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ORIGINAL ARTICLE

Intratumoral inflammation is associated with more aggressive prostate cancer

Joseph C. Klink · Lionel L. Bañez · Leah Gerber · Amy Lark · Robin T. Vollmer · Stephen J. Freedland

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Abstract
Purpose Inflammation may play a role in the development and progression of many cancers, including prostate cancer. We sought to test whether histological inflammation within prostate cancer was associated with more aggressive disease.
Methods The slides of prostatectomy specimens were reviewed by a board-certified pathologist on 287 men from a Veterans Affairs Medical Center treated with radical prostatectomy from 1992 to 2004. The area with the greatest tumor burden was scored in a blinded manner for the degree of inflammation: absent, mild, or marked. We used logistic and Cox proportional hazards regression analysis to examine whether categorically coded inflammation score was associated with adverse pathology and biochemical progression, respectively.
Results No inflammation was found in 49 men (17 %), while 153 (53 %) and 85 (30 %) had mild and marked inflammation. During a median follow-up of 77 months, biochemical recurrence occurred among 126 (44 %) men. On multivariate analysis, more inflammation was associated with greater risk of positive margins, capsular penetration, and seminal vesicle invasion (all $p < 0.05$). Marked inflammation was associated with increased PSA recurrence risk when adjusting for preoperative features only (HR 2.08, 95 % CI 1.02–4.24), but not after adjusting for pathologic features.
Conclusions Inflammation within prostate cancer was associated with more advanced disease, although it is unclear whether aggressive disease caused increased inflammation or inflammation caused aggressive disease.

Keywords Inflammation · Prostate cancer · Radical prostatectomy · Adverse pathology · Biochemical progression

J. C. Klink · L. L. Bañez · S. J. Freedland
Urology Section, Department of Surgery,
Veterans Affairs Medical Center, Durham, NC, USA

J. C. Klink · L. L. Bañez · L. Gerber · S. J. Freedland
Division of Urologic Surgery and the Duke Prostate Center,
Department of Surgery, Duke University Medical Center,
Durham, NC, USA

J. C. Klink (✉)
Glickman Urological and Kidney Institute, Cleveland Clinic,
9500 Euclid Ave. Q10-1, Cleveland, OH, USA
e-mail: klinkj@ccf.org

A. Lark · R. T. Vollmer
Department of Pathology, Veterans Affairs Medical Center,
Durham, NC, USA

S. J. Freedland
Department of Pathology, Duke University Medical Center,
Durham, NC, USA

Introduction

Inflammation may play a role in the development and progression of many cancers, including prostate cancer (PC). Epidemiologic studies have correlated symptomatic prostatitis with PC development [1]. Multiple genetic and molecular mechanisms link PC with inflammation [2]. Pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and IL-17 as well as TNF-α and TGF-β, are associated with metastatic PC [3]. If inflammation promotes PC, one would expect anti-inflammatory drugs to lower PC rates and indeed there is some evidence to support this hypothesis. While some have concluded that aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs) reduce PC risk [4–8], others have not [9, 10]. Genetic studies have also revealed

links between PC and inflammation. *MSR1* is a gene associated with inflammation and immunity that has been shown in many, but not all studies to be a PC susceptibility gene [11]. The *TLR* family of genes is involved with inflammation. Mutations of the TLR-4 gene and of the TLR-1-6-10 gene cluster have been associated with PC [11]. While this evidence, taken together, makes a strong argument for a link between inflammation and PC, more direct evidence is needed to prove this hypothesis.

These basic science and epidemiologic findings are corroborated by the clinical observation that inflammatory infiltrates are often seen on prostate biopsy and radical prostatectomy specimens. This histology suggests an association between inflammatory cells and PC development. However, the link between inflammation and more aggressive disease has not been well studied. We analyzed a large cohort with adjustment for multiple preoperative and pathological variables to explore the association between tumor inflammation, adverse pathology, and biochemical recurrence (BCR) after radical prostatectomy.

