Both acute and chronic inflammation are associated with lower perineural invasion in men with prostate cancer on repeat biopsy

Andrew G. Kuang[1], J. Curtis Nickel[2], Gerald L. Andriole[3], Ramiro Castro-Santamaria[4], Stephen J. Freedland[5] and Daniel M. Moreira[1]

[1]Department of Urology, University of Illinois at Chicago, Chicago, IL, USA
[2]Department of Urology, Queen's University, Kingston, ON, Canada
[3]Division of Urologic Surgery, Department of Surgery, Washington University School of Medicine, St. Louis, MO, USA
[4]GlaxoSmithKline Inc., Global R&D, King of Prussia, Pennsylvania, PA, USA
[5]Division of Urology, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA, and the Section of Urology Durham VA Medical Center, Durham, NC, USA

Corresponding author: Daniel M. Moreira, MD, Department of Urology, University of Illinois at Chicago, 820 S Wood Street, CSN Suite 515, Chicago, IL 60612, USA; Phone: (312) 996-9331; E-mail: moreira@uic.edu

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Abstract:

Objectives: To evaluate the association between acute and chronic inflammation with the presence of perineural invasion (PNI) in prostate biopsies positive for prostate cancer (PCa).

Material and methods: Retrospective analysis of 1399 prostate biopsies positive for PCa in the Reduction by Dutasteride of PCa Events (REDUCE) study. PCa, acute and chronic prostate inflammation, and PNI were assessed by central pathology review. The association between acute and chronic inflammations with PNI was evaluated using chi-square test and Kruskal-Wallis tests, and logistic regression adjusting for clinicopathological and biochemical variables.

Results: PNI was identified in 133 (9.5%) biopsies. 267 (19.1%) biopsies had acute inflammation, 1038 (74.2%) had chronic inflammation, and 255 (18.2%) had both. The presence of acute and chronic inflammations was associated with each other (P<0.001). Chronic inflammation was associated with lower Gleason score (P=0.009) and lower tumor volume (P<0.001), while acute inflammation was associated with lower Gleason score (P=0.04), lower tumor volume (P=0.004) and higher prostate-specific antigen levels (P=0.05). In both uni- and multivariable analyses, chronic prostate inflammation was significantly associated with less PNI (univariable OR=0.54; 95% CI=0.37-0.79; P=0.001; multivariable OR=0.65; 95% CI=0.43-0.99; P=0.045). Acute prostate inflammation was associated with less PNI only in univariable analysis (univariable OR=0.51; 95% CI=0.29-0.89, P=0.018; multivariable OR=0.63; 95% CI=0.35-1.13; P=0.12).

Conclusion: Acute and chronic prostate inflammation were both associated with a lower prevalence of PNI in prostate biopsies positive for PCa. If confirmed, this suggests that inflammation and immunomodulation can serve as areas of potential therapeutic design to mitigate PNI in PCa patients.

Introduction:

Perineural Invasion (PNI) is the infiltration of cancer cells in, around, and/or through nerves and is observed frequently in prostate cancer (PCa), with studies reporting its prevalence in 7% to 43% of prostate needle biopsies with PCa [1-3]. PNI has been implicated in PCa cell proliferation and migration, and the presence of PNI in diagnostic prostate biopsies for PCa has been shown to be associated with markers of advanced and aggressive disease in patients on active surveillance or following radical prostatectomy [4-5]. One thought is that perineural space provides cancer cells with a path of lesser resistance to spread beyond the prostate [6]. Another thought is that the nerves themselves drive PCa to be more aggressive. Multiple authors have reported worse outcomes among subjects with PCa and PNI in prostate biopsies, including an increased risk of biochemical recurrence after radical prostatectomy and radiotherapy, metastatic disease progression, and mortality [2, 7-8]. Yet others have shown no association between PNI and poor PCa outcomes [9-10].

