Serum Interleukin 6 as a Prognostic Factor in Patients with Prostate Cancer

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ABSTRACT
The present study was undertaken to evaluate the prognostic significance of the serum levels of interleukin 6 (IL-6) in patients with prostate cancer. Serum IL-6 levels were measured in 74 patients with prostate cancer. The tumor was stage B in 23 patients, stage C in 14 patients, and stage D in 37 patients. Prognostic significance of tumor histology, performance status (PS), bone metastasis, serum prostate-specific antigen (PSA) level, serum alkaline phosphatase (ALP) level, serum dehydrogenase level, serum IL-6 levels, and hemoglobin on disease-specific survival was assessed using univariate and multivariate Cox's proportional hazards model analyses. Serum IL-6 was significantly correlated with the clinical stage of prostate cancer. Univariate analysis of all patients demonstrated that an extent of disease (EOD) on bone scanning ≥1, IL-6 ≥7 pg/ml, PSA ≥1, PSA >100 ng/ml, and ALP >620 IU/liter were associated with a significantly lower survival rate than their respective counterparts. In multivariate analysis, however, the only two significant prognostic factors were EOD and IL-6. In 51 patients with stage C and stage D prostate cancer, univariate analysis showed that EOD ≥1, IL-6 ≥7 pg/ml, PSA ≥1, PSA >100 ng/ml, LDH >200 IU/liter, and ALP >620 IU/liter were significantly related to survival, whereas multivariate analysis again demonstrated that EOD ≥1 and IL-6 ≥7 pg/ml were significant prognostic factors. These results indicate that the serum IL-6 level is a significant prognostic factor for prostate cancer as well as EOD.

INTRODUCTION
IL-6 is a pleiotropic cytokine that was originally identified as a T cell-derived lymphokine inducing final maturation of B cells into antibody-producing cells (1, 2). It has a variety of effects on hematopoiesis, the immune system, and acute-phase responses (3). IL-6 has been shown to regulate cell growth (1). It stimulates the growth of myeloma/plasmacytoma cells and lymphoma cells, while inhibiting the growth of myeloid leukemia cells and breast carcinoma cells. It has also been reported that IL-6 can act as an autocrine growth factor in malignancy (1). Elevated serum IL-6 levels have been associated with morbidity and disease activity in a variety of chronic diseases, including human immunodeficiency virus infection, arthritis, psoriasis, inflammatory bowel disease, and Castleman’s disease (4). Furthermore, it has been reported that human prostate cancer cells produce IL-6 (5) and that serum levels of IL-6 are elevated in patients with prostate cancer (5). Twillie et al. (4) have measured serum IL-6 in patients with advanced, hormone-refractory prostate cancer and have suggested that IL-6 is a prostate exocrine gene product, a candidate mediator of prostate cancer morbidity, and a candidate marker of disease activity. No study, to date, however, has investigated the association between serum IL-6 levels and survival in patients with untreated prostate cancer. Accordingly, the present study was undertaken to evaluate the prognostic significance of serum IL-6 in patients with prostate cancer.

MATERIALS AND METHODS
Seventy-four patients with prostate cancer, ages 57–91 years (73.3 ± 0.88 years), were examined in the present study. The present study was from a community-based urology practice. No patients had evidence of active infection or inflammatory disease, and none were under any treatment for prostate cancer at the time of examination. The diagnosis of prostate cancer was confirmed by needle biopsy or by transurethral resection of the prostate. Sixteen patients had well differentiated adenocarcinoma, 48 patients had moderately differentiated adenocarcinoma, and 10 patients had poorly differentiated adenocarcinoma. The staging procedures included clinical examination, i.v. pyelography, bone scanning, and computed tomography and/or ultrasonography and/or endorectal magnetic resonance imaging and/or magnetic resonance imaging of the abdomen and pelvic cavity. Bone metastases were detected by bone scintigraphy after i.v. injection of 25 mCi Tc-99m methylene diphosphonate. Bone lesions were graded based on the

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1 The abbreviations used are: IL-6, interleukin 6; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; PS, performance status; EOD, extent of disease; PSA, prostate-specific antigen; Hb, hemoglobin; ESR, erythrocyte sedimentation rate.
number of metastases identified on the bone scan according to the EOD system devised by Soloway et al. (6). The grades were: EOD 0, normal or abnormal due to benign bone disease; EOD 1, < 6 metastases; EOD 2, 6–20 metastases; EOD 3, >20 metastases but not a superscan; and EOD 4, superscan. The tumor was stage B in 23 patients, stage C in 14 patients, and stage D in 37 patients.

