Osteopontin and Interleukin-8 Expression is Independently Associated with Prostate Cancer Recurrence

Daniel J. Caruso,1 Adrienne J. K. Carmack,1 Vinata B. Lokeshwar,1,2,3 Robert C. Duncan,4 Mark S. Soloway,1 and Bal L. Lokeshwar1,3,5

Abstract

Purpose: Lack of reliable biomarkers limits accurate prediction of prostate-specific antigen biochemical recurrence (disease progression) in prostate cancer. The two inflammatory chemokines, osteopontin and interleukin-8 (IL-8), are associated with tumor angiogenesis and metastasis. We investigated whether osteopontin and IL-8 expression in prostate cancer correlates with disease progression.

Experimental Design: Archival prostatectomy specimens (n = 103) were obtained from patients with minimum 72-month follow-up. Osteopontin and IL-8 expression was evaluated by immunohistochemistry and graded for intensity and the area. Association of osteopontin and IL-8 staining with biochemical recurrence was evaluated by univariate and multivariate models.

Results: In tumor cells, osteopontin and IL-8 staining was higher in the recurred group (203.2 ± 78.4; 181.1 ± 89.3) than in the nonrecurred group (122.7 ± 76.6; 96.4 ± 85.6; P < 0.001). Higher osteopontin and IL-8 staining was also observed in benign areas adjacent to tumor in the recurred group, than in nonrecurred group. In univariate analysis, except age, all preoperative and postoperative variables and osteopontin and IL-8 staining scores were significantly associated with biochemical recurrence (P < 0.05). In multivariate analysis, margin status and osteopontin staining independently associated with biochemical recurrence within 72 months. Osteopontin, either alone or with IL-8 and seminal vesicle invasion, was a significant variable in predicting biochemical recurrence within 24 months. Osteopontin and IL-8 staining predicted recurrence with high sensitivity (75.5%; 73.6%) and specificity (76%; 70.6%).

Conclusion: In prostatectomy specimens, osteopontin expression is independently associated with biochemical recurrence. Both osteopontin and IL-8 may be predictors of early disease progression.

The number of organ-confined prostate cancer (CaP) cases has significantly increased due to the widespread use of prostate-specific antigen (PSA). Despite careful selection of patients, a substantial percentage of patients with localized CaP will experience disease recurrence after undergoing radical prostatectomy or radiotherapy (1, 2). Although existing variables such as Gleason sum or preoperative PSA provide some prognostic information, it is a challenge estimating prognosis in patients with CaP, considering two-thirds will have a Gleason sum of 5 to 7 and serum PSA levels of 4 to 10 ng/dL. Therefore, there is a need for more accurate prognostic markers to identify the biological potential of the tumor.

Osteopontin, a glycosylated phosphoprotein comprising ~2% of the noncollagenous proteins of the bone (3). It is involved in osteoblastic differentiation and bone formation, as well as, anchorage of osteoclasts to bone and reabsorption of bone (4). Osteopontin is overexpressed in a variety of cancers and is involved in invasion and metastasis (5). High levels of osteopontin expression are associated with a poor prognosis in breast cancer patients (6). High serum-osteopontin levels have been reported in patients with metastatic CaP (7). A recent study causally linked high osteopontin expression with CaP cell proliferation and metastasis (8). Another study showed that increased osteopontin expression correlates with Gleason sum and decreased survival in CaP patients (9). Osteopontin is also an inflammatory chemokine and has been linked to hepatic toxicity and cancer (10).

A causal link between chronic or recurrent inflammation has been suggested for genesis and progression of CaP. Interleukin-8 (IL-8), also known as the CXC ligand-8, is a member of the CXC chemokine family and is a proinflammatory cytokine. IL-8 is a common chemotactic factor regulating pathologic angiogenesis, tumor growth, and metastasis. For example, high level of IL-8 is associated with CaP invasion and metastasis, and inhibition of IL-8 production in experimental
CaP models decreases metastatic potential (11). We have recently shown that IL-8 expression in androgen dependent/ responsive CaP cells induces androgen independence and increased survival when exposed to chemotherapy drugs (12). A study involving a small number of patients has linked high serum level of IL-8 to Gleason sum and pathologic stage of CaP (13). Furthermore, an increased serum IL-8 level was linked to CaP bone metastasis (14). IL-8 gene polymorphism that correlates with elevated IL-8 expression may also be associated with increased risk for development of CaP (15, 16). However, the prognostic potential of IL-8 to predict biochemical recurrence in patients with clinically localized CaP has not been evaluated.