Materials and methods

Study population

After obtaining IRB approval, we identified men who underwent radical prostatectomy between 1992 and 2004 at the Durham Veterans Affairs Medical Center who did not receive preoperative androgen deprivation or radiation therapy. Of 442 men treated during this time, 287 men had prostatectomy specimens available for review. Men not included were similar to men included with regard to demographic, clinical, pathological, and PSA outcome data (all $p > 0.1$). BCR was defined as a single PSA >0.2 ng/ml, two concentrations at 0.2 ng/ml, or secondary treatment for an elevated postoperative PSA. Mean and median follow-up were 80 and 77 months, respectively (range 1–181 months), among nonrecurrent men.

Evaluation of inflammation

The H&E-stained slides from the prostatectomy specimen of each patient were reviewed by a single pathologist (RV). The slide with the greatest tumor burden was identified for each patient, and that slide was reviewed for the degree of inflammation in a blinded manner by a separate board-certified pathologist (AL). Each slide received a score of 0 ("No inflammation," Fig. 1a), 1 ("Mild inflammation," estimated less than or equal to 10 % inflammation within the tumor, Fig. 1b), or 2 ("Marked inflammation," estimated greater than 10 % inflammation within the tumor, Fig. 1c). These levels of inflammation are similar to those reported previously [12] with the "No inflammation" and "Mild inflammation" categories defined identically between the studies, but "Moderate" and "Severe inflammation" categories in the literature lumped into one category, "Marked inflammation," in our study due to low numbers in that group. Tumor inflammation included acute and/or chronic inflammation within the stroma among adenocarcinoma as well as glandular luminal inflammation. Inflammation associated with benign glands and stroma was not evaluated in this study.

Statistical analysis

Tumors were categorized into three groups based on their inflammation score. Differences in demographic and clinicopathological factors among inflammation groups were examined using Kruskal–Wallis and χ^2 tests for continuous and categorical variables, respectively. The association between inflammation group and risk of adverse pathological features and BCR was analyzed using logistic regression and Cox proportional hazards analyses, respectively. Analyses predicting adverse pathological features were adjusted for clinical factors, including biopsy Gleason score, preoperative PSA, race (African American or non-African American), surgery year, age at surgery, prostatectomy specimen weight, and number of prior biopsies. In exploratory analyses, we noted that further adjustments for body mass index, percent of cores positive, and clinical stage did not alter the association between inflammation and adverse pathology. Given that many men were missing these data, these factors were not included in our final models. Adverse pathological features were defined as pathological Gleason score $\geq 4 + 3$, positive margins, extracapsular extension, and/or seminal vesicle invasion. For analyses predicting BCR, we used log-rank to test the overall trend. We then ran two separate multivariate models: one adjusted for only the clinical factors listed above plus clinical stage and a second adjusted for clinical and pathological factors including pathological Gleason score, positive margins, positive lymph nodes, and pathologic stage (T2, T3, and T4). Gleason score (2–6, $3 + 4$, and $\geq 4 + 3$) and stage were analyzed as categorical variables, while age, surgery year, logarithmically transformed PSA, and prostate weight were analyzed as continuous variables. Statistical analyses were performed using STATA 10.1 (STATA Corp, College Station, TX).

Results

Clinical and pathological characteristics

No inflammation was found in 49 (17 %), while 153 (53 %) and 85 (30 %) had mild and marked inflammation.

