Circulating Cytokine Levels in Prostate Cancer Patients Undergoing Radiation Therapy: Influence of Neoadjuvant Total Androgen Suppression

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Abstract. Background: The purpose of this study was to investigate the immunological impact of combining neoadjuvant total androgen suppression (TAS) with radiotherapy (xRT) in the treatment of prostate cancer by monitoring blood cytokine levels. Patients and Methods: Participants were stage I-II prostate cancer patients receiving xRT alone (n=18) or TAS+xRT (n=19) under the procedures outlined in RTOG protocols #94-08 and #94-13. Peripheral blood samples were collected immediately prior to TAS (xRT+TAS group), immediately prior to xRT, 24 hours after initiation of xRT, and weekly during xRT. Samples were monitored for the immunoregulatory cytokines interleukin (IL)-1β, IL-6 and transforming growth factor (TGF)β using ELISA procedures. Results: Following initiation of xRT, both patient groups demonstrated an immediate elevation of the proinflammatory cytokines IL-1β and IL-6 in their plasma. These cytokine levels appeared to peak after 1-2 weeks of xRT before returning toward pre xRT levels. In contrast, the profibrotic cytokine TGFβ appeared to decrease immediately following initiation of xRT, but, subsequently, underwent two distinct waves of elevation, occurring at 1-2 weeks and 5-6 weeks into the xRT. Surprisingly, while the temporal pattern of plasma cytokine response was similar in both treatment groups, the magnitude of cytokine expression was noticeably different, appearing to be significantly affected by the addition of TAS. Indeed, administration of neoadjuvant TAS appeared to bring about a marked elevation of IL-1β and IL-6 and a significant reduction in TGFβ when compared to patients receiving xRT alone. Conclusion: The precise mechanisms underlying this TAS-related increase of the proinflammatory cytokines IL-1β and IL-6 and decrease of the profibrotic cytokine TGFβ remain unclear. However, previous reports have documented that androgens tend to be immunosuppressive in nature. It is conceivable, therefore, that administration of TAS shifts the ratio of proinflammatory and profibrotic cytokines toward a more immunostimulatory state.

Over the past two decades, several studies have focused upon investigating the prognostic potential of circulating cytokines to serve as biomarkers of radiotherapy (xRT)-induced side effects and complications (1-11). Despite numerous studies, however, a precise understanding of the cytokine changes occurring either as a function of the neoplastic disease or its treatment with xRT remains elusive. Some of this uncertainty stems from differences among individual patients with respect to their inherent radiosensitivity profiles and/or their intrinsic circulating cytokine levels. However, an additional factor obscuring precise definition of cytokine response to radiation is whether or not the xRT is administered in combination with other therapeutic modalities.

In the case of prostate cancer, xRT is frequently administered in conjunction with androgen suppression therapy. Indeed, the use of androgen suppression therapy in combination with xRT has become a popular recourse for the treatment of prostate cancer (12-19). However, androgens are known to be potent modulators of the immune response. As such, the immunological consequences of androgen suppressive therapy need to be considered (18, 19), since they could add to, or even synergize with, the potential immunosuppressive actions of radiation to produce serious clinical ramifications. Historically, for example, combining xRT with antiandrogen regimens using estrogenic compounds such as diethylstilbestrol and diethylstilbestrol diphasphate brought about concern because of these compounds’ reported
ability to reduce natural killer lymphocyte (NK cell) activity and tumor-associated immunity (20-22), and, more recently, use of the luteinizing-hormone releasing hormone (LHHRH) analogs in combination with androgenic compounds such as flutamide has been speculated to alter the immunological status of patients by inducing a heightened inflammatory state (23, 24). Unfortunately, the immunological consequences of androgen suppressive therapy, both alone and in combination with xRT, are not well defined. Given the obvious clinical impact of prostate cancer, however, there is clearly a need for more investigation into this area. To this end, our laboratory has undertaken a series of studies to better define the immunological impact of combining neoadjuvant total androgen suppression (TAS) with xRT by monitoring cellular and humoral immune parameters within the peripheral blood of consenting stage I-II prostate cancer patients undergoing a regimen of xRT. Recently, we reported that the administration of neoadjuvant TAS in combination with xRT appeared to influence T lymphocyte response in these patients. Results demonstrated significantly less radiation-induced suppression of T-cell subsets in patients receiving both TAS and xRT than was seen in patients receiving xRT alone (25). Herein we report on the findings obtained from monitoring proinflammatory interleukins (IL-1β and IL-6) and profibrotic transforming growth factor (TGFβ) blood cytokine levels in this cohort of prostate cancer patients. Similar to our studies on lymphocyte response, the results suggest that addition of TAS to the xRT regimen significantly alters the blood cytokine profile observed.

