Review Article

Is *Trichomonas vaginalis* a Risk Factor for Prostate Cancer? A Systematic Review and Meta-analysis

Abstract

Clinical studies have shown that patients exposed to the protozoan *Trichomonas vaginalis* (TV) may present an increased risk to develop prostate cancer (PCa). However, since data from other studies and meta-analyses did not provide so far univocal results this issue remains controversial. In this systematic review, we examined the current molecular, cellular and clinical evidence in favor or against a possible association between TV prostatitis and the incidence of PCa. Electronic database search, title/abstract screening and full-text reading yielded a total of 17 clinical articles and meta-analyses and 12 articles showing the results of preclinical investigations. Preclinical evidence points to the involvement of TV in proliferative disorders in prostate cells, involving an array of immune cell mediators. Five clinical case-control studies documented a significantly increased odds for PCa in patients with a positive TV serostatus, whereas seven other studies showed nonsignificant results. Our meta-analysis including 12 studies retrieved up to June 1, 2021, did not evidence a significant association between a positive TV serostatus and PCa of any grade (odds ratio [OR], 1.14; 95% confidence interval [CI]: 0.84–1.53). Moreover, we could not find a significant association between advanced/lethal PCa and TV exposure (OR, 1.18; 95% CI: 0.70–2.00). In conclusion, the association between a positive TV serostatus and PCa remains uncertain. Studies focused on a large sample of documented cases of symptomatic, clinical TV chronic prostatitis are warranted to make a conclusive statement in this regard.

Keywords: *Carcinogenesis, oncogenesis, prostate, prostate cancer, prostatitis, Trichomonas vaginalis*

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Introduction

Chemical or physical genotoxic agents can cause-together with epigenetic modulators-the vast majority of human cancers.[1] However, carcinogenesis may also occur when human tissues are colonized by microorganisms which may cause chronic infection and sustained inflammation. This is known to occur in a significant fraction of cancer cases (up to 20%).[2,3] Among viruses, the Human Papillomavirus, the Epstein-Barr virus, the Hepatitis B and C virues, the Kaposi's sarcoma-associated herpesvirus and others are the cause of several forms of cancer.[4] Among bacteria, the linkage between *Helicobacter pylori* and gastric cancer or mucosa-associated lymphoid tissue lymphoma is well established.[5]

The persistent seroprevalence of protozoans such as *Cryptosporidium parvum, Toxoplasma gondii, Blastocystis hominis, Plasmodium falciparum* and *Trichomonas vaginalis* (TV) is also reputed to be involved in the genesis of human cancers.[6]

TV is a flagellated anaerobic protozoan parasite that can infect male and female mucosae. TV is the causative agent of the most common nonviral sexually transmitted infection (STI), affecting over 150 million people worldwide.[7] In women, TV can cause vaginitis, characterized by vaginal discharge, and *colpitis macularis*, a herythematous, micro-hemorragic inflammation of the cervix. In addition, trichomonal infection has been suggested to concur to the onset of cervical cancer, likely by enhancing the oncogenic potential of Human Papillomavirus.[8] In men, TV can cause symptomatic or asymptomatic infections of the lower urinary tract and accessory glands, and trichomonal prostatitis is an occasional finding in a fraction of patients.[9] Notably, prostatitis is suspected to be a risk factor for prostate cancer (PCa).[10,11]

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The role of TV in the genesis of PCa is still controversial and data from clinical studies and meta-analyses did not provide so far univocal results.[12]

In this systematic review, we examined the current molecular, cellular and clinical evidence in favor or against a possible association between TV prostatitis and the incidence and severity of PCa. Wherever possible, we performed a meta-analysis of clinical data.

Patients and Methods

Eligibility criteria

In the preclinical section of the review, we included only full-text articles written in English reporting the results of laboratory investigation focusing on TV, PCa, carcinogenesis and proliferative disorders. In the clinical section of the review we included only full-text articles written in English, reporting case-control studies evaluating the relationship between exposure to TV, assessed with serological methods, and a diagnosis of PCa. Patients of any ethnicity with a history of PCa of any grade, lethal or nonlethal, were eligible for the present review.

Outcomes

The single clinical outcome considered for this review is the association between the serological evidence of exposure to TV and a documented diagnosis of PCa of any grade.

Search strategy and study selection

Retrieval of published reports of case-control studies and preclinical reports was performed by searching international databases (e.g. Medline, Embase, etc., for PubMed: (Trichomonas [Title/Abstract]) AND (Prostate [Title/ Abstract]) AND (Cancer [Title/Abstract] OR carcinoma [Title/ Abstract]). All searches were assessed as up to date on June 1, 2021. Articles included in the present review are referred to by the first author and year of publication.

Quality assessment

The quality of individual clinical studies was assessed by two researchers using the case-control study version of the Newcastle–Ottawa Scale (NOS). The thresholds for converting NOS scores to Agency for Healthcare Research and Quality (AHRQ) standards were:

- Good quality: 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain
- Fair quality: 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain
- Poor quality: 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/ exposure domain.