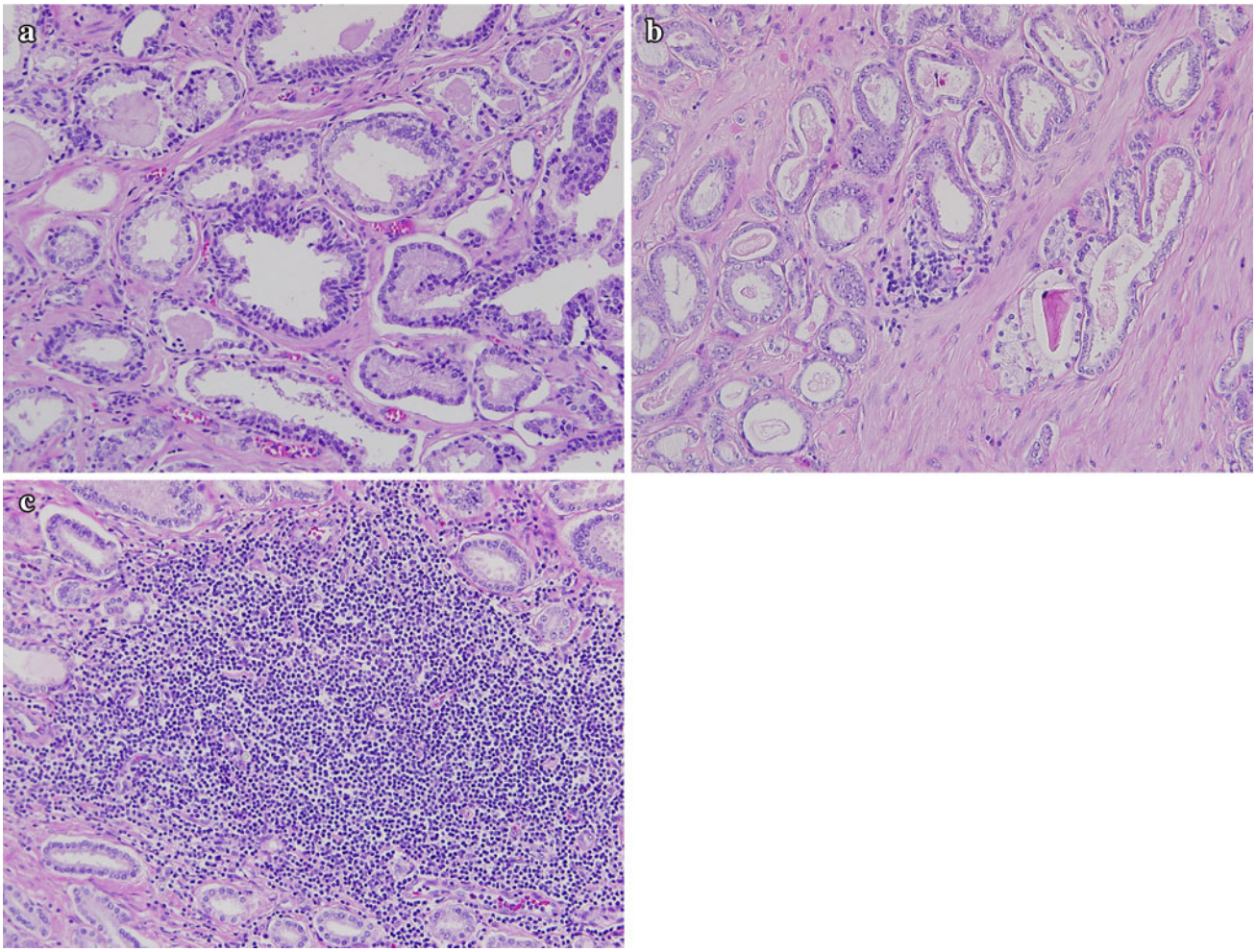

Fig. 1 Representative images of prostate adenocarcinoma with no inflammation (**a**), mild inflammation (**b**), and marked inflammation (**c**)

Inflammation group was not significantly related to most clinical and pathological characteristics (Table 1). However, men with more inflammation were significantly more likely to have higher clinical stage, higher PSA, higher percent of cores positive, and positive margins. The time between biopsy and surgery was similar between all inflammation grades. Of the 287 patients, 260 had no prior biopsies, 21 had one prior biopsy, three had two prior biopsies, and three had three prior biopsies. There was no correlation between number of previous biopsies and inflammation grade on chi-square ($p = 0.9$).

On univariate analysis, increasing inflammation grade was associated with progressively higher risk of positive margins (OR 2.26–3.56), capsular penetration (OR 3.12–3.19), seminal vesicle invasion (OR 4.08–6.83), and a trend toward high-grade disease (OR 1.76–2.55) (Table 2). After adjusting for multiple clinical characteristics, more inflammation remained significantly associated with risk of capsular penetration (OR 2.95), positive margins (OR 3.67), and seminal vesicle invasion (OR 6.21) in the marked inflammation group.

Biochemical recurrence

Biochemical recurrence occurred among 126 (44 %) men: 12 (24 %) with no inflammation, 71 (46 %) with mild inflammation, and 43 (51 %) with marked inflammation. Overall, increasing inflammation grade was associated with increasing BCR (log-rank, $p = 0.02$, Fig. 2). When inflammation grade was analyzed as a categorical variable on crude analysis, mild inflammation and marked inflammation were associated with 2.18 (95 % CI 1.16–4.14, $p = 0.02$)- and 2.55 (95 % CI 1.31–4.97, $p = 0.006$)-fold greater BCR versus no inflammation.

