likely to have detectable levels of antibodies. Because case and control subjects were matched on age (range = 40–84 years at blood collection) and timing of infection is more likely to be related to age than calendar time, this misclassification would likely be nondifferential with respect to case–control status and thus lead us to underestimate the true effect estimate.

Two additional biases also warrant attention. First, we found that the association between *T vaginalis* infection and incidence of prostate cancer was stronger among men diagnosed within 5 years of blood collection. Biomarkers most strongly associated with disease occurring early in a study typically raise concerns about reverse causation (ie, because of the influence of early preclinical disease on the measured biomarker). However, in this study and in all studies of prostate cancer, biological heterogeneity and the impact of PSA testing on the type of prostate cancers diagnosed are important considerations. Consequently, the men who were diagnosed with prostate cancer earlier in our follow-up, before the introduction of PSA testing in 1986, are more likely to be clinically relevant. Thus, the association observed among case subjects who were diagnosed early in follow-up is consistent with the strong association between infection and advanced-stage or lethal disease. For reverse causation to account for our study findings, the carcinogenic process would have to lead to higher levels of detectable antibodies. Although no data have been obtained to support or contest the assumption that levels of antibodies against *T vaginalis* increase during cancer development, tumorigenesis is known to alter adaptive immune response (19). Second, our findings could be influenced by detection bias if men with *T vaginalis* infection were more likely to be diagnosed with prostate cancer. To address this possibility, we investigated the relationship of antibody levels to baseline PSA levels but found no association. However, we cannot rule out other urologic symptoms that could bring about diagnosis. Conservatively, serological history of infection with *T vaginalis* may be a marker of clinically relevant disease, as suggested by the association between infection and development of

bony metastases or prostate cancer death. More research is required to establish this association.

Disease heterogeneity could also largely explain the apparent discrepancy between our findings and those of a recent study using data from 616 case subjects and 616 matched control subjects sampled from the Prostate Cancer Prevention Trial, a randomized trial of finasteride in 18 882 men, which found no association between *T vaginalis* seropositivity and the incidence of prostate cancer (20). We found that a *T vaginalis*–seropositive status was principally associated with aggressive, potentially lethal disease. In contrast, most prostate cancers that were analyzed in the Prostate Cancer Prevention Trial were diagnosed at an early stage as a result of annual PSA screening and end-of-study prostate biopsy (21). Evidence is accumulating that the risk factors for lethal and indolent prostate cancer may differ. In an analysis in the Health Professionals Follow-up Study that examined 10 risk factors for total or advanced prostate cancer supported by existing literature (22), only four factors were found to have a statistically significant association with overall incidence: African American race, positive family history, higher tomato sauce intake (inversely), and α-linolenic acid intake. By contrast, recent smoking history, taller height, higher body mass index, positive family history, and high intakes of total energy, calcium, and linolenic acid were all statistically significantly associated with fatal prostate cancer. Consistent with our study, these results suggest that there may be multiple biological pathways that contribute to particular subgroups of prostate cancer.

The proportions of case subjects and control subjects with high seropositivity for antibodies against *T vaginalis* were somewhat higher in this study (24.5% of case subjects and 21.4% of control subjects) than in the Prostate Cancer Prevention Trial (15.2% of case subjects and 15.0% of control subjects) or the Health Professionals Follow-up Study (13% of case subjects and 9% of control subjects) (12). Assays for all three studies were prepared under the direction of the same microbiologist (J. F. Alderete) and used an enzyme-linked immunosorbent assay to detect antibodies against α-actinin protein from *T vaginalis*. In both the Prostate Cancer Prevention Trial and the Physicians' Health Study studies, known seropositive and seronegative control samples were used to determine absorbance score cut points, which were then applied to study case subjects and control subjects. In the Health Professionals Follow-up Study, absorbance score cut points were based on previous serological findings (23,24), because serum samples from positive and negative control subjects were not available. Furthermore, absolute readings of the enzyme-linked immunosorbent assays in all three studies could be influenced by the specific technician conducting the assay and the fact that the laboratory was relocated in December 2007. Thus, differences in assay sensitivity may account for some of the variation in distribution of *T vaginalis* seropositivity across these three studies, especially given that demographic characteristics do not appear to explain the observed variability. All three studies included men from across the United States. Although African American race and lower socioeconomic status are generally associated with higher rates of sexually transmitted infections (25), including *T vaginalis* infections (3), the study with the highest proportion of men with a seropositive status (ie, the Physicians' Health Study) has the smallest proportion of African Americans (<1%) and a relatively high socioeconomic status because all participants are physicians. Further, the mean age at blood collection in all three studies was similar (ie, 66 years in Health Professionals Follow-up Study, 64 years in Prostate Cancer Prevention Trial, and 59 years in Physicians' Health Study).

Because other sexually transmitted infections occur concurrently with *T vaginalis* infections, we cannot rule out the possibility that *T vaginalis* is acting as a marker for another infection. However, two studies (5,6) report that concomitant sexually transmitted infections, including those by *N gonorrhoeae* and *C trachomatis*, occur only in 10%–20% of case subjects, making it unlikely that these particular sexually transmitted infections could account for the observed association. Furthermore, the previous study in the Health Professionals Follow-up Study investigated other common sexually transmitted infections, including those by *N gonorrhoeae*, *C trachomatis*, *Treponema pallidum*, and human papillomavirus, and found no association with prostate cancer, except for an inverse association for human herpesvirus type 8 infection (26,27). Nested case–control studies using data from the Nordic biobank consortium found no association between prostate cancer risk and human papillomavirus types 16, 18, and/or 33 (28), herpes simplex virus-2, or human herpesvirus type 8 (29); however, these studies observed a statistically significant inverse association with serological evidence of *C trachomatis* infection (30). A study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (20) found that seroprevalence of *C trachomatis*, human papillomavirus-16 and -18, herpes simplex virus-2, cytomegalovirus, and human herpesvirus type 8 were not individually associated with prostate cancer risk among white men. Men with one or more sexually transmitted infections, however, had a modest increase in risk of developing prostate cancer (OR = 1.3, 95% CI = 1.0 to 1.6), indicating that the measured infections could perhaps be serving as proxies for another infection such as *T vaginalis*.

Although our study may elucidate one mechanism by which local prostatic inflammation could arise and lead to downstream events that influence prostate cancer development and progression, studies that focus on local response to infection in the prostate are needed to determine whether *T vaginalis* is a causal agent. Nonetheless, in light of the limited understanding of factors that lead to lethal prostate cancer, our finding of an association between *T vaginalis* serostatus and aggressive prostate cancer is noteworthy. If our findings are confirmed, *T vaginalis* could serve as a marker for adverse outcomes in patients for prostate cancer or, more optimistically, as a target for secondary chemoprevention.

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Notes

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