Publication bias was investigated by visually assessing funnel plots and by performing both the Egger's regression test and the Begg's rank correlation analysis.

We outlined the results of our meta-analyses on a summary of findings table, rating as well the quality of the pooled evidence according to GRADE criteria.[13]

Clinical data collection and statistical analysis

Data extraction was performed using standard extraction forms by two authors. To analyze dichotomous data we calculated crude (unadjusted) odds ratios (OR), the 95% confidence intervals (CIs) and Z statistics. For meta-analysis, we adopted a random-effects model and the inverse variance weighting method. Heterogeneity was assessed by calculating the I^2 value.

The *Meta-Essentials* Excel workbook 1.5 (Erasmus Research Institute of Management, Erasmus University, Rotterdam, The Netherlands) was used to perform pooled analyses, heterogeneity calculations, the Egger's/Begg's tests, and to draw funnel and forest plots.

Results

A simple term search of electronic databases, up to June 1, 2021, retrieved 47 citations. After title and abstract screening 32 records were selected. After full-text reading, we included 17 articles reporting the outcome of clinical studies and meta-analyses and 12 articles showing the results of preclinical investigations.

Preclinical evidence

The hypothesis of a link between the exposure to TV and PCa, derived from clinical observations, has fostered in the last decade extensive investigation at the cellular and molecular levels. Since inflammation is a known contributor to carcinogenesis, TV-induced activation of inflammatory cells and of inflammatory mediator release have been investigated.

It is recognized that inflammation caused by TV may involve the activation of the innate immune response receptor toll-like receptor 4 (TLR4) and inflammatory cytokine release.[14] Chen *et al*. investigated whether the expression of a TLR4 single-nucleotide polymorphism (rs4986790) generating the 896A>G (Asp299Gly) mutation, which decreases the inflammatory response, could modify the association between TV infection and PCa risk. This large nested case–control study, performed on 1382 subjects, provided evidence that PCa patients carriers of the 896A>G variant showed a higher odds (OR, 4.16; 95% CI, 1.32–13.1) of being seropositive for TV compared to homozygous carriers of the wild-type genotype.[15] The authors concluded that SNP carriers may mount a weak immune response against TV, thus increasing the likelihood of establishing sustained TV inflammatory prostatitis.

The human macrophage migration inhibitory factor (MIF), a pro-inflammatory regulator of innate immunity involved in cancer growth and invasion, seems to be implicated in TV-induced changes in prostate cells. MIF acts by protecting

macrophages from activation-induced apoptosis, thus promoting sustained inflammation. A major breakthrough in research was the finding that TV secretes TvMIF, a protozoan homolog (47% homology) of human MIF.[16] It was also demonstrated that TvMIF binds the human CD74 receptor with high affinity and is as potent as HuMIF in activating extracellular-signal-regulated kinase (ERK) in both benign prostatic hyperplasia (BPH)-1 cells and PC3 human metastatic PCa cells. Moreover, TvMIF promoted both the growth and invasive phenotypes of these cell lines.[16]

Han *et al*. demonstrated that prostate epithelial cells can promptly respond to the presence of TV by producing the cytokines interleukin (IL)-6, CCL2 and CXCL8, by inducing polarization of tumor-associated M2 macrophages, which in turn upregulate M2 markers such as IL-10, transforming growth factor-β, CD36, CD206, and arginase-1, finally promoting the proliferation and migration of a panel of human PCa cells (PC3, DU145 and LNCaP). In addition, TV-conditioned TRAMP-C2 PCa cells stimulated the accumulation of M2 macrophages in the prostate of mice *in vivo,* thus supporting a pivotal role of M2 in this process.[17]

Interestingly, the interplay between TV and macrophages may involve adipocytes, which are paracrine secretors of adipokines and other inflammatory mediators involved in cancer development and progression.[18,19]

Which cellular processes may be involved in the signaling pathways activated in the prostate by TV infection? The hypothesis that TV could act by inducing epithelial-mesenchymal transition (EMT) in prostate cells was investigated by Han *et al*. EMT is a process whereby cells of the epithelial lineage loose polarity and adhesion properties, thus acquiring the capacity to migrate and invade surrounding tissues, and to gain survival capacity and resistance to apoptosis. In oncogenesis, cells that undergo EMT can evolve into cancer stem cells by acquisition of stem cell-like properties. In an elegant study, Han *et al*. could demonstrate that human prostate epithelial cells stimulated with TV produced IL-6 in a pathogen load-dependent fashion. Expression of TLRs 2 and 4 also increased upon TV stimulation, which in turn triggered the expression of downstream TLR effectors like MAPKs p38, c-JUN N-terminal kinase, ERK, of nuclear factor-kappa B (NF-κB), and of JAK2 and STAT3 proteins. Concurrently, the authors documented a pathogen load-dependent decrease of epithelial markers (E-cadherin, cytokeratin, ZO-1) coupled to an increase of mesenchymal markers (N-cadherin, vimentin, fibronec-tin-1, β-catenin, snail1, matrix metalloproteinase-9). This was associated with expression of EMT-associated STAT3 and p-IκB proteins.[20] A subsequent study by the same research group provided additional information about the molecular mechanism of TV-triggered EMT. It was shown that stimulation by TV induced in human prostate epithelial RWPE-1 cells the production of IL-1 β, IL-6, CCL2, CXCL8, prostaglandin-E2, and COX2. The TV-conditioned medium of RWPE-1 cells was then able to markedly increase the malignant phenotype of a panel of PCa cells (PC3, DU145, and LNCaP) by enhancing their proliferation, migration and invasiveness. This effect was accompanied by EMT, demonstrated by a reduction in epithelial markers and an increase in mesenchymal markers. *In vivo*, xenograft tumors exposed to the TV-conditioned medium showed increased expression of cyclin D1 and PCNA, as well as features of EMT.[21]

