Original Study

Elevated Serum Cytokines and *Trichomonas vaginalis* Serology at Diagnosis Are Not Associated With Higher Gleason Grade or Lethal Prostate Cancer

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Abstract

New prognostic biomarkers in prostate cancer are needed to identify patients with low-risk localized prostate cancer who would benefit from earlier curative and aggressive treatments. We found no association between circulating cytokine levels that regulate both innate and adaptive immunity or proinflammatory chemokines nor *Trichomonas vaginalis* seropositivity and risk of intermediate- to high-risk or lethal prostate cancer.

Background: Inflammation and infections have been associated with prostate cancer progression. We assessed whether elevated serum cytokines or *T. vaginalis* seropositivity at the time of diagnosis was associated with higher grade or lethal prostate cancer. **Patients and Methods:** Men with localized or metastatic prostate cancer were included in this study. Cytokine serum levels including interleukin (IL)-1α, IL-1β, IL-2, IL-6, IL-8, monocyte chemotactic protein 1 (CCL-2), tumor necrosis factor α, and growth-regulated oncogene α (CXCL-1) using a multiplex enzyme-linked immunosorbent assay and *T. vaginalis* serology were measured in blood samples at diagnosis. **Results:** A total of 324 patients were identified at time of localized disease and 118 at time of metastatic disease. Of the 189 patients with localized disease and clinical follow-up data (median, 73 months), 28 developed lethal disease. There was no association between circulating cytokine levels above median concentrations nor *T. vaginalis* seropositivity and risk of intermediate- to high-risk or lethal prostate cancer. **Conclusion:** Higher levels of serum cytokine levels and *T. vaginalis* seropositivity at diagnosis are not associated with high-grade or lethal prostate cancer and do not aid risk stratification of localized prostate cancer.

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Introduction

Men with low-risk localized prostate cancer are presented with the choice between active surveillance and definitive treatment. There is a major clinical need to identify additional prognostic biomarkers beyond grade, prostate-specific antigen (PSA), and clinical stage, which will parse out those patients with seemingly low-risk clinicopathologic features who have a higher risk of developing metastatic prostate cancer and would benefit from earlier curative treatments.

Evidence supporting a role of inflammation in prostate carcinogenesis is abundant,[1] but it still remains unclear, and sexually transmitted infections are one possible origin.[2] *T. vaginalis* has been

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reported to infect the prostatic gland and could result in chronic inflammation.[3] Furthermore, cytokines, which are released by different immune cells in response to injury, also play an important role in cancer development and progression.[4] Different cytokines have been described to be involved in tumor invasion or angiogenesis.[5] On the basis of this rationale, we hypothesized that markers such as elevated cytokine levels and evidence of prior *T. vaginalis* infection may add to our prognostic information and decision making for localized prostate cancer. We performed serum cytokine profiling (interleukin [IL]-1α, IL-1β, IL-2, IL-6, tumor necrosis factor (TNF)-α, IL-8, growth-regulated oncogene α (GRO-α), and monocyte chemotactic protein 1 (MCP-1)) and *T. vaginalis* serology to determine whether elevation of one or more cytokines and/or *T. vaginalis* infection was associated with more aggressive or lethal prostate cancer.

Patients and Methods

Study Population

Two different cohorts of patients with localized disease were included: the Dana-Farber Cancer Institute Gelb Center cohort (DFCI; n = 189) and the Early Detection Research Network cohort (EDRN; n = 306). The DFCI cohort is a case series of prostate cancer patients diagnosed at Dana-Farber Cancer Institute from 1976 to 2007, the details of which have been already published.[6,7] The DFCI cohort comprised 161 patients who did not experience relapse of metastatic disease during follow-up and 28 patients who had lethal prostate cancer, defined as metastatic relapse in any sites (lymph nodes, bone, or visceral metastasis). The EDRN cohort was part of a National Cancer Institute initiative and was funded for biomarker identification and validation.[8] Patients were included at the time of first prostate biopsy.[9] The EDRN cohort included 86 intermediate- to high-risk prostate cancers (Gleason $\geq$ 7 or PSA $\geq$ 10), 49 low-risk cases (Gleason = 6, PSA < 10), and 171 controls without cancer (low-risk or no prostate cancer, n = 220). A third cohort included patients from CHARTEED, a phase 3 trial for metastatic hormone-sensitive prostate cancer patients (E3805 cohort; n = 118).[10] All patients consented to institutional review board–approved protocols.

