Association between Tumor Necrosis Factor in Serum and Cachexia in Patients with Prostate Cancer

Jun Nakashima, Masaaki Tachibana, Munehisa Ueno, Akira Miyajima, Shiro Baba, and Masaru Murai

Department of Urology, Keio University School of Medicine, Shinjukuku, Tokyo 160, Japan

ABSTRACT

The present study was undertaken to evaluate the relationship between serum tumor necrosis factor (TNF) and cachexia in patients with prostate cancer. TNF levels were determined in 110 serum samples from prostate cancer patients by an enzyme immunoassay. Serum TNF activity was positive in 76% of the patients with relapsed disease, whereas only 11% of the untreated patients and 0% of the patients in remission as a result of endocrine therapy were positive. The serum total protein and albumin levels, hemoglobin levels, and body mass index of the patients with elevated serum TNF levels were significantly lower (P < 0.05) than the corresponding values in patients with undetectable serum TNF levels. The serum TNF levels of patients with serum albumin levels of <3.5 g/dl, serum total protein levels of <7.0 g/dl, hemoglobin levels of <11.0 g/dl, and a body mass index of <21 kg/m² were significantly higher (P < 0.05) than the values in their respective counterparts. There was a significant correlation between the detectability of serum TNF and performance status (P < 0.05). Patients with elevated serum TNF levels had a significantly higher mortality rate (P < 0.05) than those with undetectable serum TNF levels. These findings suggest that TNF may be one of the factors contributing to the complex syndrome of cachexia in patients with prostate cancer.

INTRODUCTION

Because wasting and weight loss are among the most common systemic complications of cancer, an understanding of the mechanism of cancer cachexia would obviously benefit a large number of patients. Cancer cachexia is characterized by weight loss, anorexia, anemia, metabolic abnormalities, wasting, and eventual death. It has been reported that the rapid wasting seen in cancer patients is due to an imbalance between metabolic demands and inadequate dietary replenishment (1). Many patients with prostate cancer develop bone metastases and have a decreased PS. Prostate cancer with progression is often associated with a debilitating state of anorexia, weight loss, and accelerated malnutrition called cachexia, even if the progression site is bone alone. Although the involvement of a variety of mechanisms has been proposed in the pathogenesis of cachexia, the pathophysiology of cachexia in cancer patients remains to be elucidated. TNF was discovered in 1975 as a factor in the serum of Bacillus Calmette-Guérin-primed endotoxin-treated animals that was capable of eliciting hemorrhagic necrosis of tumors (2). Later, Beutler et al. (3, 4) isolated cachectin, a factor presumed to be involved in the pathogenesis of cachexia, and showed TNF to be a molecule homologous to cachectin. The present study was designed to investigate the relationship between serum TNF and cachexia in patients with prostate cancer.

PATIENTS AND METHODS

Patient Population. One hundred ten samples from patients with prostate cancer were examined in the present study. No patient had evidence of an active infection or inflammatory disease at the time of examination. The diagnosis of prostate cancer was histologically confirmed by examination of needle biopsy or transurethral resection specimens in every case. Twenty-two patients had well-differentiated adenocarcinoma, 63 had moderately differentiated adenocarcinoma, and 25 had poorly differentiated adenocarcinoma. There were 63 patients with untreated disease, 26 patients in remission as a result of endocrine therapy, and 21 patients with relapsed bone metastatic disease. The staging evaluation of the 63 patients with untreated disease revealed stage A in 3 patients, stage B in 21 patients, stage C in 12 patients, and stage D in 27 patients. The criteria for remission included any of the following: (a) reduction or disappearance of tumor masses; or (b) decrease in the number, size, or relative intensity of metastatic areas on successive bone scans. In addition, there must be no new sites of disease and no deterioration in the symptoms or PS. Any of the following events was considered evidence of tumor progression: (a) the appearance of any new metastasis; (b) increase in the number, size, or relative intensity of metastatic areas on successive bone scans; or (c) significant cancer-related deterioration in symptoms or PS.

TNF Assay and Laboratory Studies. Blood specimens were collected in nonheparinized tubes, and sera were separated for TNF assay. Serum TNF activity was determined with an
enzyme immunoassay specific for human TNF (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). Standard solutions (100 ml) containing purified recombinant human TNF-α or serum were incubated with 100 ml of anti-rHu-TNF-α antibody at 37°C for 1 h. β-galactosidase-labeled rHu-TNF-α solution (200 ml) was added and goat antirabbit IgG was used to separate bound- from free-labeled antigen. After addition of the substrate (2-nitrophenyl-β-D-galactopyranoside), absorbance at 410 nm was measured, and the concentration of TNF in the samples was determined by referring to the standard curve. The lower limit of detection with this assay system is 0.29 units/ml.