IL-8 and osteopontin have been coexpressed in a variety of tumors, and also, increased IL-8 levels lead to increased osteopontin expression in some benign conditions, suggesting deregulation of IL-8 and osteopontin during disease progression (17). However, such IL-8–mediated increase of osteopontin expression has yet not been reported in tumors. In this study, we investigated osteopontin and IL-8 expression in archival radical prostatectomy specimens from 103 CaP patients with a mean follow-up of 96.3 months. Our results show that both osteopontin and IL-8 expression is elevated in CaP cells and associates with biochemical recurrence.

### Materials and Methods

**Specimens and study patients.** We analyzed 103 CaP specimens obtained from patients who underwent a radical prostatectomy with bilateral pelvic lymphadenectomy between 1992 and 2001. This study was conducted under a protocol approved by the University of Miami’s Institutional Review Board. During 1992 to 2001, a total of 911 patients underwent radical prostatectomy, of which 695 did not receive neoadjuvant androgen deprivation therapy. A minimum 5-y follow-up was available on 381 patients. The pathology resource selected 117 blocks, containing CaP tissues, representing the major Gleason score, seminal vesicle invasion (SVI), and lymph node status. Thus, no special tissue-processing procedures were undertaken for this study. We sectioned specimens for 10 slides from each block. Two slides, one stained for osteopontin and IL-8 staining, were scored. The remaining slides were available for the optimization of osteopontin and IL-8 antibody concentration for staining, determination of nonspecific staining, and repeating the staining in case of any discrepancies. The slides were incubated with either anti-osteopontin or anti–IL-8 antibodies (affinity-purified IgG; Sigma-Aldrich and R&D Systems, respectively) at 4°C.

<table>
<thead>
<tr>
<th>Progression</th>
<th>Age (y)</th>
<th>PSA (ng/mL)</th>
<th>Clinical stage</th>
<th>Gleason sum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical recurrence* (n = 53)</td>
<td>Mean, 63.8 ± 5.9</td>
<td>Mean, 11.5 ± 8.8</td>
<td>T1c, 23 (43.3%)</td>
<td>5 = 1 (1.9%)</td>
</tr>
<tr>
<td></td>
<td>Median, 65.5</td>
<td>Median, 8.9</td>
<td>T2a, 10 (18.9%)</td>
<td>6 = 4 (7.5%)</td>
</tr>
<tr>
<td></td>
<td>Range, 51-73</td>
<td>Range, 1.4-41.3</td>
<td>T2b, 20 (37.7%)</td>
<td>7 = 20 (37.7%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 = 17 (32.1%)</td>
<td>8 = 17 (32.1%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 = 11 (20.8%)</td>
<td>9 = 11 (20.8%)</td>
</tr>
<tr>
<td>No biochemical or clinical recurrence (n = 50)</td>
<td>Mean, 61.7 ± 7.1</td>
<td>Mean, 6.6 ± 3.0</td>
<td>T1c, 32 (64%)</td>
<td>4 = 1 (2%)</td>
</tr>
<tr>
<td></td>
<td>Median, 63</td>
<td>Median, 6.2</td>
<td>T2a, 10 (20%)</td>
<td>5 = 4 (8%)</td>
</tr>
<tr>
<td></td>
<td>Range, 41-75</td>
<td>Range, 0.5-15.5</td>
<td>≥T2b, 8 (16%)</td>
<td>6 = 12 (24%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 = 29 (58%)</td>
<td>7 = 29 (58%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>8 = 3 (6%)</td>
<td>8 = 3 (6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 = 1 (2%)</td>
<td>9 = 1 (2%)</td>
</tr>
</tbody>
</table>

NOTE: The information on the postoperative variables (Gleason sum, margin status, EPE, SVI, and lymph node status) was assessed by pathologic examination of the surgical specimens immediately after surgery, and therefore, these variables are considered as baseline covariates. Three of the patients without recurrence had an unspecified T2 clinical stage.