Inflammation is commonly present in prostate biopsies and radical prostatectomy specimens [11]. Previous studies have indicated that between 35% to 100% of prostate biopsies show some degree of inflammation [12-14]. The association of inflammation and PCa has been controversial, but recent work suggests that inflammation in negative biopsies may be protective and associated with less aggressive tumors among patients eventually diagnosed for PCa. It has been historically thought that inflammatory and immune system mechanisms inhibit carcinogenesis by targeting and acting upon tumor-specific and tumor-associated antigens [15]. Studies have found that both acute and chronic inflammation in negative biopsies were significantly associated with lower PCa risk in repeat prostate biopsies [14]. Moreover, chronic prostate inflammation in men with initial negative biopsy for PCa correlates with a lower tumor volume among men in whom a repeat prostate biopsy shows PCa [16].

Although PNI is usually seen in cases of PCa presenting aggressive and advanced tumors, the factors associated with ability of PCa to permeate nerves is not completely understood. Moreover, it has remained unknown whether benign prostate histological findings, like inflammation, are correlated with PNI in PCa. Therefore, we sought to evaluate the association of acute and chronic prostate inflammation with PNI among men with a positive biopsy for PCa

in the Reduction by Dutasteride of PCa Events (REDUCE) study. The REDUCE study was a clinical trial where men with a baseline negative prostate biopsy for cancer were followed with prostate biopsies every 2 years for 4 years and, importantly for this analysis, included central pathology review of all biopsy slides. Given that prostate inflammation has been shown to correlate with less aggressive and extensive tumors, we hypothesize that prostate inflammation will be inversely associated with PNI.

Methods:

The methodology of the REDUCE study was previously published [17]. In summary, men aged 50-75 years who had serum PSA ≥ 2.5 or 3.0 ng/mL based on age (50-60 and 60-75 years, respectively) but ≤ 10 ng/mL and one single negative prostate biopsy within 6 months prior to enrollment were included. Men with history of PCa, high-grade intraepithelial neoplasm, atypical small acinar proliferation, prostate volume greater than 80 mL, history significant for previous prostate surgery, or an International Prostate Symptom score ≥ 25 or ≥ 20 on alpha-blockers were excluded from the study. The subjects were randomized in a double-blind method to given daily oral doses of either dutasteride 0.5 mg or placebo and then followed every 6 months for 4 years. All prostate biopsies were read centrally at Bostwick laboratories. This laboratory had no access to randomization codes. At the time of randomization, 2 and 4 years after enrollment, prostate volume was measured using ultrasonography. At the 2- and 4-year mark, at least ten-core transrectal ultrasound-guided prostate biopsies were performed regardless of PSA levels. PCa, acute and chronic inflammation, and PNI were coded as present or absent. Acute inflammation was determined based on the presence of neutrophilic infiltrate while chronic inflammation consisted mostly of lymphocytes, plasma cells, and macrophages. PNI was coded as present or absent based on the presence of infiltration of cancer into nervous tissue. All pathology slides were reviewed by two independent pathologists. Discrepancies were settled by consensus. The REDUCE protocol was approved by the institutional review board at all involved research sites, and all participants gave written informed consent. A total of 11,840 prostate biopsies, including 2-year, 4-year, and off-protocol biopsies, were obtained from the 8,231 men participating in the study (Figure 1). Of these, 2,261 (19.1%) biopsies were positive for PCa. Given that some subjects had more than one positive prostate biopsy, only the first positive biopsy (2-year, 4-year, and off protocol) was analyzed, which included 2,132 (94.3%) biopsies. Inflammation and PNI were measured in the same biopsy specimen. We excluded 733 biopsies (34.4%) due to missing data in one of the variables of interest (see below), leading to a final study sample of 1,399 (65.6%) biopsies.

Univariable comparisons of baseline characteristics between men with acute and chronic prostate inflammations were performed using chi-squared test for categorical data and Kruskal-

Wallis rank test for continuous variables. The association of acute and chronic prostate inflammation with the presence of PNI was tested using chi-squared test in univariable analysis. Logistic regression was used to test the multivariable association between acute and chronic prostate inflammation with presence of PNI, controlling for the following covariates: age (continuous, in years), race (White or other), geographic region (North America, Europe, or other), body mass index (BMI, continuous in kg/m^2), digital rectal examination (DRE, scored as normal or abnormal), Gleason score (2-6, 7, or 8-10), prostate volume (continuous, in cm^3), tumor volume (continuous, in μL), biopsy number (continuous), prostate serum antigen (PSA, continuous, in ng/mL), and treatment arm (dutasteride or placebo). All statistical analyses were two-tailed and performed using Stata 12.0 (StataCorp, College Station, TX). A P value < 0.05 was considered to indicate statistical significance.