Laboratory studies included analysis of serum TP, albumin and total cholesterol in 110 samples, and the Hgb concentration and hematocrit in 108 samples. PS was assessed in accordance with the Eastern Cooperative Oncology Group performance assessment scale. BMI was calculated by using the formula: weight (kg)/height² (m²).

**Statistical Analysis.** Data on PS, BMI, serum albumin, serum TP, serum total cholesterol, Hgb concentration, and TNF are reported as mean values ± SE. TNF levels that were nondetectable were assigned a value equal to the lower limit of detection for the assay. The patients with prostate cancer were divided into two groups on the basis of their serum TNF levels. Variables of different groups were compared by using the Mann-Whitney test. The independence of fit of categorical data were analyzed by the χ² test. The Spearman-rank correlation test was used to evaluate the correlation between the variables. A Kaplan-Meier survival analysis was carried out in patients with detectable serum TNF levels and with undetectable serum TNF levels. The log-rank test was used to evaluate differences between the two survival curves. P < 0.05 was considered statistically significant.

**RESULTS**

Twenty three of the 110 samples had detectable serum levels of TNF; in 87 samples, TNF levels were undetectable. The serum TNF activity was positive in 16 (76%) of the 21 patients with relapsed disease, 7 (11%) of the 63 untreated patients, and none (0%) of the 26 patients in remission as a result of endocrine therapy. The seven untreated patients included none (0%) of the 3 stage-A patients, 2 (10%) of the 21 stage-B patients, 1 (8%) of the 12 stage-C patients, and 4 (15%) of the 27 stage-D patients (Table 1). There was a significant association between the disease status and the positive rate of the serum TNF activity (P < 0.05). The two groups (87 samples and 23 samples) were similar with respect to the age of the patients (72.74 ± 0.88 years in patients with undetectable serum TNF levels; 75.52 ± 1.66 years in patients with detectable serum TNF levels).

In patients who had elevated serum TNF levels, serum TP and albumin levels were 6.41 ± 0.17 g/dl (n = 23) and 3.29 ± 0.12 g/dl (n = 23), respectively, and were significantly lower (P < 0.05) than in patients with undetectable serum TNF levels [TP, 7.17 ± 0.06 g/dl (n = 87); albumin, 3.85 ± 0.05 g/dl (n = 87); Fig. 1]. Patients with serum albumin levels lower than 3.5 g/dl and serum TP levels lower than 7.0 g/dl had significantly lower survival rates than others.

**Table 1** Disease status and parameters

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>TNF (ng/ml)</th>
<th>TP (g/dl)</th>
<th>Hgb (g/dl)</th>
<th>PS</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>range</td>
<td>mean ± SE</td>
<td>mean ± SE</td>
<td>mean ± SE</td>
<td>mean ± SE</td>
<td></td>
</tr>
<tr>
<td><strong>Un treated patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>2–7.1</td>
<td>2.19 ± 0.09</td>
<td>11</td>
<td>7.19 ± 0.06</td>
<td>13.14 ± 0.26</td>
</tr>
<tr>
<td>Stage A</td>
<td>3</td>
<td>2–2</td>
<td>2 ± 0</td>
<td>0</td>
<td>7.30 ± 0.50</td>
<td>14.13 ± 1.04</td>
</tr>
<tr>
<td>Stage B</td>
<td>21</td>
<td>2–7.1</td>
<td>2.28 ± 0.24</td>
<td>10</td>
<td>7.22 ± 0.11</td>
<td>13.04 ± 0.48</td>
</tr>
<tr>
<td>Stage C</td>
<td>12</td>
<td>2–3.25</td>
<td>2.10 ± 0.10</td>
<td>8</td>
<td>7.25 ± 0.10</td>
<td>12.80 ± 0.56</td>
</tr>
<tr>
<td>Stage D</td>
<td>27</td>
<td>2–3.85</td>
<td>2.18 ± 0.09</td>
<td>15</td>
<td>7.14 ± 0.09</td>
<td>13.24 ± 0.41</td>
</tr>
<tr>
<td>Patients with remission as a result of endocrine therapy</td>
<td>26</td>
<td>2–2</td>
<td>2 ± 0</td>
<td>0</td>
<td>7.08 ± 0.11</td>
<td>12.13 ± 0.40</td>
</tr>
<tr>
<td>Patients with relapsed bone metastatic disease after endocrine therapy</td>
<td>21</td>
<td>2–8.14</td>
<td>4.37 ± 0.45</td>
<td>76</td>
<td>6.37 ± 0.20</td>
<td>9.46 ± 0.45</td>
</tr>
</tbody>
</table>

a TNF positive rate, the % of patients with detectable TNF levels.

b Endocrine therapy included castration with antiandrogens and luteinizing hormone-releasing hormone analogue with antiandrogens.
Fig. 2 Scatter plot of serum levels of TNF and serum albumin levels. A significant correlation was found between the serum TNF levels and the albumin levels (P < 0.05 by Spearman rank correlation test).