Abbreviations: EPE, extraprostatic extension of tumor; SVI, seminal vesicle invasion.

*Out of the 53 patients who experienced biochemical recurrence, 47 recurred within 72 mo and 29 recurred within 24 mo.
Table 1. Preoperative and postoperative variables of the study patients (Cont’d)

<table>
<thead>
<tr>
<th>EPE</th>
<th>Margin</th>
<th>SVI</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+) = 32 (60.4%)</td>
<td>(+) = 40 (75.5%)</td>
<td>(+) = 16 (32%)</td>
<td>(+) = 4 (7.6%)</td>
</tr>
<tr>
<td>(-) = 21 (39.6%)</td>
<td>(-) = 13 (26%)</td>
<td>(-) = 37 (69.8%)</td>
<td>(-) = 49 (92.5%)</td>
</tr>
<tr>
<td>(+) = 7 (14%)</td>
<td>(+) = 14 (28%)</td>
<td>(+) = 2 (4%)</td>
<td>(+) = 0 (0%)</td>
</tr>
<tr>
<td>(-) = 43 (86%)</td>
<td>(-) = 36 (72%)</td>
<td>(-) = 48 (56%)</td>
<td>(-) = 50 (100%)</td>
</tr>
</tbody>
</table>

Osteopontin and IL-8 Predict Cancer Recurrence

for 16 h. The concentration of antibodies used for osteopontin and IL-8 staining were 0.25 and 2 µg/mL, respectively. The slides were developed using the Dako LASB kit (DakoCytomation) and 3,3'-diaminobenzidine staining. The slides were counterstained with hematoxylin and mounted, as described before (18). To determine the reproducibility of staining, 25 slides were restained twice and 10 slides were restained thrice and scored independently in a blinded fashion by both readers. For both readers, the differences in the staining scores obtained by restaining the slides twice or thrice were within 10% of the initial staining score.

Slide grading Two readers independently evaluated all slides in a blinded fashion (i.e., the readers were blinded to whether the slides were from the recurred or the nonrecurred group during the staining procedure and also when evaluating the slides). Osteopontin and IL-8 staining of tumor cells in each slide was graded for intensity (0-3+) and percentage of the specimen stained with a particular intensity. A final score was then determined by multiplying the intensity score and the percentage of the specimen. For example, if a specimen exhibited a staining distribution of 50% 2+ and 50% 1+, then the final score is 150 (i.e., 2 × 50 + 1 × 50). Therefore, the weighted scores ranged between 0 and 300. Of the 103 stained slides, there was a discrepancy in 15 slides. These discrepancies were resolved by both readers reexamining those slides simultaneously. In addition, 20% (21 slides) of the slides were also evaluated using the Image Analysis software and these results were comparable with the readers’ scores.

Statistical analysis Based on the availability, in this study, 117 patients per block were selected and 103 were ultimately included in the analysis. Among these 103 patients, 47 had biochemical recurrence within 72 mo after prostatectomy and 56 patients were recurrence free at 72 mo or more. Therefore, based on the way the specimens were collected for analysis, the study design is that of a cohort study. To determine the correlation of each preoperative and postoperative variables, and IL-8 and osteopontin staining scores (analyzed as continuous variables) with biochemical recurrence, we did logistic regression single-variable analysis. Because this is a cohort study, we did the Cox proportional hazard analysis on the entire cohort, for determining the early predictors of progression, Gleason sum was included in the model either as a continuous variable or as a stratified variable (≤8 or ≥8), and the intensity scores of osteopontin and IL-8 were included as continuous variables.

Receiver operating curves were generated to determine the association between staining scores (i.e., for IL-8 or osteopontin) and biochemical recurrence within 72 mo. Cutoff values selected by a statistical program (JMP 6 Software from SAS) were used for defining high- or low-grade expression of osteopontin (cutoff, 160) and IL-8 (cutoff, 138). The program selects the cutoff limit that yields the highest Sensitivity (1-Specificity) value. A staining score above the cutoff value was considered as a true positive if the patient had biochemical recurrence, and the score lower than the cutoff value was considered as a true negative if the patient had no biochemical recurrence. The sensitivity and specificity for osteopontin and IL-8 staining inferences were calculated as described before (19). Monte-Carlo crossvalidation was done to obtain the mean ± SD and 95% CI for the sensitivity and specificity for osteopontin and IL-8 staining scores.