Results:

The overall median age, BMI, PSA and prostate volume for our sample was 67 years, 26.9 kg/m^2, 5.2 ng/dL, and 39.4 cm^3, respectively. A total of 1281 (91.6%) of the subjects were Caucasian. There were 84 (6.0%) with abnormal DRE. A majority, 921 (65.8%) subjects, were from Europe, with 299 (21.4%) from North America and 179 (12.8%) from other parts of the world. A total of 267 (19.1%) of biopsies had acute inflammation, and 1038 (74.2%) had chronic inflammation, and 255 (18.2%) had both acute and chronic inflammations (Table 1). Chronic inflammation was associated with lower Gleason score (P = 0.009) and lower tumor volume (P = 0.0001) but was unrelated to PSA, prostate volume, DRE, race, geographic region, BMI, or age (Table 2). Acute inflammation was associated with lower Gleason score (P = 0.04), lower tumor volume (P = 0.004) and higher PSA levels (P = 0.05) but was not associated with prostate volume, DRE, age, BMI, race, or geographic region (Table 3). The presences of both acute and chronic inflammation were associated with each other (P < 0.001).

A total of 133 (9.5%) biopsies were positive for PNI (Table 4). Of the 267 total biopsies positive for acute prostate inflammation, 15 (5.6%) were positive for PNI. Of the 1038 biopsies positive for chronic inflammation, 83 (8.0%) had PNI. In univariable analysis, both acute and chronic prostate inflammation were significantly associated with lower presence of PNI (acute inflammation: OR = 0.51; 95% confidence interval [CI] = 0.29-0.89; P = 0.018; chronic inflammation: OR = 0.54; 95% CI = 0.37-0.79; P = 0.001). After adjustments for age, race, geographic region, BMI, DRE, Gleason score, prostate volume, tumor volume, biopsy number, PSA levels, and treatment arm in multivariable analyses, chronic inflammation remained significantly associated with less PNI (OR = 0.65; 95% CI = 0.43-0.99; P = 0.045) while acute prostate inflammation was no longer significantly associated with PNI (OR = 0.63; 95% CI = 0.35-1.13; P = 0.12).

Discussion:

Although the clinical implications of prostate inflammation are still debatable, inflammatory infiltrate is a common histological finding observed in 35 to 100% of the prostate biopsies done for PCa screening [12-14]. In our study of prostate biopsies positive for PCa, the presence of acute and chronic inflammations was 19.1% and 74.2%, respectively, which is like that described in previous studies [14, 16]. We found that chronic inflammation was associated with higher prostate volume, lower Gleason score, and lower tumor volume while acute inflammation was associated with lower tumor volume and higher PSA. These findings agree with previous studies reporting that chronic baseline prostate inflammation in men with initial negative PCa biopsy is associated with lower tumor volume and lower Gleason scores in a repeat biopsy positive for PCa [16]. In our study, 9.5% of the biopsies positive for PCa had histological evidence of PNI. Although the factors associated with PNI are not completely understood, some studies suggest that PNI in prostate surgical specimens is associated with worse PCa outcomes [2, 7-8]. However, it is unclear whether benign histological findings such as acute or chronic inflammation correlate with the presence of PNI. In this study, we found that both acute and chronic prostate inflammation were significant correlated with lower prevalence of PNI.

While there are well-established models of inflammation leading to carcinogenesis in hepatocellular, cervical, esophageal and gastric carcinomas, the role of inflammation in PCa development has remained unclear. Although some authors have reported higher odds of PCa in cases in which at least one biopsy core had the presence of moderate or severe inflammation [18, 19], a recent meta-analysis of 25 studies concluded that histological inflammation on prostate needle biopsies was associated with a lower risk of subsequent PCa diagnosis [20]. Beyond cancer risk, for those who are eventually diagnosed with PCa Moreira *et al.* found the presence of chronic inflammatory infiltrate in the biopsy was associated with lower tumor volumes [16]. In addition, our present findings of less PNI in men with prostate inflammation reinforce the hypothesis that inflammation is associated with less aggressive tumors. Thus, although there is conflicting evidence in the literature, most studies suggest a potential protective role of inflammation on prostate cancer carcinogenesis and aggressiveness.