After adjustments for clinical characteristics, marked inflammation was associated with higher BCR (HR 2.08, 95 % CI 1.02–4.24, $p = 0.04$) (Table 2). After adjustment for clinical and pathological variables, inflammation was no longer associated with BCR (HR 1.25–1.38, $p > 0.4$).

Given the strong association between positive surgical margins and inflammation, an exploratory analysis was performed on BCR in the subset of patients with negative surgical margins (Table 2). The sample size was small

Table 1 Demographic, clinical, and pathological features by amount of inflammation

	None	Mild	Marked	*p* value*
No. pts (%)	49 (17)	153 (53)	85 (30)	
Mean age at surgery ± SD	60.9 (6.5)	63.0 (6.3)	62.7 (6.0)	0.10
Median year of surgery	2000	2000	2000	0.60
BMI categories (%)				0.47†
≤24.9 kg/m^2	13 (30)	48 (38)	16 (23)	
25.0–29.9 kg/m^2	20 (47)	46 (36)	31 (45)	
30.0–34.9 kg/m^2	9 (21)	27 (21)	18 (26)	
≥35.0 kg/m^2	1 (2)	7 (5)	4 (6)	
Clinical stage (%)				0.02†
T1	34 (77)	84 (60)	37 (49)	
T2	10 (23)	55 (40)	39 (51)	
Preoperative PSA				0.03
Mean (SD)	7.8 (6.1)	11.1 (9.3)	10.2 (9.9)	
Median	6.5	8.2	7.1	
Days from biopsy to RP Mean (SD)	65 (49)	82 (93)	82 (104)	0.61
Biopsy Gleason Sum (%)				0.43†
2–6	35 (72)	92 (60)	53 (63)	
3 + 4	7 (15)	33 (22)	13 (15)	
≥4 + 3	6 (13)	28 (18)	18 (21)	
% Positive biopsy cores				0.002
Mean (SD)	29 (18)	37 (22)	44 (24)	
Median	25	33	40	
Pathologic Gleason Sum (%)				0.08†
2–6	16 (33)	27 (18)	19 (22)	
3 + 4	24 (49)	87 (57)	38 (45)	
≥4 + 3	9 (18)	38 (25)	28 (33)	
Positive surgical margins (%)	22 (45)	98 (64)	63 (74)	0.02
Seminal vesicle invasion (%)	2 (4)	25 (16)	19 (23)	0.20
Extracapsular extension (%)	6 (12)	45 (30)	27 (32)	0.13

* *p* value computed using Kruskal–Wallis test except where noted

† *p* value from chi-square

(none = 25, mild = 51, and marked = 18) in this subset, limiting statistical power. The association of inflammation with BCR did not reach statistical significance ($p > 0.2$) after adjustment for clinical characteristics (HR 2.09–2.33) or after adjustment for clinical and pathological variables (HR 2.06–2.76). However, it should be noted that the HRs for recurrence were actually slightly higher than in the overall analysis, suggesting our conclusions may hold even in men with negative margins though admittedly due to small numbers the confidence intervals were large and the comparisons did not reach statistical significance.

To test whether BCR may be affected by presence or absence of inflammation instead of by the degree of inflammation, we performed an exploratory analysis after grouping inflammation as "any" (mild + marked) versus "none." On log-rank analysis, inflammation was a significant predictor of biochemical recurrence ($p = 0.007$). On multivariate 1, the trend remained, but lost significance ($p = 0.08$). On multivariate 2, results were similar to prior analyses (nonsignificant).

Discussion

Prostate cancer has been associated with inflammation in epidemiologic, molecular, and pharmacological studies, but the exact relationship is unclear. We tested the association of histological inflammation in PC with adverse pathology and BCR by grading the degree of inflammatory infiltrate in radical prostatectomy specimens. On multivariate analysis, more inflammation was associated with more advanced disease. On univariate analysis and after adjustment for only preoperative variables, marked inflammation was also associated with increased BCR.