Patients and Methods

Patients. Thirty-seven prostate cancer patients receiving wide-field pelvic (WFP) and prostate boost (PB) xRT were entered into this study. Eighteen patients received xRT alone, while nineteen received neoadjuvant TAS + xRT. Eligibility was based on the following: histologically confirmed, locally confined adenocarcinoma of the prostate, with or without significant risk for lymph node involvement based on a clinical stage of T1-T2, a grade of T1cN0M0 and prostate-specific antigen (PSA) of ≤21 ng/ml and Gleason score ≤6. Eighteen of the 37 patients were also entered into Radiation Therapy Oncology Group (RTOG) protocols RTOG 94-08 or RTOG 94-13 (11, 25). The 19 patients who were not on protocol were treated according to procedures outlined in RTOG 94-13. For our normal control group, 15 consenting healthy male adults were enrolled, ranging from 53-79 years of age, with no history of previous cancer or antiandrogen therapy. Blood was obtained from patients entered into this study before TAS (if TAS was administered), immediately preceding the initiation of xRT, and at designated intervals throughout the xRT. During widefield pelvic xRT, blood was drawn from the patients after they had received accumulated doses of 1.8, 9, 18, 27, 36 and 45 Gy. During prostate boost xRT, blood was drawn after patients had received accumulated doses of 54 Gy and 63 Gy. At each sampling time, 5-10 ml of peripheral blood was drawn into blindly-coded 5-7 ml vacutainer tubes containing powdered sodium heparin (14 Units/ml blood). The blood samples were immediately placed on ice for transport to the laboratory, aliquoted into conical 15 ml tubes, and centrifuged (3000 x g x 20 min) to separate out the plasma. This process was expedited so that from blood draw to plasma separation, the process was never more than 10 minutes in duration. The platelet-free plasma layer was separated from the blood, transferred into coded cryotubes and frozen at −70˚C until they were analyzed. Analysis was carried out under blind conditions and in accordance with the guidelines established by the ECU committee for handling biohazardous material.

Assessment of plasma cytokines. Plasma concentration of the cytokines IL-1β, IL-6 and TGFβ were measured using the sandwich enzyme-linked immunosorbent assays (ELISAs) which are commercially available in kit form (Quantikine™) from R&D Systems (Minneapolis, MN, USA). In all cases, the assays were performed according to the manufacturer’s instructions. The assay kits were chromogen-based and cytokine concentration (color) was quantified using a Titrertek Multiskan MCC/340 plate reader at the appropriate wavelengths dictated by the specific instructions of each kit. Each assay was run against a standard curve with a full range predetermined for each cytokine and sample source.

Statistical methods. Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was used for assessment of patient group differences in age, disease stage, PSA levels and Gleason scores.
The nonparametric Kruskal-Wallis test and Tukey’s HSD (honestly significant difference) test were used to assess pre-treatment and post-treatment blood cytokine concentrations. Pearson’s product moment correlation coefficients were used to determine if any linear relationships existed among clinical patient variables such as age, clinical stage of disease, or PSA concentration and plasma cytokine concentrations. A p-value of <0.05 was taken as significant.

Results

Characteristics of control and patient populations. Clinical characteristics of the patient population contributing to this study are summarized in Table I. Patients selected for this study presented without metastasis-positive nodes or any evidence of metastatic disease, and, based on clinical stage, the distribution of patients in both treatment groups favored the T2 over the T1 stage by a ratio of approximately 2:1. ANOVA revealed no evidence of statistical differences between the two treatment groups for PSA levels, Gleason scores, nor the mean age of individuals receiving xRT vs. xRT+TAS. The mean age of the healthy controls, while slightly younger than the mean ages of the two patient groups, did not prove to be statistically different.