Serum levels of PSA were assayed by E-Test Tosoh II PA. Serum ALP was measured using 4-nitrophenyl-phosphate as a substrate, and serum LDH was measured using i-lactate as a substrate. Blood for the measurement of serum IL-6 was collected into nonheparinized tubes, and serum was separated within 1 h of blood collection. The serum was stored at −80°C and then thawed just prior to testing. IL-6 determination was performed according to the manufacturer’s instructions using an R&D Systems Quantikine enzyme-linked immunoadsorbent assay kit. The interval between the measurement of the serum IL-6 level and the initiation of treatment was 0–90 days (median, 23 days).

Seven of 23 patients with stage B disease were initially treated with radiation therapy or radical prostatectomy, and the remaining 16 patients were treated with endocrine therapy, using castration (medical castration using a luteinizing hormone-releasing hormone analogue or surgical castration) with or without antiandrogens. All of the patients with stage C and stage D disease were treated using immediate endocrine therapy. No local treatment was performed for stage C patients. The median time of follow-up and the follow-up range were 41 months and 8–85 months, respectively.

The patients were arbitrarily divided into groups according to tumor histology (well and moderately differentiated adenocarcinoma versus poorly differentiated adenocarcinoma), PS (PS = 0 versus PS ≥1), bone scan findings (EOD 0 versus EOD ≥1 or EOD =1 versus EOD ≥2), pretreatment serum PSA levels (<100 ng/ml versus ≥100 ng/ml), pretreatment serum ALP levels [less than or equal to twice the upper limit of normal (≥620 IU/liter) versus greater than twice the upper limit of normal (>620 IU/liter)], pretreatment serum LDH levels [less than or equal to the upper limit of normal (≥200 IU/liter) versus greater than the upper limit of normal (>200 IU/liter)], pretreatment serum IL-6 levels (<7 pg/ml versus ≥7 pg/ml), and pretreatment Hb levels (Hb >12 g/dl versus Hb ≤12 g/dl).

Variables of two different groups were compared using Student’s t test. The influence of tumor histology, PS, bone metastasis, serum PSA, serum ALP, serum LDH, serum IL-6, and Hb on disease-specific survival was assessed. Survival curves were constructed by the Kaplan-Meier method. Cox’s proportional hazards model was used for univariate and multivariate analyses of survival. To obtain a multivariate model with the maximum precision of the important variables, a stepwise selection procedure was used. In all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

The mean serum level of IL-6 in all patients with prostate cancer was 7.20 ± 2.18 pg/ml. The IL-6 level in patients with stages B, C, and D prostate cancer was 2.56 ± 0.41 pg/ml, 2.54 ± 0.47 pg/ml, and 11.85 ± 4.25 pg/ml, respectively, whereas the serum PSA level in these respective groups was 50.12 ± 13.88 ng/ml, 62.94 ± 12.77 ng/ml, and 660.07 ± 207.44 ng/ml, respectively. Serum IL-6 levels in patients with well, moderately, and poorly differentiated adenocarcinoma were 5.33 ± 2.03 pg/ml, 6.37 ± 2.47 pg/ml, and 14.16 ± 10.79 pg/ml, respectively.

The patients were arbitrarily divided into two groups according to the serum IL-6 level (≥7 pg/ml versus <7 pg/ml). The former group was 72.67 ± 2.60 years old, and the latter group was 73.63 ± 0.96 years old, and the two groups were similar with respect to age ($P = 0.727$). The serum PSA, LDH, and ALP levels in the patients with serum IL-6 levels ≥7 pg/ml were 1059.88 ± 587.13 ng/ml, 234.88 ± 59.92 IU/liter, and 790.40 ± 278.80 IU/liter, respectively, which were significantly higher than those in patients with serum IL-6 levels <7 pg/ml (260.27 ± 90.86 ng/ml, 167.79 ± 5.44 IU/liter, and 324.47 ± 43.97 IU/liter, respectively; $P < 0.05$).

To identify the variables of potential prognostic significance in all of the patients with prostate cancer, univariate analysis of each variable was performed in relation to the survival time. The difference in prognosis was assessed by examining the relative hazard and $P$ for each variable. This analysis demonstrated that patients with EOD ≥1, IL-6 ≥7 pg/ml, PSA ≥100 ng/ml, and ALP >620 IU/liter had a significantly lower survival rate than their respective counterparts ($P < 0.05$; Table 1). Tumor histology, LDH, and the Hb