Statistical analyses were carried out using the JMP Software Program (version 6.0; SAS Institute).

Results

Localization of osteopontin and IL-8 in CaP tissues

Because CaP is a slow disease to progress, a longer follow-up is necessary to make clinically meaningful predictions regarding biochemical recurrence. Therefore, in this study, we examined the prognostic potential of osteopontin and IL-8 in archival radical prostatectomy specimens from patients with CaP on whom there was a minimum 72-month follow-up. It is noteworthy that the preoperative and postoperative variables of the patients selected for the study (n = 103) were not significantly different from the total number of patients on whom there was 6-year follow-up (n = 381) or those who were lost to follow-up before 6 years (n = 314). The variables compared were age, mean PSA, Gleason sum (P > 0.05; Mann-Whitney test in each case) and margin status, EPE, and SVI (P > 0.05; χ² test, in each case). A trend-based χ² analysis for
clinical stage ($P > 0.05$; degrees of freedom, 1; $\chi^2$, 1.532) also showed no difference between the group of patients on whom there was 6-year follow-up and those who were lost to follow-up before 6 years. Therefore, the study patients ($n = 103$) were most likely not different from those who were not included in the study.

As shown in Fig. 1A, very little osteopontin staining is observed in specimens from patients with Gleason sum 6, 7, or 8 CaP, who did not have biochemical recurrence ($a$, $c$, and $e$). However, high-grade staining is observed in CaP specimens from patients who had biochemical recurrence ($b$, $d$, and $f$). Figure 1B ($a$, $c$, and $e$) shows low-grade IL-8 staining in specimens from patients with Gleason sum 6, 7, or 8 CaP, who did not have biochemical recurrence. Contrarily, high-grade staining is observed in CaP specimens from patients who had biochemical recurrence (Fig. 1B, $b$, $d$, and $f$).

Figure 1C shows the IL-8 and osteopontin staining intensity scores for both the recurred and nonrecurred patients. The differences in the mean intensity scores among recurred and nonrecurred groups for osteopontin and IL-8 staining were statistically significant ($P < 0.001$; degrees of freedom, 1; unpaired $t$ test).

We also compared the staining intensity in areas adjacent to the tumors, for both IL-8 and osteopontin. As shown in Fig. 1D,
for both osteopontin and IL-8, the normal-benign glands adjacent to the tumor cells show high-grade staining, if the specimen was obtained from a patient who recurred (a and c). However, the normal-benign glands adjacent to tumor cells do not stain for osteopontin or IL-8, if the specimen was obtained from a patient who did not recur (b and d). In the normal and benign glands, the intensity scores (mean ± SD) for osteopontin and IL-8 staining in the recurred group (169.1 ± 89.9 and 157.5 ± 91.6, respectively) were 2.8- and 6.0-fold higher than those in the nonrecurred group (57.5 ± 65 and 27.2 ± 50.7). Logistic regression analysis showed that the differences in the intensity scores of IL-8 and osteopontin staining in normal specimens from patients who had biochemical recurrence and those who did not are statistically significant (IL-8: χ², 11.8; P < 0.001; odds ratio, 1.01; 95% CI, 1.00-1.01; osteopontin: χ², 10.0; P = 0.002; odds ratio, 1.02; 95% CI, 1.01-1.03). In addition, the analyses also showed that the staining intensity in the normal glands correlated with staining intensity in tumor cells for both IL-8 (Spearman r = 0.853; 95% CI, 0.724-0.924; P ≤ 0.001) and osteopontin (Spearman r = 0.862; 95% CI, 0.734-0.931; P ≤ 0.001). This suggests that osteopontin and IL-8 expression in the benign glands associates with biochemical recurrence.