Table 2 Risk of adverse pathology and biochemical recurrence with increasing inflammation relative to no inflammation

	OR (95 % CI)	*p* value
Adverse pathology		
High-grade disease		
Crude		
Mild	1.76 (0.76–4.09)	0.19
Marked	2.55 (1.05–6.17)	0.04
Multivariate*		
Mild	1.36 (0.56–3.29)	0.50
Marked	2.24 (0.89–5.62)	0.09
Positive surgical margins		
Crude		
Mild	2.26 (1.16–4.38)	0.02
Marked	3.56 (1.68–7.55)	0.001
Multivariate*		
Mild	1.85 (0.93–3.67)	0.08
Marked	3.67 (1.67–8.03)	0.001
Extracapsular extension		
Crude		
Mild	3.12 (1.24–7.86)	0.02
Marked	3.19 (1.21–8.45)	0.02
Multivariate*		
Mild	2.52 (0.98–6.50)	0.055
Marked	2.95 (1.10–7.91)	0.03
SV invasion		
Crude		
Mild	4.08 (0.92–18.04)	0.06
Marked	6.83 (1.52–30.77)	0.01
Multivariate*		
Mild	3.38 (0.74–15.36)	0.12
Marked	6.21 (1.34–28.76)	0.02
	HR (95 % CI)	*p* value
Biochemical recurrence		
Log-rank		0.02
Multivariate 1		
Mild	1.64 (0.83–3.28)	0.15
Marked	2.08 (1.02–4.24)	0.04
Multivariate 2		
Mild	1.25 (0.66–2.37)	0.49
Marked	1.38 (0.70–2.72)	0.36
Biochemical recurrence in subset with negative surgical margins ($n = 94$, *none* $= 25$, *mild* $= 51$, *marked* $= 18$)		
Log-rank		0.27
Multivariate 1		
Mild	2.09 (0.56–7.90)	0.28
Marked	2.33 (0.44–12.33)	0.32
Multivariate 2		
Mild	2.06 (0.57–7.38)	0.27
Marked	2.76 (0.55–13.75)	0.22
Biochemical recurrence with inflammation categorized as none versus any		
Log-rank		0.007
Multivariate 1	1.80 (0.93–3.51)	0.08
Multivariate 2	1.29 (0.69–2.41)	0.42

Multivariate *: adjusted for PSA, year of surgery, race, age at surgery, prostate weight, and number of prior biopsies

Multivariate 1: adjusted for clinical characteristics: PSA, year of surgery, race, age at surgery, biopsy Gleason score, and clinical stage

Multivariate 2: adjusted for clinical and pathological characteristics: PSA, year of surgery, race, age at surgery, pathological Gleason score, positive surgical margins, lymph node metastases, and pathological stage

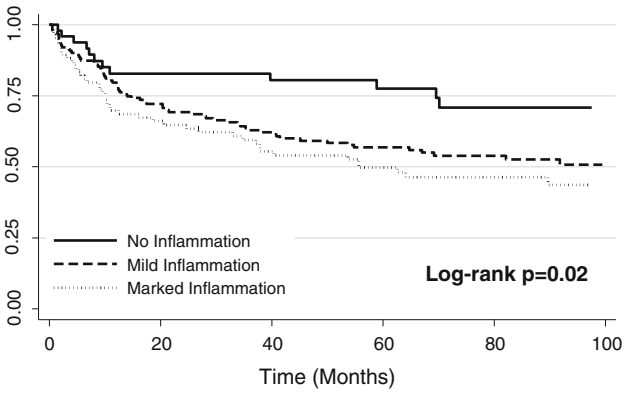

Fig. 2 Kaplan–Meier estimates of PSA-free survival stratified by amount of inflammation in the tumor

These findings suggest that grade of inflammation in preoperative biopsy specimens could be used to risk-stratify men with prostate cancer and this should be specifically addressed in future studies. While our results suggest inflammation is associated with aggressive PC, it is unclear whether aggressive disease caused increased inflammation or inflammation caused aggressive disease or they are etiologically unrelated.