The key role of IL-6 as a mediator of TV was also confirmed by Sutcliffe *et al*., who, as a result of their and others' evidence, proposed a model whereby TV-induced production of IL-6 leads to transcriptional activation of the STAT3-PIM1-HMGA1 cascade. Activation of the PIM1 effector HMGA1 proto-oncogene would then be a principal actor in prostate carcinogenesis through pathways involving COX2 and prostate-specific membrane antigen.[22]

The signaling responses characterized by Han *et al*. and Sutcliffe *et al*. were consistently confirmed by cytokine array, Reactome, and STRING analyses. Up to 23 cytokines were upregulated within 24 h of exposure of RWPE-1 cells to TV. Among the cytokines, upregulation of IL-6 was confirmed, together with IL-8, NF-κB, STAT3 and COX2. STRING further confirmed the role of MIF described above, and of PIM-1. Tumor proliferation markers Ki67, PCNA, and Bcl-2 were also upregulated. Finally, EMT was confirmed by assessment of downregulation of E-cadherin, upregulation of vimentin, and activation of focal adhesion kinase. Interestingly, human DU145 PCa cells were more sensitive to TV-activated signaling compared to normal, nontumorigenic, nonneoplastic human RWPE-1 prostatic epithelial cells.[23]

Although macrophages appear to play a key role in prostate cell growth and progression, neutrophils are also involved in the inflammatory response to TV and in inflammatory tissue damage. It was shown that neutrophils can activate *trogocytosis* to kill and clear TV infection in response to various inflammatory mediators.[24] However, this may involve inflammatory tissue damage and the release of mediators that may concur to neoplastic transformation. In addition to macrophages and neutrophils, activated mast cells were found to play a role in the stimulation of cellular proliferation in the prostate in the presence of TV. Kim *et al*. demonstrated that trichomonads could stimulate BPH-1 benign prostate hyperplasia cells to produce various chemokines (CCL2, IL-1b, IL-6, CXCL8), that activated mast cells, which in turn stimulated the proliferation of prostate stromal cells.[25]

Taken together, the evidence emerging from preclinical investigation performed so far supports a model whereby IL-6, secreted by polarized M2 macrophages in response

to stimuli exerted by TV, would be part of a complex signaling pathway also involving innate immune system pattern recognition receptors such as TLR4. The cascade would include transcriptional activation of the HMGA1 oncogene, directly contributing to cancer progression via pathways involving COX-2. The phenotypic expression of these molecular events would be inflammation, EMT, and promotion of various steps in prostatic oncogenesis.

Despite the fact that several experts demonstrated that TV can trigger a number of molecular and cellular events leading to the acquisition of phenotypic features highly suggestive of neoplastic transformation, others have provided experimental evidence of an anti-proliferative and pro-apoptotic effect of TV on PCa cells. Zhu *et al*. showed that the TV-conditioned medium inhibits the growth of two human PCa cell lines (PC-3 and DU145) and that this effect correlated with upregulation of p21, downregulation of Bcl-2, and induction of apoptosis. The authors concluded that TV may not be associated with the development of PCa, but rather that TV infection might be potentially beneficial and therapeutic to patients in an early neoplastic state.[26] Although these considerations are in contrast with the evidence provided by other research groups, they are worthy of careful thought and suggest the existence of some controversy in this matter.

Clinical evidence, meta-analysis, and quality assessment of clinical data

Table 1 summarizes the characteristics and outcomes of the clinical studies included in the present review.