### Table 1 Relative hazards of survival in univariate and multivariate analyses in all patients

<table>
<thead>
<tr>
<th></th>
<th>Relative hazards</th>
<th>$P$</th>
<th>95% confidence interval</th>
<th>$P$</th>
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<tr>
<td>PS ≥1</td>
<td>3.704</td>
<td>0.0030</td>
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<tr>
<td>EOD ≥1</td>
<td>11.236</td>
<td>&lt;0.0001</td>
<td>3.247–30.303</td>
<td>&lt;0.0001</td>
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<tr>
<td>Poorly</td>
<td>2.066</td>
<td>0.1598</td>
<td></td>
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</tr>
<tr>
<td>IL-6 ≥7 pg/ml</td>
<td>4.950</td>
<td>0.0009</td>
<td>2.874–10.11</td>
<td>0.0476</td>
</tr>
<tr>
<td>PSA ≥100 ng/ml</td>
<td>6.993</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP &gt;620 IU/liter</td>
<td>7.299</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb &lt;12 g/dl</td>
<td>1.691</td>
<td>0.1789</td>
<td></td>
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</tr>
<tr>
<td>LDH &gt;200 IU/liter</td>
<td>1.575</td>
<td>0.3547</td>
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### Table 2 Relative hazards of survival in univariate and multivariate analyses in patients with stages C and D prostate cancer

<table>
<thead>
<tr>
<th></th>
<th>Relative hazards</th>
<th>$P$</th>
<th>95% confidence interval</th>
<th>$P$</th>
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<td>PS ≥1</td>
<td>3.145</td>
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<tr>
<td>EOD ≥1</td>
<td>11.905</td>
<td>0.0009</td>
<td>9.709–21.69</td>
<td>0.0029</td>
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<tr>
<td>Poorly</td>
<td>2.667</td>
<td>0.0956</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 ≥7 pg/ml</td>
<td>6.897</td>
<td>0.0008</td>
<td>3.676–11.17</td>
<td>0.0249</td>
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<td>PSA ≥100 ng/ml</td>
<td>8.130</td>
<td>0.0009</td>
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<tr>
<td>ALP &gt;620 IU/liter</td>
<td>5.650</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb &lt;12 g/dl</td>
<td>1.889</td>
<td>0.1744</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt;200 IU/liter</td>
<td>3.185</td>
<td>0.0233</td>
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value were not significantly correlated with the prognosis in this univariate analysis. The relative importance of each variable was then determined by multivariate Cox’s proportional hazards model analysis. Stepwise inclusion of variables in the model showed that the significant prognostic factors were EOD and IL-6 (Table 1).

In 51 patients with stage C and stage D prostate cancer, univariate analysis showed that EOD ≥1, IL-6 ≥7 pg/ml, PS ≥1, PSA >100 ng/ml, ALP >620 IU/liter, and LDH >200 IU/liter were significantly related to survival (Table 2). Cox’s proportional hazards analysis using a stepwise inclusion of variables demonstrated that EOD ≥2 and IL-6 ≥7 pg/ml were significant prognostic factors (Table 2).

In 37 patients with stage D prostate cancer, univariate analysis showed that EOD ≥2, IL-6 ≥7 pg/ml, PSA >100 ng/ml, and ALP >620 IU/liter were significantly related to survival (Fig. 1). Cox’s proportional hazards analysis using a stepwise inclusion of variables demonstrated that EOD ≥2 and IL-6 ≥7 pg/ml were significant prognostic factors (Table 3).

**DISCUSSION**

Human prostate cancer cell lines secrete IL-6 in vitro (4), and prostate cancer explants also secrete IL-6 (4). More recently, it has been demonstrated that IL-6 acts as a paracrine growth factor and as an autocrine growth factor for prostate cancer cells (7). Aside from prostate cancer, it has been reported that IL-6-induced lymphoblastoid tumorigenicity is due possibly to the inhibitory effect on tumor immunity of very high concentrations of this cytokine (8) and that natural killer cell dysfunction induced by IL-6 production from tumor cells is a novel mechanism of tumor escape from immune surveillance (8). Therefore, it seems possible that IL-6 stimulates the growth of prostate cancer cells via autocrine and/or paracrine mechanisms and might contribute to tumor escape from immune surveillance, resulting in disease progression through the increased production of IL-6.

Furthermore, it has been reported that serum levels of IL-6 are elevated in patients with malignant tumors, including patients with prostate cancer (5, 9). Patients with metastatic non-small cell lung cancer (10) and patients with metastatic renal cell carcinoma (11, 12) have higher serum IL-6 levels than those without disseminated disease. On the other hand, it has been reported that in patients with epithelial ovarian cancer, serum IL-6 levels were not correlated with tumor stage (13). In the present study, serum IL-6 levels were significantly higher in patients with stage D prostate cancer than in patients with stage