Association of preoperative and postoperative variables and osteopontin/IL-8 staining with biochemical recurrence

Univariate analysis. Because in this study, all patients had minimum 72-month follow-up, we categorized the patients as those who recurred before 72 months and those who did not recur but had minimum 72-month follow-up. We used the logistic regression analysis to determine the association of each of the preoperative (i.e., age, PSA, and clinical stage) and postoperative (i.e., Gleason sum, margin, EPE, SVI, and lymph node status) variables, as well as staining inferences of osteopontin and IL-8, with biochemical recurrence. As shown in Table 2, except age, all other preoperative and postoperative variables, as well as osteopontin and IL-8 staining inferences, were significant in predicting biochemical recurrence.

Multivariate analyses. To determine the smallest number of variables that can independently associate with biochemical recurrence within 72 months, we did multivariate analysis using the Cox proportional hazard model. Initially, we did the Cox analysis using only the preoperative and postoperative

![Table 2. Univariate analysis of preoperative and postoperative variables and immunohistochemical staining inferences](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>χ²</th>
<th>P</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason Sum</td>
<td>15.06</td>
<td>&lt;0.001*</td>
<td>2.72 (1.71-4.74)</td>
</tr>
<tr>
<td>Gleason ≥ 7</td>
<td>10.09</td>
<td>0.001*</td>
<td>1.92 (1.368-2.697)</td>
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<tr>
<td>Gleason &gt; 8</td>
<td>24.69</td>
<td>&lt;0.001*</td>
<td>5.22 (2.056-13.263)</td>
</tr>
<tr>
<td>Age</td>
<td>2.61</td>
<td>0.11</td>
<td>0.98 (0.89-1.01)</td>
</tr>
<tr>
<td>Preoperative PSA</td>
<td>8.27</td>
<td>&lt;0.004</td>
<td>1.13* (1.05-1.25)</td>
</tr>
<tr>
<td>Stage</td>
<td>11.5</td>
<td>&lt;0.001*</td>
<td>2.95 (1.318-6.599)</td>
</tr>
<tr>
<td>EPE</td>
<td>24.14</td>
<td>&lt;0.001*</td>
<td>3.78 (1.89-7.532)</td>
</tr>
<tr>
<td>Positive surgical margin</td>
<td>24.0</td>
<td>&lt;0.001*</td>
<td>2.85 (1.766-4.611)</td>
</tr>
<tr>
<td>SVI</td>
<td>12.52</td>
<td>&lt;0.001*</td>
<td>5.13 (1.371-19.186)</td>
</tr>
<tr>
<td>Lymph node status</td>
<td>3.926</td>
<td>0.0475*</td>
<td>9.18</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>13.43</td>
<td>&lt;0.001*</td>
<td>1.01* (1.00-1.01)</td>
</tr>
<tr>
<td>IL-8</td>
<td>11.91</td>
<td>&lt;0.001*</td>
<td>1.01* (1.00-1.01)</td>
</tr>
</tbody>
</table>

NOTE: Logistic regression single-variable analysis was used to determine the association of preoperative (age, preoperative PSA, and clinical stage) and postoperative (Gleason sum, margin, EPE, SV invasion, and lymph node status) variables and osteopontin and IL-8 staining inferences with biochemical recurrence. *Statistically significant. 1Change in odds ratio per unit change in the variable.