Adverse pathology risk was greater for men with marked inflammation than for those with mild inflammation based on increasing odds ratios and decreasing *p* values (Table 2). BCR on crude and multivariate analysis also showed this trend (Table 2). Given the discrepancy between univariate and multivariate for the mild group, we explored which covariate had the greatest influence on attenuating the association between mild inflammation and poor outcome. We found that adding PSA to the multivariate model resulted in the greatest shift in HR for mild inflammation, suggesting the worse outcomes noted in univariate analyses were in part attributable to higher PSA levels in this group. While mild inflammation was

consistently linked with adverse pathology and poor outcome, these associations did not reach significance and larger more well-powered studies are needed to better refine the association between the amount of inflammation and PC aggressiveness, as the cut-points in the literature separating mild versus marked disease may not have be optimal.

While several studies attempted to link histological inflammation and PSA levels [13, 14], few have studied histological inflammation in PC specimens. Irani et al. [15] graded inflammation within malignant tissue as high (confluent inflammatory cells or disruption of the glandular epithelium) versus low (all others) in radical prostatectomy specimens. The authors concluded that high-grade inflammation surrounding malignant glands was associated with BCR. This study had a few men with high-grade inflammation: only 15 of 161. Our study was more robust as it included 53, 154, and 86 men in the none, mild, and marked inflammation groups. Another limitation of the Irani et al. study is that it did not examine the association between inflammation and adverse pathological findings, but our study demonstrates that inflammation grade is associated with specific adverse pathologic features on multivariate analysis, providing further evidence to support the hypothesis that intratumoral inflammation is linked with aggressive PC. Another study demonstrated that more CD4+ lymphocytes within PC tissue from radical prostatectomy ($n = 11$) or transurethral resection ($n = 69$) were associated with a higher risk of PC death [16]. Since most patients in this study did not have a prostatectomy, pathologic variables were not assessed.

A recent study of PC specimens obtained at TURP graded inflammation as none, mild, moderate, or severe—very similar to the grading system used in the current study [12]. They found that prostatic intraepithelial neoplasia (PIN), and perhaps moderate/severe chronic inflammation especially in the presence of postatrophic hyperplasia, may be associated with increased risk of PC-specific death. Nonomura et al. examined prostate biopsies of 71 men treated primarily with androgen deprivation therapy for tumor-associated macrophages via CD68 staining. In a manner similar to our study, they counted the macrophages within the tumor, not in surrounding benign tissue. They found that the number of tumor-associated macrophages was associated with PSA failure and progression-free survival. Tumor-associated macrophage number was also associated with higher PSA and clinical T stage [17]. While each of these studies investigated the link between inflammation and aggressive PC by a different approach, they all support the conclusion of our study.

Inflammatory mediators may promote the development of more aggressive PC. Alternatively, more aggressive tumors may be more immunogenic. Pro-inflammatory cytokines, such as IL-1, IL-6, IL-8, and IL-17 as well as TNF-α and TGF-β, are associated with metastatic PC [3]. Perhaps the cell proliferation promoted by these cytokines leads to unchecked cell multiplication. Supporting this view is the observation that reducing inflammation with medications reduces PC risk in some studies [8]. Alternatively, the immune system may react more strongly to more aggressive tumors. When the immune response to metastatic PC is upregulated by an autologous active cellular immunotherapy product, survival is prolonged [18]. Whether the inflammatory cells are the cause or the effect remains to be determined, and thus, we simply conclude from our study that they were associated with advanced disease.

Our study has several limitations. First, this is a retrospective study. The findings will need to be confirmed by prospective trials and expanded by basic science investigations into the molecular mechanisms connecting inflammation and PC. Second, though early BCR correlates with PC-specific survival [19], our findings should be confirmed using more distant end points such as metastasis and survival. Third, we did not distinguish between types of inflammatory cells. Differentiating between acute and chronic inflammation may have prognostic importance and should be investigated by further studies.

Conclusions

Inflammation within PC was associated with advanced disease after radical prostatectomy. This may be due to inflammatory mediators promoting development of aggressive PC. Alternatively, more aggressive tumors may be more immunogenic. These findings corroborate growing evidence linking inflammation and aggressive PC, but prospective studies are needed to determine the clinical utility of inflammation as a prognostic marker of high-risk disease or as a therapeutic target.

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Conflict of interest No conflict of interest exists for any of the authors.

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