Fig. 4 Scatter plot of serum levels of TNF and Hgb levels. A significant correlation was found between the serum TNF levels and the Hgb levels (P < 0.05 by Spearman rank correlation test).

Fig. 3 The relationship between detectability of serum TNF and Hgb levels. Hgb levels in patients with elevated serum TNF levels and with undetectable serum TNF levels were 10.21 ± 0.58 g/dl (n = 23) and 12.71 ± 0.23 g/dl (n = 85), respectively. The difference is statistically significant (P < 0.05).

Fig. 5 The relationship between serum levels of TNF and PS. Serum TNF levels in PS ≥ 1 and PS0 patients were 4.00 ± 0.40 units/ml (n = 28) and 2.07 ± 0.03 units/ml (n = 80), respectively. The difference is statistically significant (P < 0.05).

Higher (P < 0.05) serum TNF levels [3.41 ± 0.36 units/ml (n = 31) and 3.06 ± 0.26 units/ml (n = 47), respectively] than the patients with serum albumin levels of 3.5 g/dl or higher and serum TP levels of 7.0 g/dl or higher [2.23 ± 0.09 units/ml (n = 79) and 2.19 ± 0.09 units/ml (n = 63), respectively; Fig. 2]. On the other hand, serum total cholesterol levels did not differ in the patients with detectable serum TNF levels and with undetectable serum TNF levels [177.18 ± 9.51 mg/dl (n = 23) versus 186.55 ± 3.77 mg/dl (n = 87); P = 0.256].

Patients with elevated serum TNF levels had significantly (P < 0.05) lower Hgb [10.21 ± 0.58 g/dl (n = 23) versus 12.71 ± 0.23 g/dl (n = 85)] and hematocrit values [30.75 ± 1.62% (n = 23) versus 38.01 ± 0.63% (n = 85)] than the patients with undetectable serum TNF levels (Fig. 3). The patients with Hgb concentrations below 11.0 g/dl had significantly higher serum TNF levels [3.47 ± 0.33 units/ml (n = 37)] than the patients with Hgb concentrations of 11.0 g/dl or higher [2.10 ± 0.04 units/ml (n = 71); P < 0.05; Fig. 4].

There was a significant association between the detectability of the serum TNF and the PS (P < 0.05). The patients with detectable serum TNF levels displayed significantly greater impairment of PS [2.35 ± 0.34 (n = 23)] than the patients with undetectable serum TNF levels [0.20 ± 0.07 (n = 85)]. The serum TNF levels of the patients showing PS1 or higher were 4.00 ± 0.40 units/ml (n = 28), and significantly higher (P < 0.05) than those of the PS0 patients [2.07 ± 0.03 units/ml (n = 80); Fig. 5].
The BMI of the patients with detectable serum TNF levels was significantly lower ($P < 0.05$) than that of the patients with undetectable serum TNF levels ($20.24 \pm 0.59$ kg/m$^2$ ($n = 20$) versus $22.60 \pm 0.28$ kg/m$^2$ ($n = 79$); Fig. 6). The serum TNF levels of patients who had a BMI lower than 21 kg/m$^2$ were $3.07 \pm 0.30$ units/ml ($n = 33$) and were significantly higher ($P < 0.05$) than in the patients who had a BMI of 21 kg/m$^2$ or higher ($2.25 \pm 0.11$ units/ml ($n = 66$); Fig. 7).

Significant correlations ($P < 0.01$) were found between the serum TNF levels and the albumin levels ($r = -0.395$; Fig. 2), TP levels ($r = -0.389$), Hgb concentrations ($r = -0.393$; Fig. 4), hematocrit ($r = -0.399$), PS ($r = 0.661$), and BMI ($r = -0.338$; Fig. 7). Ten (43.5%) of the 23 TNF-positive patients died within 3 months after determination of their TNF levels versus only 3 (3.4%) of the 87 TNF-negative patients. As shown in Fig. 8, after the determination of their TNF levels, the survival rates of the patients with elevated serum TNF levels and the patients with undetectable serum TNF levels were significantly different ($P < 0.05$).

**DISCUSSION**

It has been reported that the administration of recombinant TNF induces anorexia, weight loss, and the depletion of whole-body protein in mice (5), and Blick et al. (6) reported a significant decrease in serum albumin levels in patients with disseminated cancer who completed therapy with recombinant TNF. The present study showed that patients with detectable serum TNF levels had significantly lower levels of serum albumin and TP. It thus seems quite reasonable to speculate that TNF mediates, at least in part, the decrease in both the serum albumin level and the TP level because TNF-α has been reported to inhibit albumin gene expression and albumin synthesis in the liver of tumor-bearing mice (7).