Influence of tumor on circulating cytokine levels. Elevated circulating cytokine levels have been reported in several types of cancers and have sometimes correlated with disease progression or prognosis. It was of interest, therefore, to ascertain if any disease-related changes in plasma cytokine concentrations were present in the prostate cancer patients entered into this study. As can be observed in Figure 1, mean plasma TGFβ levels in the two patient groups were indeed significantly elevated when compared to the levels found for the healthy control group (1.6-fold increase in the xRT patient group and a 1.7-fold increase in the xRT+TAS patient group), suggesting the possibility of a disease-related modulation of this cytokine. Additionally, while not statistically significant, mean plasma concentrations of IL-1β and IL-6 prior to treatment in both groups of prostate cancer patients also appeared to be slightly increased over levels observed in the healthy controls. In contrast, intergroup comparison of mean cytokine expression between

Table I. Characteristics of control and patient populations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data±range</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>Sample size</td>
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</tr>
<tr>
<td>Age (years)</td>
<td>63.4±6.2 (53-79)</td>
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<tr>
<td>Patient groups</td>
<td>xRT vs. xRT+TAS</td>
</tr>
<tr>
<td>Sample size</td>
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<tr>
<td>Age (years)</td>
<td>69.2±7.4 (58-79)</td>
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<tr>
<td>Clinical stage</td>
<td>T1b,cN0M0 vs. T2a,bN0M0</td>
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<tr>
<td>Sample size</td>
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<tr>
<td>PSA (ng/ml)</td>
<td>14.3±7.2 (3.5-20.2)</td>
</tr>
<tr>
<td>Gleason score</td>
<td>6 (4-7)</td>
</tr>
</tbody>
</table>

The mean±SD. Values in parentheses represent the range of patient data for each parameter.

![Image](https://via.placeholder.com/150)

**Figure 1.** Results demonstrating the influence of prostate cancer on circulating cytokine levels. Data represent the mean plasma levels (±SEM) for the cytokines IL1-β, IL-6 and TGFβ in a healthy control group (controls) and two prostate cancer patient groups (xRT and xRT+TAS) prior to any treatment (pretreatment levels). Mean cytokine levels in the patient groups that were observed to be statistically different from healthy controls (p<0.05) are signified by an asterisk. No statistical differences in pretreatment plasma cytokine levels between the two patient treatment groups were found.

![Image](https://via.placeholder.com/150)

**Figure 2.** Results demonstrating the influence of neoadjuvant total androgen suppression (TAS) therapy on circulating cytokine levels. Data represent the mean plasma levels (±SEM) for the cytokines IL1-β, IL-6 and TGFβ in the healthy control group and the xRT+TAS patient group prior to any treatment (Pre TAS) and following 60 days of androgen suppression (Post TAS). Mean cytokine levels in prostate cancer patients that were observed to be statistically different from healthy controls (p<0.05) are signified by an asterisk. Mean cytokine levels in prostate cancer patients that were observed to be statistically different (p<0.05) following TAS therapy (Pre TAS vs. Post TAS) are signified by a cross.
the two treatment groups of prostate cancer patients revealed very similar circulating levels for the three cytokines studied. Finally, attempts to correlate changes in cytokine expression among the prostate cancer patients with such clinical variables as age, stage of disease, or PSA concentration produced no significant correlations (data not shown).

Influence of total androgen suppression (TAS) on circulating cytokine levels. Because androgens are known to be potent immune modulators, it was of interest to determine what effect, if any, that total androgen suppression (TAS) may have upon the levels of circulating cytokines. To accomplish this, plasma cytokine concentrations in prostate cancer patients prior to treatment (pre TAS) were compared to those following 60 days of TAS (post TAS) therapy (post TAS levels were assessed from blood drawn from patients immediately prior to initiation of their xRT regimen). The results displayed in Figure 2 clearly demonstrate that two months of TAS significantly altered the circulating levels of all three cytokines monitored. Mean plasma levels of the proinflammatory cytokines IL-1β and IL-6 demonstrate significant elevation in the post TAS samples when compared to pre TAS values, while the concentration of the profibrotic cytokine TGFβ is so reduced that levels are not statistically different from those observed in the healthy control group.