**Table 3** Relative hazards of survival in univariate and multivariate analyses in patients with stage D prostate cancer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate</th>
<th>Multivariate</th>
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<tr>
<td></td>
<td>Relative hazards</td>
<td>P</td>
</tr>
<tr>
<td>PS ≥1</td>
<td>2.513</td>
<td>0.0681</td>
</tr>
<tr>
<td>EOD ≥2</td>
<td>5.587</td>
<td>0.0031</td>
</tr>
<tr>
<td>Poorly</td>
<td>2.667</td>
<td>0.0950</td>
</tr>
<tr>
<td>IL-6 ≥7 pg/ml</td>
<td>4.348</td>
<td>0.0106</td>
</tr>
<tr>
<td>PSA ≥100 ng/ml</td>
<td>5.988</td>
<td>0.0184</td>
</tr>
<tr>
<td>ALP &gt;620 IU/liter</td>
<td>4.202</td>
<td>0.0046</td>
</tr>
<tr>
<td>Hb &lt;12 g/dl</td>
<td>1.909</td>
<td>0.2011</td>
</tr>
<tr>
<td>LDH &gt;200 IU/liter</td>
<td>2.183</td>
<td>0.1386</td>
</tr>
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</table>

**Fig. 1** Disease-specific survival according to EOD, IL-6, PSA, and ALP in patients with stage D prostate cancer. Patients with an EOD ≤1 showed a significantly shorter survival than those with an EOD ≥2 (top left). Patients with a serum IL-6 level ≥7 pg/ml showed a significantly shorter survival than those with a serum IL-6 level <7 pg/ml (top right). Patients with a serum PSA level >100 ng/ml showed a significantly shorter survival than those with a serum PSA level ≤100 ng/ml (bottom left). Patients with a serum ALP level >620 IU/liter showed a significantly shorter survival than those with a serum ALP level ≤620 IU/liter (bottom right).
B and stage C prostate cancer, supporting the previous report that patients with metastatic prostate cancer had significantly elevated serum IL-6 levels compared with other prostate cancer patients (5).

It has been reported that elevated serum IL-6 levels are associated with a poor prognosis in patients with metastatic renal cell carcinoma (11), in patients with non-small cell lung cancer (10), and in patients with ovarian cancer (14). In addition, esophageal squamous cell carcinoma patients with serum IL-6 levels $\geq 7$ pg/ml had a significantly lower survival rate than those with serum levels $< 7$ pg/ml (15). On the other hand, in patients with epithelial ovarian cancer, serum IL-6 levels were not correlated statistically with overall survival (13). Therefore, the prognostic significance of IL-6 remains to be fully elucidated in patients with malignancy. To our knowledge, no previous study has investigated the prognostic significance of this cytokine in patients with prostate cancer.

In the present study, univariate analysis demonstrated that EOD, IL-6, PSA, and ALP were associated with a poor prognosis in stage D prostate cancer patients. It was reported that in univariate analysis, ALP, ESR, and EOD were significantly related to prostate cancer death in patients with bone metastases (16). Matzkin et al. (17) have reported that in a univariate analysis PS, EOD, Hb, ALP, and PSA were all significant prognostic factors in patients with metastatic prostate cancer, whereas multivariate analyses showed that only ALP and Hb were significant prognostic variables. On the other hand, it has been reported that multivariate analyses showed ALP and PS to be significant prognostic factors in patients with metastatic prostate cancer (18), whereas Hb was not shown to be a significant prognostic factor (18, 19). In the present study, multivariate analyses showed that PS, ALP, PSA, and Hb were all insignificant as prognostic variables. This may be partly because these variables probably reflect the metastatic burden and interact with EOD and partly because most patients with prostate cancer now begin therapy before a decrease in PS, as suggested previously (20), and probably a decrease in Hb. Johansson et al. (19) have reported that metastasis, tumor stage, and ESR are significantly related to survival on multivariate analysis in patients with advanced prostate cancer. However, the clinical relevance of ESR is unclear (19), and they have no clear hypothesis on what aspect of tumor behavior the ESR reflects (19).

It is interesting to note that IL-6 induces the synthesis and secretion of acute-phase proteins such as fibrinogen and C-reactive protein by hepatocytes (21) as well as indirectly increasing the $\alpha_2$-globulin levels, resulting in an increase of the ESR. In addition, serum IL-6 levels have been reported to show a significant correlation with ESR (12). In the present study, the variables with a significant influence on survival in patients with advanced prostate cancer according to multivariate analysis were EOD and serum IL-6, which may explain the previous report that ESR is an indicator of death in patients with advanced prostate cancer (22).

These results indicate that the serum IL-6 level may be associated with the prognosis of patients with prostate cancer, suggesting that IL-6 and EOD are both prognostic factors that can be used to identify patients with a poor prognosis who may benefit from more aggressive management.

REFERENCES


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