Tessitore et al. (8) have reported that the total plasma protein decreased in rats transplanted with a fast-growing ascites hepatoma but that the total cholesterol increased. In contrast, it has been reported that repeated injections of TNF produced a significant decrease in serum cholesterol in humans (6). Feingold et al. (9, 10) have reported that TNF increases hepatic synthesis of cholesterol in experimental animals (9) and that serum cholesterol levels increase in experimental animals after a single injection of TNF (10). However, Feingold and Grunfeld (11) later reported that serum cholesterol levels were not elevated after repeated injections of TNF, suggesting that the initial increase in serum cholesterol may not persist. In the present study, no significant difference in serum total cholesterol levels was found between patients with detectable serum TNF levels and those with undetectable serum TNF.

In recent years, elevated levels of TNF have been reported in AIDS patients with both secondary infections and weight loss (12), and it has been reported that circulating levels of TNF are increased in cachectic patients with chronic heart failure (13).
McMurray et al. (14) also confirmed increased circulating concentrations of TNF in a significant proportion of patients with chronic heart failure and low body weight. In addition, weight loss is also considered to be an established effect of TNF in laboratory studies (5, 15). In agreement with these reports, the present study demonstrated a significant correlation between the detectability of serum TNF and the BMI in patients with prostate cancer.

Levine et al. (13) have demonstrated lower Hgb and hematocrit values in chronic heart failure patients with high TNF levels than patients with low TNF levels. In the present study, significant decreases in Hgb concentration and hematocrit were found in patients with detectable serum TNF levels. Repeated injections of TNF have also been reported to produce anemia in humans (6) and experimental animals (5). It is conceivable that TNF may explain in part the anemia in patients with cachexia because TNF is reported to inhibit the production of erythropoietin (16) and the proliferation of hematopoietic cells (17).

Higher serum TNF levels have been found in breast cancer patients with progressive disease than in those without recurrence (18). Correlations between detectable serum TNF and higher mortality rates have been demonstrated in meningococcal (19, 20), severe falciparum malaria (21), and in elderly nursing home patients (22). In the present study, a higher percentage of the patients with relapsed disease were positive for serum TNF activity than the patients with untreated disease or in remission as a result of endocrine therapy. In addition, patients with detectable serum TNF levels had a higher early mortality rate after determination of TNF. Thus, it seems likely that serum TNF levels increase in prostate cancer patients with end-stage disease.

Stovroff et al. (23) investigated the relationship between TNF production and both tumor burden and host cachexia in tumor-bearing rats. Their findings suggested that host macrophages are activated in response to malignant tumors before there are clinical signs of cachexia and that elevated levels of TNF production are associated with both tumor necrosis and host cachexia as the tumor progresses. It has also been suggested that the TNF that is produced by normal host cells stimulated by the presence of tumor may influence the development of cachexia (24). In addition, Oliff et al. (15) and Tracey et al. (25) transplanted a TNF-α-secreting Chinese hamster ovary cell tumor into nude mice, and both groups reported significant wasting and cachexia. Antibodies to TNF/cachectin have been successfully used to block the lethal sequelae of endotoxin (26) and cerebral malaria (27). In the study by Sherry et al. (28) anti-TNF antibodies significantly reduced the extent of carcass protein and fat loss in a murine sarcoma model. Teng et al. (29, 30) have reported that soluble TNF receptors and anti-TNF antibodies prevented weight loss in nude mice bearing a TNF-secreting tumor and that, after continuous treatment with soluble TNF receptors, the TNF transgenic mice grew to normal size.

It has been reported that serum TNF levels were increased in a significant proportion of patients with cardiac cachexia but there was an overlap in the serum TNF levels between cachectic and noncachectic patients (14). It may be possible that patients vary in their sensitivity to TNF or acquire a tolerance to it. It has also been suggested that the half-life of TNF in the bloodstream is short; its biological effects may be related more to the duration of exposure to the cytokine than to its absolute level in the circulation (13). Although Balkwill et al. (32) have reported elevated serum TNF levels in cancer patients and no correlation between weight loss and serum TNF activity, serum TNF levels were reported to be significantly elevated in patients with stage IV breast cancer who developed weight loss, suggesting that TNF may play a role in the development of cachexia in patients with advanced breast cancer (33). In the present study, a significant correlation was found between elevated serum TNF levels and cachexia in patients with prostate cancer. Although this correlation does not prove a causal relationship, our findings suggest that TNF may be one of the factors contributing to the complex syndrome of cachexia in patients with prostate cancer.

ACKNOWLEDGMENTS

We are indebted to Dainippon Pharmaceutical Co., Ltd., Osaka, Japan, for providing the enzyme immunoassay kits for TNF.

REFERENCES

Association between tumor necrosis factor in serum and cachexia in patients with prostate cancer.

J Nakashima, M Tachibana, M Ueno, et al.