Evaluation of Serum Markers

Blood from baseline at time of diagnosis was evaluated for serum cytokines and *T. Vaginalis* seropositivity. We investigated cytokines that regulate both innate and adaptive immunity (IL-1α, IL-1β, IL-2, IL-6, and TNF-α) and proinflammatory chemokines (IL-8, GRO-α, and MCP-1) using a previously validated single multiplex electrochemiluminescence platform (Meso Scale Discovery, Gaithersburg, MD; Fichorova Laboratory, Brigham and Women's Hospital, Boston, MA).[11] *T. vaginalis* was assayed by enzyme-linked immunosorbent assay (Alderete Laboratory, Washington State University, WA), which detects immunoglobulin G antibodies against recombinant *T. vaginalis* α-actinin.[12]

Statistical Analysis

Serum marker levels were categorized above or below the median values and by highest versus other quartiles. *T. vaginalis* was scored as positive for values > 2 and negative for $\leq$ 2, on the basis of prior literature.[13,14] In the DFCI cohort, we assessed the association between cytokine levels and *T. vaginalis* seropositivity with risk of developing a metastatic relapse by conducting multivariate analyses adjusting for age and D'Amico risk group. For the EDRN cohort, we used logistic regression to examine the association between cytokine levels and *T. vaginalis* status with risk of being diagnosed with an intermediate- to high-risk prostate cancer versus low-risk prostate cancer and no cancer. Differences were tested by the Wilcoxon rank sum test for numeric covariates and generalized chi-square test for categorical variables. For the E3805 cohort, we only measured *T. vaginalis* serology and estimated its prevalence at the time of metastasis. Given the samples available as part of a retrospective study, if we assume that 82% of patients with lethal prostate cancer of the DFCI cohort had a higher level of a certain marker and 36% of patients with non-lethal prostate cancer had a higher level of the same marker, then there is at least 80% power to detect a 46% difference in rate of positivity with 1-sided alpha of 0.05 by a chi-square test. Software used for the DFCI cohort was SAS 9.4 and for EDRN was SAS 9.3. $P < .05$ was planned to be considered nominally significant.

Results

Table 1 presents patient characteristics at diagnosis for the DFCI cohort (n = 189) with localized disease. Median follow-up was 73 months (range, 2-182 months), during which time 28 men developed metastases. There was no association between cytokine levels or *T. vaginalis* seropositivity with risk of developing lethal prostate cancer when analyzed by above and below the median (Table 1, Figure 1). In the EDRN cohort, there was also no association between levels of any of the cytokine or *T. vaginalis* positivity and risk of intermediate- to high-risk localized prostate cancer (Table 2, Figure 1). Cytokine level distributions for both cohorts are presented in Figure 1. All analyses done by upper quartile versus lower quartiles also yielded consistently null results (data not shown).

The prevalence of *T. vaginalis* at the time of blood draw was similar among men diagnosed with localized disease (15% from DFCI and EDRN cohort; Tables 1 and 2) and at the time of metastasis (15% from E3805 cohort; data not shown).

Discussion

Many preclinical and clinical studies support the hypothesis that inflammation is involved in prostate carcinogenesis. With this in mind, we sought to determine whether markers of inflammation can serve as prognostic biomarkers at time of diagnosis of localized prostate cancer or metastasis. Our results analyzing data from 3 separate cohorts failed to show any association of cytokine levels with intermediate- to high-risk disease or metastatic disease. Specifically, in the DFCI cohort with follow-up data, we found no association between higher levels of any of the 8 individual cytokines or *T. vaginalis* seropositivity at time of diagnosis with clinically localized disease with higher grade or greater risk of relapse with metastatic disease. In the second cohort, where the EDRN patients just have baseline information, we found no association between cytokine serum levels or *T. vaginalis* seropositivity with intermediate- to high-risk disease versus low-risk cancer or no cancer at time of biopsy. In a third analysis, we found no difference in the percentage of *T. vaginalis* seropositivity in samples from men with metastatic disease versus clinically localized prostate cancer.