The Sutcliffe 2006, Al-Mayah 2013, and Saleh 2021 case-control studies, based on a broad array of sample sizes, demonstrated a significant association between seropositivity to TV and PCa of any grade.[27-29] The Stark 2009 study, a follow-up analysis of previous findings demonstrating a higher risk for advanced disease upon exposure to TV,[27] demonstrated a significant association between a positive serostatus and advanced, metastatic, and lethal disease.[30] Accordingly, Saleh *et al*. could demonstrate a positive and significant correlation between the TV-IgG density and the stage of PCa [Table 1].[29]

In 2019 The Kim *et al*. study presented a significant association between TV seropositivity and a cumulative PCa/benign prostate hyperplasia endpoint. From the study report, we could extract the PCa data; according to our own calculation, a significant OR for the PCa-TV association was found in this small study (OR, 12.6; 95% CI, 1.51–105.56, P = 0.004). Notably, the study was likely biased by a marked difference in the mean age at presentation (controls: 40 y, cases, 73 y).[31]

The remaining studies, which also included two analyses focusing on men of African descent, known to have an increased predisposition to PCa,[32,33] failed to demonstrate an association between TV seropositivity and any grade, high-grade or lethal PCa.[15,34-36] Incidentally, a study performed in 2008 on PCa tissue specimens obtained from a group of 30 patients allowed to identify, by 16S rDNA amplification or by organism-specific polymerase chain reaction, diverse viral and bacterial species, but failed to detect the presence of TV.[37]

Contrary to any other finding, the Shui *et al*. 2016 study documented a protective activity of TV exposure against PCa (adjusted OR, 0.51; 95% CI, 0.28–0.93, P = 0.03).[38]

Meta-analysis

Twelve case–control studies (nested or nonnested) showed sufficient homogeneity and were included in a meta-analysis.[15,27-36,38] The PCa cases were 4752, of which 878 were previously exposed to TV (seropositive cases), whereas controls were 6369, of which 1133 had a history of TV exposure (seropositive controls). Pooled analysis resulted in a nonsignificant crude OR of 1.14 (95% CI: 0.84–1.53; Z = 0.94, P = 0.34). Figure 1a shows the forest plot of this meta-analysis. We calculated an I^2 value of 68%, indicating "substantial" heterogeneity according to Cochrane criteria.

Visual inspection of the funnel plot [Figure 2 Panel A] suggested asymmetry of the data distribution, which was confirmed by the Egger's and Begg's tests (P = 0.013 and P = 0.02, respectively).

The median score of the NOS was 6* (range: 0–9), and the mode was 6*. When the NOS scores were converted to AHRQ standards, 2 studies were rated as "poor," 1 study were rated as "fair" and 9 studies were rated as "good" [Table 2].

Since some studies evidenced a significant OR for a history of TV exposure in lethal or bone-metastatic cases (e.g. 30), we performed a subgroup analysis by extracting from five studies the data relative to highly advanced, bone-metastatic or lethal cases.[29,30,32,33,38] The lethal/metastatic PCa cases were 485, of which 127 were previously exposed to TV, whereas controls were 2424, of which 690 had a history of TV exposure. Again, analysis resulted in a nonsignificant OR (OR, 1.18; 95% CI: 0.70–2.00, Z = 0.88, P = 0.37), and substantial heterogeneity (I^2 = 51.83%) [Figure 1, Panel B]. The funnel plot and asymmetry tests did not indicate the presence of significant publication bias (Egger's test: P =0.5; Begg's test: P =0.32) [Figure 2, Panel B].

The findings of this meta-analysis are summarized in Table 3. According to GRADE criteria, the quality of the evidence is poor, mainly due to the retrospective design of the included studies and to the presence of risk of bias.

Discussion

TV is a common infective agent of the urogenital tract. It is transmitted through sexual intercourse and may cause symptoms similar to those of other STIs within a month

Table 1: Characteristics, assessments and outcomes of the clinical studies included in this review

Author (reference)	*n* total	*n* cohorts	Method	Results and statistical significance (*P*)
Sutcliffe 2006	1382	691 prostate cancer patiens 691 controls PSA tested	ELISA for detection of anti-TV IgG PSA testing Questionnaire data	TV (+) PCa versus control (0.07) TV (+) PCa/race versus control (<0.05) TV (+) PCa/familial PCa versus control (<0.05) TV (+) PCa/STI versus control (<0.05) TV (+) PCa/prostatitis versus control (<0.05) TV (+) PCa and irregular aspirin (<0.05) TV (+) PCa and age of diagnosis (>0.05) TV (+) PCa and screening (>0.05)
Stark 2009	1346	673 prostate cancer patients 673 matched controls	ELISA for detection of anti-TV IgG Questionnaire data	TV (+) and total PCa risk (>0.05) TV (+) and risk of advanced PCa (<0.05) TV (+) and death from cancer risk (<0.05) TV (+) and metastasis risk (<0.05) TV (+) and lethal PCa risk (<0.05)
Groom 2012	158	96 prostate cancer patients 62 matched controls	ELISA for detection of anti-TV IgG Questionnaire data	TV (+) PCa versus control (>0.05)
Shui 2016	327	146 metastatic or fatal PCa 181 age-matched control	ELISA for detection of anti-TV IgG	TV (+) and metastasis risk (>0.05) TV (+) and lethal PCa risk (>0.05) TV (+) and decreased risk of advanced PCa (<0.05)
Sutcliffe 2009	1232	616 PCa diagnosed on any biopsy after visit 2 616 controls matched by age, treatment arm, and family history of PCa	ELISA for detection of anti-TV IgG	TV (+) PCa versus control (>0.05) TV (+) PCa and aspirin use (>0.05) TV (+) PCa and minerals use (>0.05) TV (+) PCa and ethnicity/race (>0.05) TV (+) PCa and family history (>0.05)
Fowke 2016	793	296 PCa cases 497 race-matched controls	ELISA for detection of anti-TV IgG PSA testing Questionnaire data	TV (+) PCa versus control (>0.05) TV (+) PCa/race versus control (>0.05) TV (+) and Gleason score 7–10 (>0.05) TV (+) and Stage 2–4 PCa (>0.05)
Marous 2017	2342	Caucasians (c) 438 Gleason 7 487≥Gleason 8/Stage III and IV African-Americans (a) 109<Gleason 7 92≥Gleason 7 1216 controls	ELISA for detection of anti- TV IgG PSA testing Questionnaire data DRE	TV (+) PCa (a) versus PCa (c) (<0.0001) (c) TV (+) and Gleason score>7 (>0.05) (a) TV (+) and total PCa risk (>0.05) TV (+) PCa and ethnicity/race (>0.05)
Vicier 2019	307	189 localized PCa 118 metastatic PCa	ELISA for detection of anti-TV IgG	TV (+) and lethal PCa (>0.05) TV (+) and high-grade PCa (>0.05) TV (+) and stratification risk of local PCa prostate cancer (>0.05)
Tsang 2019	1485	736 PCa patients (PHS) 749 PCa patients (HPFS)	ELISA for detection of anti-TV IgG	TV (+) and death of PCa risk (>0.05) TV (+) and any cancer death risk (>0.05)