The exact biological mechanisms linking the presence of acute and chronic inflammation with less PNI are not entirely clear. However, it is conceivable that the presence of inflammatory infiltrate correlates with the activity of the immune system inhibiting carcinogenesis. This is called, immunosurveillance, the process in which the host's immune system identifies malignant cells and eliminates them before tumor development can complete. It has been proposed that a correlation between inflammation and immunosurveillance may lead to immunomodulation that can lower PCa risk and tumor size in repeat prostate biopsies [14]. One possibility could be that immunosurveillance serves as the mechanism by which inflammation helps prevent the spread of PCa cells which may include the perineural space in PNI. For example, robust lymphocytic infiltration has been reported to be positively correlated with patient survival in melanoma, head and neck, ovarian, urothelial, colorectal, lung, hepatocellular, gallbladder, esophageal, and breast cancers [21]. Research studying tumor-associated macrophages in human PCa suggests that presence of tumor associated macrophages in PCa tissues is inversely associated with PCa clinical stage [22].

In addition to the immunosurveillance hypothesis, it is possible that the association between inflammation and less PNI is mediated by other factors, such as differences in patient and disease characteristics between subjects with inflammation and those without it at baseline. To study this further, we performed multivariable analyses adjusting for patient demographics and PCa characteristics. Results remained significant, except for acute prostate inflammation, though the magnitude of the inverse association was little changed. These results suggest that the link between inflammation and less PNI is not related to imbalances in baseline characteristics such as age, PSA levels and prostate volume.

The clinical implications of our findings are manifold. First, our results support the idea that inflammation and immunomodulation can serve as areas of potential therapeutic development for treating PCa and minimalizing PNI. For example, there are already examples of immunotherapy to treat PCa. Sipuleucel-T is a currently approved autologous cellular immunotherapy which has been shown to increase survival of men with metastatic castration-resistant PCa [23]. In addition, anti-CTLA-4 (ipilimumab) and anti-PD-1 (nivolumab) therapies that target immune checkpoints have been shown to increase immune cell infiltration in prostate

tumors and potentially improve survival of metastatic PCa [24]. Second, our findings support the idea that meticulous pathological review for the presence of inflammatory cells in prostate specimens may be useful in PCa risk stratification as subjects with prostate inflammation seem to have less aggressive PCa including a lower prevalence of PNI.

The REDUCE study provides data based on a diverse patient population due to its broadly ranging and multicentric geographic scope. Its major strengths include a prospective data acquisition and central pathology review. Reports in the past have indicated that histological prostate inflammation is associated with elevated PSA and traditionally patients have been selected for biopsy according to a PSA criterion [12, 25]. Thus, it is conceivable that for a given PSA level, those with prostate inflammation have less aggressive disease (and potentially less PNI) than their no-inflammation counterparts, given that inflammation rather than PCa is potentially the reason for some of the elevated serum PSA. The REDUCE study is designed to minimize such a bias since study biopsies were performed regardless of PSA levels. Despite these strengths, our study is not devoid of limitations. First, the inclusion criterion excluded men with PSA levels greater than 10 ng/mL. As such, future studies expanding this inclusion criterion could elucidate the association of inflammation and PNI among patients with higher PSA levels. Second, patients in our study were followed for a maximum of only 4 years; thus, long-term implications of the relationship between inflammation and PNI on PCa disease progression and other oncologic outcomes could not be studied. Third, an analysis on race influencing the association between acute and chronic inflammation and PNI could not be performed due to a limited sample size of non-Caucasian men. Fourth, currently not all pathologists evaluate or report acute and chronic inflammation in prostate cancer biopsies and there is still no consensus on how the two types should be evaluated or reported. However, studies that analyze the degree of interobserver agreement in the evaluation of prostate cancer acute and chronic inflammation as well as perineural invasion show moderate to high interobserver agreement [26, 27]. Finally, although our results suggest an association of inflammation and less PNI, causality could not be inferred from our findings. Further studies evaluating the biology of inflammation and PNI as well as the temporal sequence of inflammation and PNI can help better elucidate this relationship.