Table 1 Clinical Characteristics and *Trichomonas vaginalis* and Cytokine Results for Dana-Farber Cancer Institute Gelb Center Cohort (N = 189)

Characteristic	Lethal Prostate Cancer		Univariate *P*/ Multivariate *P*
	No (N = 161)	Yes (N = 28)	
Age at diagnosis (years), median (Q1, Q3)	61 (55-70)	63 (56-71)	—
PSA at diagnosis (ng/mL), median (Q1, Q3)	6 (4.60-9.4)	9.4 (5.1-23.7)	—
Biopsy Gleason Score			—
≤ 6	59 (37)	3 (11)	
7	70 (43)	10 (36)	
≥ 8	31 (19)	15 (54)	
Missing	1 (1)	0	
T Stage			—
T1	112 (70)	12 (43)	
T2	31 (19)	11 (39)	
T3/T4	3 (2)	1 (4)	
Tx/unknown	15 (9)	4 (14)	
N Stage			—
N0	67 (42)	12 (43)	
N1	1 (1)	2 (7)	
Nx/unknown	93 (58)	14 (50)	
Local Therapy			—
RP	70 (43)	7 (25)	
RT	89 (55)	19 (68)	
RP + RT	1 (<1)	0	
No/unknown	1 (<1)	2 (7)	
***T. vaginalis* Score**			1.00/.94
0-2	136 (84)	24 (86)	
3-4	25 (16)	4 (14)	
IL-1α			1.00/.90
Median or lower	89 (55)	16 (57)	
Greater than median	72 (45)	12 (43)	
IL-1β			.69/.59
Median or lower	82 (51)	13 (46)	
Greater than median	79 (49)	15 (54)	
IL-2			.84/.67
Median or lower	80 (50)	13 (46)	
Greater than median	81 (50)	15 (54)	
IL-6			.54/.32
Median or lower	82 (51)	12 (43)	
Greater than median	79 (49)	16 (57)	
IL-8			.42/.25
Median or lower	83 (52)	12 (43)	
Greater than median	78 (48)	16 (57)	
MCP-1			.42/.29
Median or lower	83 (52)	12 (43)	
Greater than median	78 (48)	16 (57)	
TNF-α			.48/.33
Median or lower	119 (74)	23 (82)	
Greater than median	42 (26)	5 (18)	
GRO-α			.69/.76
Median or lower	82 (51)	13 (46)	
Greater than median	79 (49)	15 (54)	