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Table 1: Continued

Author (reference)	*n* total	*n* cohorts	Method	Results and statistical significance (*P*)
Breyer 2016	38340	Men with possible BPH without PCa	ELISA for detection of anti-TV IgG PSA testing Questionnaire data DRE	prevalent nocturia and (+) TV (<0.05) large prostate and (+) TV(<0.05) prevalent BPH/LUTS and (+) TV (<0.05)
Kim 2019	241	139 BPH patients 44 PCa patients 58 controls	ELISA for detection of anti-TV IgG radiologic evaluation (MRI and bone scan) Questionnaire data	TV (+) PCa versus BPH (>0.05) TV (+) PCa/BPH versus control (<0.05)
Langston 2019	732	732 healthy young men	ELISA for detection of anti-TV IgG PSA testing	TV (+) and levels of PSA (>0.05)
Saleh 2013	246	126 PCa patients 120 matched controls	ELISA for detection of anti-TV IgG PSA testing	TV (+) and PCa (0.0150) TV-IgG density score and PSA correlation, r=0.7, (<0.0001) TV-IgG density score and PCa stage correlation, r=0.5, (<0.05)
Al-Mayah 2021		50 PCa patients 40 matched controls	ELISA for detection of anti-TV IgG PCR of TV DNA in PCa specimens	TV (+) PCa versus controls (<0.05) TV DNA (+) PCa versus controls (>0.05)

Pca: Prostate cancer, TV: *Trichomonas vaginalis*, PSA: Prostate specific antigen, STI: Sexually transmitted infections, PHS: Polygenic hazard score, HPFS: Health professional's follow-up study, DRE: Digital rectal examination, IgG: Immunoglobulin G, PCR: Polymerase chain reaction, MRI: Magnetic resonance imaging, BPH: Benign prostatic hyperplasia, LUTS: Lower urinary tract symptoms

after exposure. Up to 50% of patients will not develop any symptom but will remain infectious. Although the prostate gland is believed to serve as a parasite reservoir in men's trichomoniasis, the association between trichomonads and prostatic diseases has been under discussion.

In this review, we summarized the data emerging from preclinical investigations performed both at the cellular and molecular level. In general, these data are suggestive of a possible link between the exposure to TV and the onset of growth abnormalities in prostate cells-including EMT-that may be linked to the development of a neoplastic phenotype.

Early research by Sutcliffe *et al*. documented a statistically significant association between a TVs serostatus and prostate inflammation[34] in men, confirming previous laboratory observations. In 1986, Gardner *et al*., positively identified trichomonads in the prostatic urethra, glandular lumina, submucosa, and stroma. Concomitant foci of nonspecific acute and chronic inflammation, as well as intraepithelial vacuolization, suggested an association with the infection.[39]

In the clinical practice, cases of acute or chronic prostatitis related to TV are infrequently reported. Probably, TV prostatitis may be overlooked due to limited culture-based assays routinely performed in hospital microbiology laboratories.[40] In fact, by using specific tests researchers found a 16%–18% incidence of TV prostatitis.[41]

Worldwide investigation to assess an association between a positive TV serostatus (a marker of exposure to the protozoan) and PCa was fostered by the findings of Sutcliffe *et al*. on a large population of patients, showing a significantly increased odds for PCa of any grade on exposure to TV.[27] These results were confirmed by the same group in later studies focusing on advanced/lethal PCa,[30] as well as by other independent studies performed more recently in smaller patient populations.[28,29,31] However, other clinical studies failed to find a significant increase of the risk for PCa in patients with a positive TV serostatus.[15,32-36] Thus, clinical data were not univocal, the issue has remained controversial and meta-analyses-including ours-have been performed to obtain additional information in this respect.