In conclusion, among men with a positive biopsy for PCa following initial negative baseline biopsy, acute and chronic inflammations were associated with lower presence of PNI. Although the mechanisms for this association remain unclear, protective mechanisms such as those proposed by immunosurveillance could provide one explanation. Thus, therapies targeting the inflammatory response may serve as a potential means to mitigate PNI in PCa patients.

Conflict of Interests:

Gerald L. Andriole Jr is a member of the Consulting and Advisory Board at GlaxoSmithKline, and he has received speaker fees and research support from GlaxoSmithKline. Ramiro Castro-Santamaria is an employee at GlaxoSmithKline, PLC. Stephen J. Freedland received research support from GlaxoSmithKline. The remaining authors declare that they have no relevant conflicts of interest.

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Table 1: Baseline patient population characteristics

Characteristic	Overall
Patients, N (%)	1,399 (100)
Median age (IQR) in years	67.0 (9.0)
Median BMI (IQR) in Kg/m²	26.9 (4.4)
Race, N (%):	
White	1,281 (91.6)
Other	118 (8.4)
Geographic region, N (%):	
North America	299 (21.4)
Europe	921 (65.8)
Other	179 (12.8)
Type of biopsy, N (%):	
2-year	871 (62.3)
4-year	388 (27.7)
Off-protocol	140 (10.0)
DRE, N (%):	
Normal/enlarged	1,315 (94.0)
Abnormal	84 (6.0)
Median prostate volume (IQR) in cm³	39.4 (25.8)
Median PSA (IQR) in ng/dL	5.2 (5.2)
Median biopsy tumor volume (IQR) in µL	0.8 (2.1)
Median biopsy volume sampled (IQR) in µL	125.8 (40.1)
Treatment arm, N (%):	
Placebo	799 (57.1)
Dutasteride 0.5 mg	600 (42.9)
Gleason score, N (%):	
2-6	981 (70.1)
7	372 (26.6)
8-10	46 (3.3)
Acute inflammation, N (%):	
No	1,132 (80.9)
Yes	267 (19.1)
Chronic inflammation, N (%):	
No	361 (25.8)
Yes	1,038 (74.2)

BMI: body-mass index; DRE: digital rectal exam; PSA: prostate-specific antigen; PV: prostate volume; IQR: interquartile range.

Table 2: Baseline patient characteristics by chronic prostate inflammation

Characteristic	Chronic inflammation		P value
	No	Yes	
DRE, N (%):			0.6
Normal	337 (93.4)	978 (94.2)	
Abnormal	24 (6.7)	60 (5.8)	
Race, N (%):			0.6
White	333 (92.2)	948 (91.3)	
Other	28 (7.8)	90 (8.7)	
Geographic region, N (%):			0.3
North America	76 (21.1)	223 (21.5)	
Europe	247 (68.4)	674 (64.9)	
Other	38 (10.5)	141 (13.6)	
Treatment arm, N (%):			0.001
Placebo	234 (64.8)	565 (54.4)	
Dutasteride 0.5 mg	127 (35.2)	473 (45.6)	
Type of biopsy, N (%):			0.4
2-year	224 (62.1)	647 (62.3)	
4-year	107 (29.6)	281 (27.1)	
Off-protocol	30 (8.3)	110 (10.6)	
Gleason score, N (%):			0.009
2-6	231 (63.4)	750 (72.3)	
7	118 (32.7)	254 (24.5)	
8-10	12 (3.3)	34 (3.3)	
Biopsy number, N (%):			0.2
2	235 (65.1)	711 (68.5)	
3	115 (31.9)	307 (29.6)	
4	11 (3.1)	17 (1.6)	
5	0 (0)	3 (0.3)	
Median PSA (IQR) in ng/mL	5.6 (3.2-8.7)	5.2 (2.8-8.0)	0.08
Median BMI (IQR) in Kg/m^2	26.8 (24.7-29.2)	27.0 (24.7-29.1)	0.8
Median prostate volume (IQR) in cm^3	37.8 (26.7-52.7)	40.0 (28.6-54.2)	0.06
Median age (IQR) in years	66.0 (61.5-71.1)	67.0 (62.0-71.0)	0.7
Median tumor volume, (IQR) in µL	1.2 (0.4-3.1)	0.8 (0.2-2.0)	<0.001
Median biopsy volume sampled, (IQR) in µL	120.3 (98.2-140.7)	127.5 (108.3-146.8)	0.001