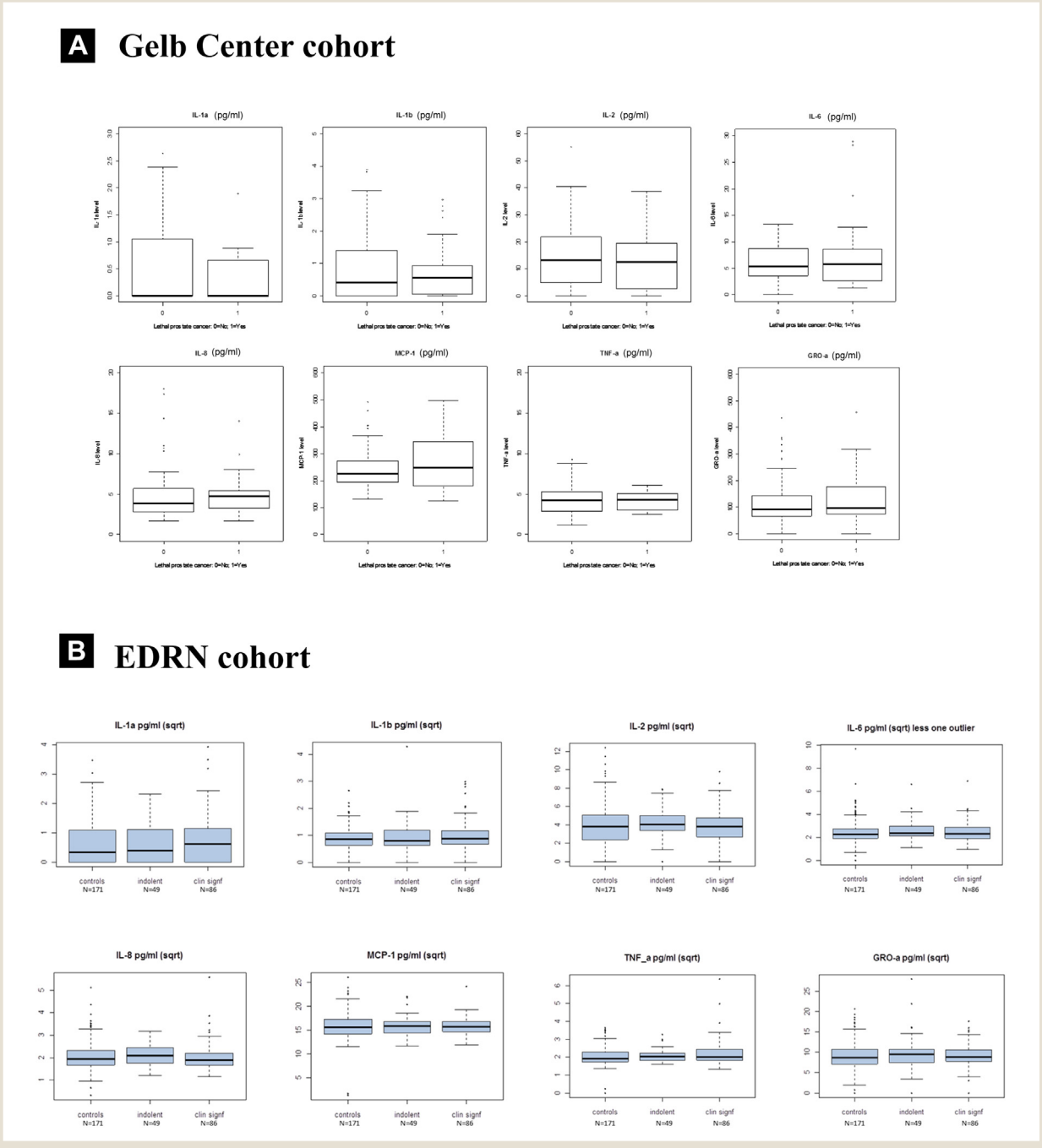
Data are presented as n (%) unless otherwise indicated.
Abbreviations: GRO-α = growth-regulated oncogene α; IL = interleukin; MCP-1 = monocyte chemoattractant protein 1; PSA = prostate-specific antigen; Q = quartile; RP = radical prostatectomy; RT = radiotherapy; TNF-α = tumor necrosis factor α.

This is in contrast with prior studies, which showed an association between elevated cytokines and *T. vaginalis* seropositivity in prediagnostic blood and subsequent development of lethal prostate cancer.[13-16] We have previously shown that elevated cytokine levels in men with metastatic prostate cancer were significantly associated with shorter time to castration resistance and overall survival in a cohort of 122 men.[15] Specifically, we identified that high levels of IL-8, TNF-α, and MCP-1 in men initiating androgen deprivation therapy for metastatic hormone-sensitive prostate cancer were clearly associated with shorter time to castration resistance and importantly with overall survival. No association with level of IL-1β, IL-2, or IL-6 was demonstrated. Interestingly, IL-6 has been reported in other studies to be a prognostic biomarker in castration-resistant prostate cancer and to correlate with chemotherapy resistance in this setting.[17] Preclinical work has also shown IL-6 supports progression to metastasis[18] and IL-8 supports development of androgen-independent prostate cancer development.[19]

T. vaginalis seropositivity was previously described to be associated with higher risk of prostate cancer in a large cohort of 691 patients[14] and in another cohort of 673 men in a study that found a statistically significant correlation between *T. vaginalis* seropositivity and risk of extraprostatic prostate cancer.[13]

The work presented here was limited by having only 28 cases who developed lethal prostate cancer and by using heterogenous cohorts to compare rates of *T. vaginalis* seropositivity. However, the lack of any trends across different clinically relevant cohorts makes false-negative results unlikely. A perfect study would be to prospectively analyze enrolled low-risk patients who are receiving active surveillance and look for associations with early progression. Other limitations are the retrospective analysis of prospectively obtained cohorts and the use of 3 separate cohorts with different baseline features and methods of ascertainment. It should be highlighted that this work only pertains to localized

Figure 1 Cytokine Distribution. Box Plots Suggest No difference Between Lethal and Nonlethal Prostate Cancer (A), or Between Low-Risk Prostate Cancer (GS = 6, PSA < 10) or No Cancer and Intermediate- to High-Risk Significant (GS ≥ 7, PSA ≥ 10) Prostate Cancer in any cytokine (B)