Summary of the main results

Our meta-analysis including 12 studies retrieved up to June 1, 2021, did not evidence a significant association between a positive TV serostatus and PCa of any grade. Moreover, we could not find a significant association between advanced/lethal PCa and TV exposure. Thus, the involvement of TV in the genesis of PCa remains unknown. However, once foci of chronic infection are

Table 2: Assessment of the quality of studies included in the meta-analysis according to the Newcastle-Ottawa Scale

Study ID (first author, year)	Selection				Comparability	Exposure			Score, AHRQ rating
	Is PCa definition adequate?	Representativeness of cases	Selection of controls (hospital control bias)	Definition of controls	Comparability cases- controls	Ascertainment of exposure (recall bias)	Same method of ascertainment for cases and controls	Nonresponse rate	
Sutcliffe 2006	Questionnaire- based assessment of PCa. In most cases data also extracted from cancer registry, with no independent validation	Obviously representative*	No hospital controls*	No history of PCa*	Age- matched, adjusted for a number of different covariates*	De novo serological assessment of exposure (seropositivity)*	Yes*	Unknown	6*, good
Stark 2009	PCa assessment not disclosed	Obviously representative*	No hospital controls*	No history of PCa*	Controls were matched to cases by age, smoking status and follow-up time*	Serologically assessed seropositivity*	Yes*	Unknown	6*, good
Sutcliffe 2009	Cases were men with a pathologically confirmed diagnosis of PCa. Independent validation was performed*	Consecutive accrual (randomized study)*	No hospital controls*	A negative prostate biopsy at end of study*	Age -matched, adjusted for a number of different covariates*	De novo serological assessment of exposure (seropositivity)*	Yes*	Unknown	7*, good
Al-Mayah 2013	Cases were men with a pathologically confirmed diagnosis of PCa*	Obviously representative*	No hospital controls*	No history of PCa*	Significant age difference between cases and controls	Serologically assessed seropositivity*	Yes*	Unknown	6*, good
Chen 2013	Cases were men with a pathologically confirmed diagnosis of PCa*	Obviously representative*	No hospital controls*	No history of PCa*	Matched for age, PSA test frequency and date	Serologically assessed seropositivity*	Yes*	Unknown	6*, good
Shui 2016	Cases were men with a pathologically confirmed diagnosis of PCa*	Obviously representative*	Community controls*	No history of PCa*	Age -matched, adjusted*	Serologically assessed seropositivity*	Yes*	Unknown	7*, good
Fowke 2016	Cancer and death registry, no independent validation	Obviously representative*	Community controls*	No history of PCa*	Age and ethnicity-matched, adjusted for age at diagnosis, race, income*	Serologically assessed seropositivity*	Yes*	Unknown	6*, good
Marous 2017	Cases were men with a pathologically confirmed diagnosis of PCa*	Consecutive accrual (randomized study)*	Community controls*	No history of PCa*	Age and ethnicity-matched, adjusted*	Serologically assessed seropositivity*	Yes*	Unknown	7*, good

Contd...

Table 2: Continued

Study ID (first author, year)	Selection				Comparability	Exposure			Score, AHRQ rating
	Is PCa definition adequate?	Representativeness of cases	Selection of controls (hospital control bias)	Definition of controls	Comparability cases- controls	Ascertainment of exposure (recall bias)	Same method of ascertainment for cases and controls	Nonresponse rate	
Vicier 2019	Cases were extracted from various studies and registries, no independent validation	Unclear	Controls might include cases with low-stage PCa	Controls might include cases with low-stage PCa	Unclear	Serologically assessed seropositivity*	Yes*	Unknown	2*, poor
Tsang 2019	Cases were extracted from various studies and registries, no independent validation	Unclear	No hospital controls*	No history of PCa*	Unclear	Serologically assessed seropositivity*	Unclear: Seroprevalences evaluated with different cutoffs	Unknown	3*, poor
Kim 2019	Cases were men with a pathologically confirmed diagnosis of PCa or BPH*	Obviously representative*	Hospital controls	No history of PCa*	Controls included both healthy subjects and patients with BPH; significant age difference between cases and controls	Serologically assessed seropositivity*	Yes*	Unknown	5*, fair
Saleh 2021	Cases were men with a pathologically confirmed diagnosis of PCa*	Obviously representative*	Hospital controls	No history of PCa*	Age-matched, adjusted*	Serologically assessed seropositivity*	Yes*	Unknown	6*, good