![Table 3. Multivariate analyses of preoperative and postoperative variables and immunohistochemical staining inferences](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>χ²</th>
<th>P</th>
<th>Risk ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No IL-8 and/or osteopontin included (Whole model)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>6.21</td>
<td>0.013</td>
<td>1.59 (1.10-2.34)</td>
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<tr>
<td>No IL-8 and/or osteopontin included (whole model, Gleason categorized)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>6.09</td>
<td>0.014</td>
<td>1.58 (1.10-2.33)</td>
</tr>
<tr>
<td>Gleason categorized</td>
<td>5.06</td>
<td>0.025</td>
<td>1.51 (1.06-2.18)</td>
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<tr>
<td>IL-8 (whole model; Gleason continuous)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>4.6</td>
<td>0.032</td>
<td>1.51 (1.04-2.25)</td>
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<td>IL-8 (Whole Model; Gleason categorized)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>4.75</td>
<td>0.029</td>
<td>1.52 (1.04-2.28)</td>
</tr>
<tr>
<td>Gleason categorized</td>
<td>3.88</td>
<td>0.049</td>
<td>1.46 (1.00-2.13)</td>
</tr>
<tr>
<td>IL-8 (parsimonious model; Gleason categorized)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>10.93</td>
<td>&lt;0.001</td>
<td>1.74 (1.25-2.51)</td>
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<tr>
<td>Gleason categorized</td>
<td>10.84</td>
<td>0.001</td>
<td>1.7 (1.24-2.34)</td>
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<td>Osteopontin (whole model; Gleason continuous)</td>
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</tr>
<tr>
<td>Margin</td>
<td>5.75</td>
<td>0.017</td>
<td>1.55 (1.08-2.29)</td>
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<tr>
<td>Osteopontin (whole model)</td>
<td>7.88</td>
<td>0.005</td>
<td>1.01* (1.00-1.01)</td>
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<td>Osteopontin (whole model; Gleason categorized)</td>
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<tr>
<td>Margin</td>
<td>6.83</td>
<td>0.016</td>
<td>1.55 (1.08-2.29)</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>6.75</td>
<td>0.009</td>
<td>1.00* (1.00-1.01)</td>
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<td>Osteopontin (parsimonious model; Gleason categorized)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>13.51</td>
<td>&lt;0.001</td>
<td>1.78 (1.29-2.54)</td>
</tr>
<tr>
<td>Gleason categorized</td>
<td>6.13</td>
<td>0.013</td>
<td>1.47 (1.08-2.02)</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>9.19</td>
<td>0.002</td>
<td>1.01* (1.00-1.01)</td>
</tr>
<tr>
<td>Osteopontin and IL-8 (whole model; Gleason continuous)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Margin</td>
<td>5.16</td>
<td>0.023</td>
<td>1.52 (1.06-2.25)</td>
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<td>Osteopontin</td>
<td>7.36</td>
<td>0.007</td>
<td>1.01* (1.00-1.01)</td>
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<td>Osteopontin and IL-8 (whole model; Gleason categorized)</td>
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<td>Margin</td>
<td>5.27</td>
<td>0.022</td>
<td>1.53 (1.07-2.26)</td>
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<td>Osteopontin</td>
<td>6.46</td>
<td>0.011</td>
<td>1.01* (1.00-1.01)</td>
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</tr>
<tr>
<td>Margin</td>
<td>12.16</td>
<td>&lt;0.001</td>
<td>1.81 (1.27-2.54)</td>
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<tr>
<td>Osteopontin</td>
<td>8.5</td>
<td>0.004</td>
<td>1.01* (1.00-1.01)</td>
</tr>
<tr>
<td>Gleason ≥ 8</td>
<td>4.75</td>
<td>0.029</td>
<td>1.44 (1.04-2.00)</td>
</tr>
</tbody>
</table>

NOTE: Cox proportional hazard analysis was done by including the following: (a) only the preoperative (i.e., age, PSA, and clinical stage) and postoperative (i.e., Gleason sum, EPE, margin, SV invasion, lymph node status) variables; (b) preoperative and postoperative variables and IL-8 staining score (IL-8 whole model); (c) margin, Gleason sum, and IL-8 staining score (IL-8 parsimonious model); (d) preoperative and postoperative variables and osteopontin staining score (osteopontin whole model); (e) margin, Gleason sum, and osteopontin staining score (osteopontin parsimonious model); (f) preoperative and postoperative variables, and osteopontin and IL-8 staining scores included as individual variables (osteopontin and IL-8 whole model); (g) margin, Gleason sum, and osteopontin and IL-8 staining scores (osteopontin and IL-8 parsimonious model). The whole model analyses were also done by including the Gleason sum as a categorical variable (Gleason sum <8 or ≥8). The significant variables (P < 0.05) selected by the model are shown. In all of the analyses, IL-8, osteopontin, or IL-8 and osteopontin staining scores were included as continuous variables. 1Change in risk ratio per unit change in osteopontin staining score.
variables. As shown in Table 3, in the whole model, when Gleason sum was included as a continuous variable, only the margin status reached statistical significance. However, when Gleason sum was included as a categorized variable (<8 and ≥8) both margin status and Gleason sum reached statistical significance. When Gleason sum was categorized as <7 and ≥7, it did not reach statistical significance. We then determined the statistical significance of the staining scores of IL-8 and osteopontin in the parsimonious model that included all preoperative and postoperative variables along with the staining scores. In the parsimonious model, we included margin status and Gleason sum (categorized as <8 or ≥8) and the staining scores, to determine whether IL-8 or osteopontin staining scores reach statistical significance beyond the margin status and Gleason sum. As shown in Table 3, IL-8 staining score did not reach statistical significance either in the whole model or in the parsimonious model. However, osteopontin staining score along with margin status reached statistical significance in the whole model, regardless of whether Gleason sum was included as a continuous or as a categorized variable (Table 3). In the parsimonious model, all three variables (i.e., margin, Gleason sum categorized, and osteopontin) were found to be statistically significant (Table 3). When both osteopontin and IL-8 staining scores were included in the whole model, once again, margin status and osteopontin staining score reached statistical significance regardless of whether Gleason sum was categorized or not. Categorized Gleason sum, along with margin status and osteopontin staining score, reached statistical significance in the parsimonious model. It is noteworthy that lymph node status did not reach statistical significance in any of the models. The obvious explanation for this is that there were only 4 patients in the entire cohort who had positive lymph node status and all of these patients experienced biochemical recurrence; 3 in <72 months.