BMI: body-mass index; DRE: digital rectal exam; PSA: prostate-specific antigen; IQR: interquartile range.

Table 3: Baseline patient characteristics by acute prostate inflammation

Characteristic	Acute inflammation		P value
	No	Yes	
DRE, N (%):			0.6
Normal	1,062 (93.8)	253 (94.8)	
Abnormal	70 (6.2)	14 (5.2)	
Race, N (%):			0.4
White	1,040 (91.9)	241 (90.3)	
Other	92 (8.1)	26 (9.7)	
Geographic region, N (%):			0.09
North America	244 (21.6)	55 (20.6)	
Europe	754 (66.6)	167 (62.6)	
Other	134 (11.8)	45 (16.9)	
Treatment arm, N (%):			0.1
Placebo	636 (56.2)	163 (61.1)	
Dutasteride 0.5 mg	496 (43.8)	104 (39.0)	
Type of biopsy, N (%):			0.4
2-year	713 (63.0)	158 (59.2)	
4-year	305 (26.9)	83 (31.1)	
Off-protocol	114 (10.1)	26 (9.7)	
Gleason score, N (%):			0.04
2-6	777 (68.0)	204 (76.4)	
7	317 (28.0)	55 (20.6)	
8-10	38 (3.4)	8 (3.0)	
Biopsy number, N (%):			0.3
2	770 (68.6)	176 (65.9)	
3	334 (29.5)	88 (33.0)	
4	26 (2.3)	2 (0.8)	
5	2 (0.2)	1 (0.4)	
Median PSA (IQR) in ng/mL	5.1 (2.9-8.1)	5.7 (3.3-8.3)	0.05
Median BMI (IQR) in Kg/m^2	27.0 (24.8-29.1)	26.7 (24.5-29.2)	0.3
Median prostate volume (IQR) in cm^3	39.0 (27.7-53.4)	41.2 (29.5-55.3)	0.1
Median age (IQR) in years	67.0 (62.0-71.0)	67.0 (62.0-71.0)	0.6
Median tumor volume, (IQR) in µL	1.0 (0.2-2.3)	0.8 (0.2-2.0)	0.004
Median biopsy volume sampled, (IQR) in µL	123.4 (102.8-144.5)	131.4 (115.4-148.5)	<0.001

BMI: body-mass index; DRE: digital rectal exam; PSA: prostate-specific antigen; IQR: interquartile range.

Table 4: Association of acute and chronic prostate inflammation with perineural invasion positivity

Prostate Inflammation		Perineural invasion (Positive/Total biopsies)	Univariable		Multivariable*	
			OR (95%CI)	P value	OR (95%CI)	P value
Acute						
	Absent	10.4% (118/1,132)	*ref.*	-	*ref.*	-
	Present	5.6% (15/267)	0.512 (0.293-0.891)	0.018	0.626 (0.348-1.126)	0.12
Chronic						
	Absent	13.9% (50/361)	*ref.*	-	*ref.*	-
	Present	8.0% (83/1,038)	0.541 (0.372-0.785)	0.001	0.653 (0.431-0.990)	0.045

CI: confidence interval; OR: Odds ratio; *ref*: reference.

*Adjusted for age, race, geographic region, biopsy type, biopsy number, body-mass index, digital rectal exam, Gleason score, prostate volume, prostate-specific antigen levels, tumor volume, and treatment arm.

Figure 1: Method of exclusion