Abbreviations: GRO-α = growth-regulated oncogene α; GS = Gleason score; IL = interleukin; MCP-1 = monocyte chemoattractant protein 1; PSA = prostate-specific antigen; TNF-α = tumor necrosis factor α.

prostate cancer. Nevertheless, our *T. vaginalis* findings are supported by 2 publications that also found no association between the presence of circulating *T. vaginalis* immunoglobulin G antibodies and prostate cancer in a larger cohort of patients.[20,21]

In brief, we strove to identify biomarkers of inflammation that may highlight patients with low-risk localized prostate cancer based on the current clinical tools of PSA, Gleason grade, and prostate examination who are in fact at risk for more aggressive prostate cancer and not candidates for active surveillance. Our results do not

Table 2 Clinical Characteristics and *Trichomonas vaginalis* and Cytokine Results for Early Detection Research Network Cohort (N = 306)

Characteristic	Low Risk/No Prostate Cancer (N = 220)	Intermediate- to High-Risk Prostate Cancer (N = 86)	Parametric *P*
Age at diagnosis (years) median (min, max)	63 (36-80)	65.5 (49-89)	—
PSA at diagnosis (ng/mL), median (min, max)	4.9 (0.3-43.6)	6.2 (0.9-204.6)	—
Biopsy Gleason			—
≤ 6	49 (22.3)	0	
7	0	61 (70.9)	
≥ 8	0	25 (29.1)	
no cancer	171 (77.7)	0	
cT Stage			—
T1	35 (15.9)	57 (66.3)	
T2	9 (4.1)	24 (27.9)	
T3/T4	0	2 (2.3)	
Tx/unknown	5 (2.3)	3 (3.5)	
NA	171 (77.7)	0	
cN Stage			—
N0	49 (22.3)	84 (97.7)	
N1	0	2 (2.3)	
NA	171 (77.7)	0	
***T. vaginalis* Score**			.368
0-2	177 (80.5)	73 (84.9)	
3-4	43 (19.5)	13 (15.1)	
IL-1α			.309
Median or lower	114 (51.8)	39 (45.3)	
Greater than median	106 (48.2)	47 (54.7)	
IL-1β			.799
Median or lower	111 (50.5)	42 (48.8)	
Greater than median	109 (49.5)	44 (51.2)	
IL-2			.611
Median or lower	108 (49.1)	45 (52.3)	
Greater than median	112 (50.9)	41 (47.7)	
IL-6			.87
Median or lower	109 (49.5)	43 (50)	
Greater than median	111 (50.5)	43 (50)	
IL-8			.309
Median or lower	106 (48.2)	47 (54.7)	
Greater than median	114 (51.8)	39 (45.3)	
MCP-1			.799
Median or lower	109 (49.5)	44 (51.2)	
Greater than median	111 (50.5)	42 (48.8)	
TNF-α			.309
Median or lower	114 (51.8)	39 (45.3)	
Greater than median	106 (48.2)	47 (54.7)	
GRO-α			.799
Median or lower	111 (50.5)	42 (48.8)	
Greater than median	109 (49.5)	44 (51.2)	

Data are presented as n (%) unless otherwise indicated.
Abbreviations: GRO-α = growth-regulated oncogene α; IL = interleukin; MCP-1 = monocyte chemoattractant protein 1; PSA = prostate-specific antigen; RP = radical prostatectomy; RT = radiotherapy; TNF-α = tumor necrosis factor α.

support the use of *T. vaginalis* seropositivity or elevated cytokines as metrics or biomarkers of more aggressive disease.

Clinical Practice Points

- Treatment choices for localized prostate cancer are based on Gleason grade, PSA, and clinical stage by digital rectal examination of the prostate.
- There is a major clinical need to identify additional prognostic biomarkers to select patients with localized disease who would benefit from earlier curative treatments.
- Inflammation and infections have been associated with prostate cancer progression.
- We found no association between circulating cytokine levels above median concentrations nor *T. vaginalis* seropositivity and risk of clinically significant or lethal prostate cancer.
- The systemic levels of the 8 cytokines measured in this study as well as *T. vaginalis* serology do not appear to help with risk stratification in localized prostate cancer.

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Disclosure

The authors have stated that they have no conflict of interest.

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