**Sensitivity, specificity, accuracy of osteopontin, and IL-8 expression**

To test whether there was any association between IL-8/osteopontin levels and biochemical recurrence, receiver operating characteristic curves were generated for osteopontin and IL-8 staining scores. For IL-8, the area under the receiver operating characteristic curve was 0.7458 (χ², 17.19; P < 0.001), and for osteopontin, it was 0.767 (χ², 18.3; P < 0.001). To increase the observed degree of association, cutoff values were obtained from the receiver operating characteristic curves for IL-8 (138) and osteopontin (160), respectively. These cutoff values were then used for calculating sensitivity and specificity of both markers to predict biochemical recurrence. As shown in Table 4, at 72 months, osteopontin staining has 75.5% sensitivity and 76% specificity to predict biochemical recurrence. The sensitivity (73.6%) and specificity (70.6%) for IL-8 staining are slightly lower than that for osteopontin staining. Although the sensitivity and specificity of osteopontin or IL-8 staining to predict biochemical recurrence within 72 months will require independent confirmation, the crossvalidation results presented in Table 4 strengthen the results somewhat.

We also determined the sensitivity and specificity of combined osteopontin and IL-8 staining inferences, by considering one or both marker positive as a positive inference for the combined marker (either true positive or false positive). The combined osteopontin-IL-8 marker had 95% sensitivity and 65% specificity to predict biochemical recurrence. As shown in Table 4, at 72 months, osteopontin staining has 75.5% sensitivity and 76% specificity to predict biochemical recurrence. However, when both osteopontin and IL-8 staining scores were included as continuous or as a categorized variable (Table 3). In the parsimonious model, all three variables (i.e., margin, Gleason sum categorized, and osteopontin) were found to be statistically significant (Table 3). When both osteopontin and IL-8 staining scores were included in the whole model, once again, margin status and osteopontin staining score reached statistical significance regardless of whether Gleason sum was categorized or not. Categorized Gleason sum, along with margin status and osteopontin staining score, reached statistical significance in the parsimonious model. It is noteworthy that lymph node status did not reach statistical significance in any of the models. The obvious explanation for this is that there were only 4 patients in the entire cohort who had positive lymph node status and all of these patients experienced biochemical recurrence; 3 in <72 months.

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recurrence at 72 months. Monte-Carlo crossvalidation analysis showed 86 ± 8.5% (95% CI, 84.4-88.2%) sensitivity and 60.5 ± 6.5 (95% CI, 59.61.9%) specificity for the combined marker.