AHRQ: Agency for Healthcare Research and Quality, Pca: Prostate cancer

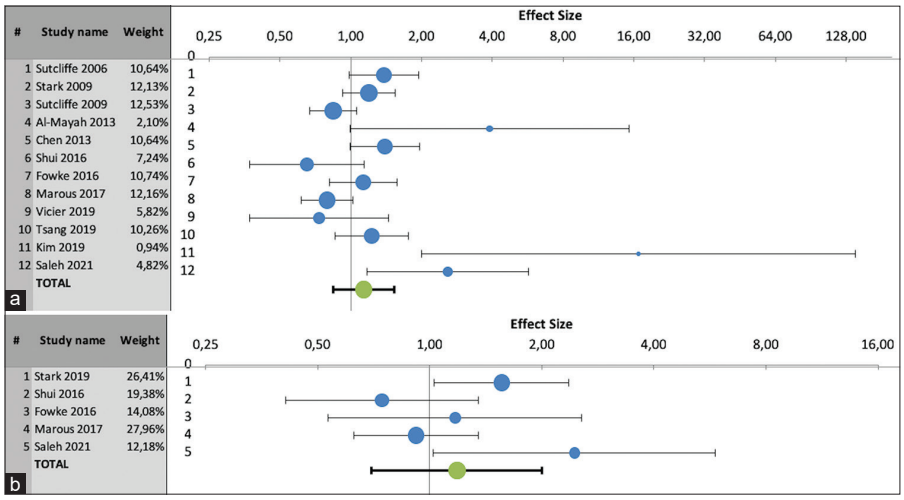

Figure 1: (a) Forest plot showing the association between a diagnosis prostate cancer (any grade or stage) and seropositivity for *Trichomonas vaginalis* in twelve case-control studies included in this review. (b) Subgroup analysis showing the association between a diagnosis of lethal or bone-metastatic prostate cancer and seropositivity for *Trichomonas vaginalis* in five case-control studies. Data to the right of the vertical no-effect line represent increased odds for prostate cancer in patients exposed to *Trichomonas vaginalis*

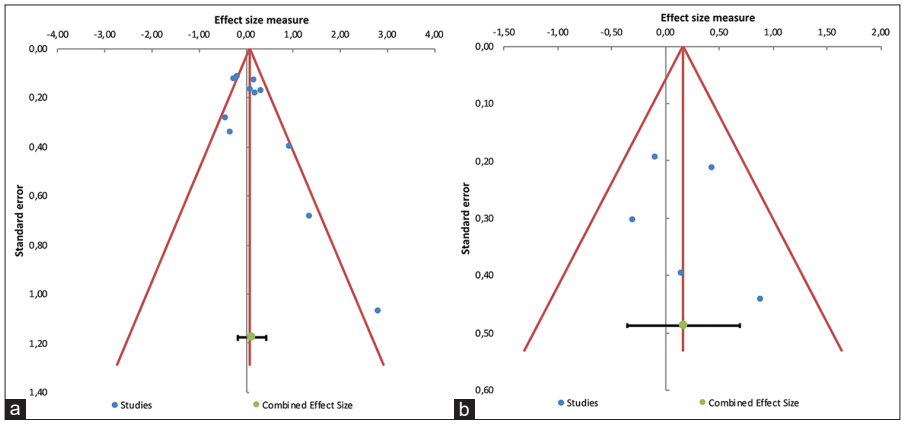

Figure 2: Funnel plots for publication bias analysis. (a) Association between any grade or stage of prostate cancer and exposure to *Trichomonas vaginalis*; (b) Subgroup analysis on the association between a diagnosis of lethal or bone-metastatic prostate cancer and exposure to *Trichomonas vaginalis*. In these plots the effect size is expressed as the natural logarithm of the odds ratio

established, the prostate microenvironment is prone to the neoplastic process, which is largely orchestrated by inflammatory cells via deregulation of the cell proliferation mechanisms, predisposing to the development of cancer and promoting all stages of tumorigenesis. Cancer cells, as well as surrounding stromal and inflammatory cells, engage in well-orchestrated reciprocal interactions to form an inflammatory tumor microenvironment.[42] Given that TV infection frequently represents as nonspecific or asymptomatic, it is conceivable that the risk of PCa may increase as a consequence of low intensity, untreated inflammation. Meta-analyses have indeed suggested that a history of CBP might be a risk factor for the subsequent development of PCa.[10,11] Yet, synergistic conditions such as excessive or low immune responses may be needed in order to trigger inflammation-induced damage to cell DNA and to affect the way cells grow and divide. This particular connection explains differences between various epidemiological studies regarding TV's tumorigenic potential and the need for new studies at the epidemiological and molecular levels.