Cytokine levels during xRT for the prostate cancer patient treatment groups. Following initiation of xRT, both patient groups demonstrated an immediate elevation of the proinflammatory cytokines IL-1β and IL-6 in their blood plasma (Figure 3). These cytokine levels appeared to peak after 1-2 weeks of xRT and then slowly decrease toward pre xRT levels throughout the remainder of the treatment regimen. In contrast, the profibrotic cytokine TGFβ appeared to decrease immediately following initiation of xRT, but subsequently, underwent two distinct waves of elevation, occurring at 1-2 weeks and 5-6 weeks into the xRT. Surprisingly, while the temporal pattern of blood cytokine response was similar in both treatment groups during the radiation regimen, the magnitude of cytokine expression was noticeably different, appearing to be significantly affected by the addition of TAS. Indeed, administration of neoadjuvant TAS appeared to bring about a marked elevation of IL-1β and IL-6 and a significant reduction in TGFβ when compared to patients receiving xRT alone.

Discussion

In recent years, several studies have focused on the elucidation of clinically useful biomarkers of xRT-induced toxicity, with the belief that the ability to identify a patient’s radiosensitivity profile could lead to more individually tailored treatments and, ultimately, improved local control and survival rates. In this regard, the possible link between altered circulating cytokine levels during xRT and the development of radiation toxicity (the cytokine cascade hypothesis) has received considerable discussion in the literature (1-11). However, most of the discussion has
centered around ascertaining the potential of circulating cytokines to predict the development of radiation-induced pneumonitis and pulmonary fibrosis following thoracic xRT for lung cancer (1, 2, 4-7, 9). Study of circulating cytokines in patients receiving a course of pelvic xRT has received much less attention. To this end, the goal of this study was to better characterize the temporal expression of the proinflammatory and profibrotic cytokines in the plasma of prostate cancer patients receiving pelvic xRT and, in particular, to ascertain the influence that neoadjuvant TAS may have upon this expression. The cytokines chosen, IL-1β, IL-6 and TGFβ1 (TGFβ), were selected on the basis of their well-documented role in the humoral response to xRT (9, 10).

Overall, the data generated during this study have revealed a number of characteristics concerning circulating cytokine levels in patients diagnosed with untreated, primary adenocarcinoma of the prostate. Among these is the observation that although no significant differences in mean plasma cytokine expression were seen between the two prostate cancer patient groups, differences were observed when the two groups of patients were compared to a representative group of healthy control individuals. Specifically, we found that plasma levels of the profibrotic cytokine TGFβ were elevated as a function of disease. In contrast, however, although IL-6 previously has been suggested to be a potential biomarker for prostate cancer (26) and both IL-1β and IL-6 have been reported to be elevated in several other types of cancer (9, 10), neither cytokine were found to vary significantly from those in healthy controls in our investigation. With regard to the presence of the cancer-related elevation of TGFβ, our data are in agreement with several earlier studies. Indeed, laboratory studies have revealed that TGFβ has a history of being correlated with prostate cancer in that it has been correlated with the development of prostate cancer in animal models (27), has been shown to accumulate in primary and metastatic prostate tissue samples processed immunohistochemically (28-30), and has been reported to be secreted by several prostate cancer cell lines in vitro (26, 27). Furthermore, clinical studies in prostate cancer patients have demonstrated elevated levels of circulating TGFβ in several (8, 11, 26, 27, 31), although not all (32), investigations. At present, the source(s) of these elevated levels of TGFβ in prostate cancer patients remains unknown, but include an endogenous production by the tumor itself and/or production by host immune cells in response to the tumor.