**Predictors of early progression**

It has been suggested that biochemical recurrence within 1 to 2 years indicates systemic disease, whereas biochemical recurrence beyond 24 months suggests local recurrence (20). The patients who had biochemical recurrence (n = 53) were categorized as those who recurred before 24 months (n = 29) and those who did not recur before 24 months (n = 24). Cox proportional hazard model, which included only the preoperative and postoperative variables, had no single variable reached statistical significance, regardless of whether Gleason sum was categorized (<8 and ≥8) or included as a continuous variable (Table 5). In the Cox model, which included IL-8 staining score and all of the preoperative and postoperative variables, no variable reached statistical significance regardless of whether Gleason sum is included as a continuous or categorical variable. Contrarily, osteopontin staining score reached statistical significance in the whole model regardless of whether Gleason sum was categorized or included as a continuous variable (Table 5). When both osteopontin and IL-8 staining scores were included in the Cox model (as separate variables), osteopontin together with IL-8 and SVI reached statistical significance (Table 5).

**Discussion**

The majority of the newly diagnosed CaP patients have clinically organ-confined disease (21). Although, nomograms based on preoperative and postoperative variables offer some information about patients’ prognosis, limited knowledge about which CaP is likely to progress, as well as when it will recur severely impedes individualized selection of therapy and subsequent prediction of outcome (22). Recent studies have shown that chemokines, cytokines, and other proteins that are associated with inflammatory processes may function in promoting tumor invasion and metastasis (22–23). Furthermore, some of these molecules, such as cyclooxygenase-2, have shown prognostic potential for predicting CaP progression (18). In this study, with a minimum of 6 years on all patients, we show that the expression of osteopontin, in radical prostatectomy specimens, is independently associated with biochemical recurrence. Furthermore, osteopontin and IL-8 may be early predictors of disease progression.

In prostate tissues, osteopontin levels have been shown to be elevated in carcinoma when compared with normal and benign prostatic hyperplasia tissues (9). Consistent with these findings, we found that benign prostate specimens stained for osteopontin with low intensity. Furthermore, we observed that osteopontin staining correlated with Gleason sum (P < 0.001; logistic regression analysis). This may explain why osteopontin staining is included in the multivariate model, Gleason sum did not reach statistical significance in predicting biochemical recurrence even when included as a categorized (<8 and ≥8). Although the overexpression of osteopontin has been previously shown to correlate with poor survival, our study is the first to report that high osteopontin staining may indicate biochemical recurrence in the future. Osteopontin was found to be independently associated with biochemical recurrence within 72 months. The high sensitivity and specificity (~76%) of osteopontin staining to predict biochemical recurrence also suggest that it may be a clinically useful marker for predicting biochemical recurrence. The observation that high intensity staining of normal glands surrounding tumor cells for both IL-8 and osteopontin proteins is an indication of the possible role of the paracrine induction of proinflammatory factors in the development of reactive stroma, which in turn, promotes tumor progression (25).

The expression of IL-8 mRNA has been shown to correlate with Gleason sum and pathologic stage (13), but whether it independently associates with biochemical recurrence has not been evaluated. In our study, IL-8 expression correlated with Gleason sum (P = 0.019; logistic regression analysis) but not with clinical stage (P = 0.8). Furthermore, IL-8 staining also does not independently associate with biochemical recurrence within 72 months.

It is noteworthy that among the group of CaP patients who recurred, osteopontin was able to distinguish between those who recurred before 24 months and those recurring after 24 months in the multivariate model. Although IL-8 staining score, when included by itself in the multivariate model, was not an early predictor of biochemical recurrence, when included in the model along with the osteopontin staining, it did contribute to recognizing those patients who will have early biochemical recurrence and, hence, may be at risk for systemic disease. This result is consistent with the role of osteopontin and IL-8 in cancer metastasis and progression.

Taken together, our study shows that osteopontin in radical prostatectomy specimens is independently associated with CaP progression, i.e., biochemical recurrence, and when combined with margin status, can stratify patients into different risk categories for developing biochemical recurrence within 6 years. In addition, osteopontin either alone or together with IL-8 and SVI may be able to stratify patients at risk for early disease progression.

**Conclusion**

Osteopontin and IL-8 expression is higher in radical prostatectomy specimens from patients who experience biochemical recurrence within 72 months. Osteopontin and, to a lesser extent, IL-8 expression are independently associated with biochemical recurrence in CaP patients and may have potential to predict early disease progression.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
References

Osteopontin and Interleukin-8 Expression is Independently Associated with Prostate Cancer Recurrence


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