Overall applicability of the evidence

The applicability of the evidence generated with this meta-analysis may be affected by issues of biologic variation due to ethnicity. In particular, between Caucasians and men of African descent there exist biologic differences that are likely to affect the pathogenesis of cancer, with the latter showing increased predisposition and higher proneness to high-grade disease when compared with the former.[43]

Moreover, patients of different nationalities or social groups may be subjected to socioeconomic conditions or attitudes

Table 3: Summary of findings (grading of recommendations assessment, development and evaluation criteria)

Patient or population: Male subjects

Settings: Case-control study

Cases: A documented diagnosis of PCa

Controls: Healthy individuals (no history of PCa)

Outcomes	Illustrative comparative risks (95% CI)		Relative effect (95% CI)	Number of participants (studies or comparisons)	Quality of the evidence (GRADE)	Comments
	Assumed risk (placebo)	Corresponding risk (antidepressants)				
PCa of any grade or stage	177.89/1000	202.79/1000 (149.42–272.17)	OR: 1.14 (0.84–1.53)	11,121 (12)	⊕⊖⊖⊖ (very low)	Reasons for downgrading Risk of bias Publication bias Observational, no ROBINS-I Reasons for upgrading None
Advanced stage PCa (lethal, bone-metastatic, etc)	284.65/1000	335.88/1000 (199.25–569.3)	OR: 1.18 (0.70–2)	2909 (5)	⊕⊕⊖⊖ (low)	Reasons for downgrading Risk of bias Observational, no ROBINS-I Reasons for upgrading None

The corresponding risk (and its 95% CI) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). GRADE working group quality of evidence, high quality: Further research is very unlikely to change our confidence in the estimate of effect, Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate, low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate, very low quality: We are very uncertain about the estimate. GRADE: Grading of recommendations assessment, development and evaluation, CI: Confidence interval; OR: Odds ratio, Pca: Prostate cancer

that make some forms of screening (e.g. frequent PSA testing) or care (e.g. CBP prolonged courses of therapy) less accessible or less feasible in some settings, such as in developing countries.

Quality of the evidence

According to GRADE criteria, the overall quality of the evidence is low, mainly due to the retrospective design of the included studies and to the presence of risk of bias.

Potential biases and confounders

Publication bias was detected by funnel plot inspection and statistics. Moreover, we hypothesize that additional generators of systematic biases may be linked to the diversity of the design of the studies included in this review. Among possible bias generators we may include the duration of exposure to TV and the assessment of TV seropositivity in patients. Moreover, no information was available concerning the time-course of cancer development. Which latency period between protozoan infection and PCa development may be considered indicative of a link between exposure and disease? In addition, a number of risk factors such as smoking, exposure to prostatic carcinogens (e.g. frequent consumption of charred meat containing the prostate carcinogen PhIP) may be additional confounders and bias generators.

It is also possible that TV infection of the prostate may be restricted to a specific patient population. Notably, Skuhala *et al*. found that TV is a major pathogen of chronic prostatitis in elderly men, suggesting that chronic decline of the immune system may also be required to propagate and provoke TV-induced constrictive pericarditis.[44] On the other side, younger, sexually-active men may be preferential targets of TV infection which might be responsible for sustained inflammation and prostate tissue alterations. Thus, in-depth research focused on different age subgroups may remove potential age-related biases.

Agreements and disagreements with other studies

A 2019 meta-analysis by Najafi *et al*., including 6 clinical studies, resulted in a OR of 1.17 (95% CI: 1.01–1.36), indicating a borderline-significant association between TV and PCa.[45]

In contrast, our updated meta-analysis including 12 studies retrieved up to June 1, 2021 did not evidence a significant association between a positive TV serostatus and PCa of any grade.

Since Stark *et al*. reported a highly significant association between advanced/lethal PCa and TV exposure in a population of in 1346 patients,[30] we performed a subgroup analysis by pooling five studies from which data about advanced/lethal disease could be extracted. Also in this case, we failed to find a significant exposure-disease association.

Conclusions

In conclusion, our meta-analysis did not provide conclusive evidence of a possible link between exposure to TV and an increased risk of PCa of any grade, as the crude OR for cancer in patients exposed to TV versus unexposed patients was not statistically significant.

Implications for practice

As far as the clinical practice is concerned, our results do not support for the moment the indication for increased surveillance of patients previously exposed to TV. However, this statement is only provisional and new clinical data from powered studies may overturn our opinion.

Implications for research

The reason underlying the different results of clinical studies may be manifold. The size of patient populations, the patients' age, the length of exposure to TV, the different ways in which PCa was staged and the ethnicity of enrolled subjects may have played a role in these discrepant findings.

Thus, whereas the results of previous trials performed by independent international research groups demonstrating a carcinogenic risk in men infected with TV should not be overlooked, novel confounder-adjusted studies performed on very large populations are warranted to provide a conclusive answer to this research question. In this regard, we believe that the simple serological assessment of a generic "exposure" to TV may act as a major confounder in such kinds of studies. In our opinion, instead of a simple serological demonstration of TV exposure, a documented history of symptomatic, clinical chronic prostatitis caused by TV may increase the likelihood of finding patients that have been truly subjected to pathological alterations of the prostatic glandular tissue caused by the protozoan. Such stringent selection may allow to exclude occasional exposure to the protozoan which may be unrelated to persistent, chronic inflammatory damage of the prostate and neoplastic transformation.

Moreover, future trials should ideally take into consideration certain potential biases and confounders, such as ethnicity, age-related issues, the degree of exposure to dietary and nondietary prostate carcinogens, and the length of a hypothetic latency period between protozoan exposure and PCa development.

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Conflicts of interest

There are no conflicts of interest.

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