As well as tumor-related alterations in circulating cytokine profiles, our data also clearly demonstrate the presence or xRT-induced changes in plasma cytokine levels. Indeed, for both patient groups monitored, the administration of xRT resulted in pronounced fluctuations of circulating cytokine levels. Specifically, all 3 cytokines underwent an immediate wave of elevation within the first 1-2 weeks of their xRT regimen. Moreover, TGFβ underwent a second strong wave of elevation near the end of the xRT regimen. The basis for these elevated waves of IL-1β, IL-6 and, in particular, TGFβ is not completely understood but may be related to the ability of cells – either those surviving within the xRT field or those mobilized to the damaged area after radiation injury – to initiate the repertoire of molecular events associated with an inflammatory reaction. Additionally, the data described herein are not inconsistent with the premises underlying the cytokine cascade hypothesis of radiation injury which postulates that cyclic rounds of elevated cytokine expression following xRT precipitate a prolonged deregulation of normal cytokine homeostasis, thereby resulting in radiation-induced fibrosis. For example, in the case of xRT for small cell lung cancer patients, development of clinical side-effects such as pneumonitis and pulmonary fibrosis have been significantly correlated with cyclic waves of circulating TGFβ elevation that are not unsimilar to those observed in this investigation (1, 2, 4, 7, 9).

Perhaps the most interesting result of these studies is the observation that administration of neoadjuvant TAS, while not altering the pattern of elevated cytokine waves observed to occur as a function of the xRT regimen, significantly influenced the magnitude of circulating cytokine levels seen. Indeed, data from these studies would suggest that administration of TAS shifts the ratio of proinflammatory and profibrotic cytokines toward a more immunostimulatory state. Specifically, results demonstrated the presence of significantly elevated levels of the proinflammatory cytokines IL-1β and IL-6 and significantly reduced levels of the profibrotic cytokine TGFβ in the xRT+TAS patient cohort when compared to patients not receiving TAS. Indeed, plasma TGFβ expression was suppressed so much in the xRT+TAS patient group that mean circulating levels, while still demonstrating the two xRT-correlated cyclic waves described in above, remained within a range that was not statistically different from that seen for healthy controls. The clinical ramification of this shifting balance of cytokines is not known and will require much more characterization before any definitive conclusions can be drawn regarding its potential benefit/detrimet. However, it is interesting to note that that the addition of TAS to conventional xRT for treatment of prostate cancer may have the potential to attenuate the increases in TGFβ following xRT that have been correlated with radiation-induced late toxicity in other types of cancers (1, 2, 4, 7-9).

Unfortunately, the precise mechanisms underlying this apparent TAS-induced cytokine shift toward a more proinflammatory state remain unclear. However, several investigations have reported that the immune response is sexually dimorphic and, as such, manipulation of the normal balance of sex steroid hormones (33-37) by such therapy as
TAS can lead to profound immunological changes. Indeed, as a rule, previous studies on the immunological consequences of the sex steroids support the concept that female sex hormones (estrogen and progesterone) tend to be immunostimulatory, while male sex hormones (androgens) tend to be immunosuppressive. It has been postulated that much of this immunological action is brought about through binding to classical hormone receptors on cells of the immunohematopoietic system (e.g. lymphocytes and macrophages). For example, studies by Olsen et al. (35-37) have elegantly demonstrated that androgens exert considerable influence on lymphocytes numbers, largely through modulation of proliferation/apoptosis. Because lymphocytes and macrophages are major sources of the cytokines studied in this investigation, it is conceivable that our observations of shifting balances between the proinflammatory and profibrotic cytokines in the xRT+TAS patients may reflect a TAS-induced reduction of the reported androgen modulation of these cell populations.

In conclusion, the results of this investigation suggest that cytokine expression in prostate cancer patients subjected to a regimen of xRT can be very complex, being influenced as a function of the disease and hormonal manipulation, as well as by the radiation regimen itself. Hence, while the number of reports documenting a correlation between cytokine expression and the development of xRT pathobiology have increased rapidly within the last fifteen years, precise characterization of the interplay between proinflammatory and profibrotic cytokines following radiation exposure remains a difficult goal, particularly within multiple, interacting cell systems being subjected to a variety of stresses as is the case in the radiation oncology clinic. Nevertheless, while additional studies will clearly be needed, hopefully, the generation of the type of information reported herein can provide additional insight into the potential cytokine interactions occurring within cancer patients, which may help build a database of knowledge upon which to ultimately develop interventional approaches that result in a significant reduction, or abrogation, of the side-effects of xRT.

References


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