Prognostic Value of IL-6 in Localized Prostatic Cancer

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Abstract. *Aim: The usefulness of interleukin 6 (IL-6) and its soluble receptor IL-6sR in the prediction of the biochemical recurrence was evaluated in patients with prostate cancer treated with radical prostatectomy. Patients and Methods: IL-6 and sIL-6R serum levels were measured in 96 patients with prostate cancer. Results: Using the log-rank test, it was evident that patients with preoperative serum levels of IL-6 higher than 1.2 pg/ml had a significantly increased probability of biochemical recurrence (p=0.031). We also observed that the Gleason score was associated with the risk of progression (p=0.033), but no relation was observed with TNM classification, PSA, % free PSA or sIL-6R. In a multivariate analysis, only IL-6 serum levels remained as a predictor of biochemical recurrence (p=0.040). Conclusion: The results presented here demonstrated the usefulness of IL-6 in predicting the biochemical progression of prostate cancer, pointing towards an association between inflammation and the aggressiveness of the tumor.*

It has been suspected for a long time that chronic inflammation has an active role in the process of carcinogenesis. Balkwill and Mantovani (1) underlined that in 1863 Virchow suggested that the lymphoreticular infiltrate reflected the origin of cancer at sites of chronic inflammation. On the other hand, in the recent past different authors (2-3) have reported that chronic or recurrent prostate inflammation may initiate and promote prostate cancer development. In this sense, several authors have evaluated the association between cytokines and prostate cancer with different methods (4-7).

Since the initial observation by Twillie *et al* (8), who described intrleukin-6 (IL-6) as a mediator of morbidity in patients with metastatic adenocarcinoma of the prostate, several studies have reported relevant data about the implication of this cytokine in prostate cancer. Culig *et al* (9-10) described that IL-6 is involved in cell growth and differentiation of prostate cancer, and more recently, Finley *et al* (11) showed the correlation of increased IL-6 content in periprostatic adipose tissue with tumour stage and grade. On the other hand, the aggressiveness and recurrence of prostate cancer has been associated with IL-6 polymorphism (12). Finally, different authors have reported on the relationship between prostate cancer prognosis and serum levels of different cytokines, such as IL-6 (7, 13-15).

The aim of our study was to evaluate the usefulness of the proinflammatory cytokine IL-6 and its soluble receptor IL-6R in the prediction of biochemical recurrence in patients with prostate cancer treated with radical prostatectomy.

Patients and Methods

Patients. Preoperative sera were obtained after informed consent from 96 non-selected patients undergoing radical retropubic prostatectomy (RRP) for adenocarcinoma of the prostate in our center from January 2005 to July 2007. Age ranged between 49 and 76 years (mean±SD: 62.91±5.79 years). Clinical data included in the study were: pathological TNM stage, Gleason score obtained in the prostatectomy specimen, total PSA and the percentage of free PSA at diagnosis. Tumours in a total of 72 patients were T2 and in 24 patients were T3 in the final pathological report. The distribution of patients according the Gleason score was: 14 in grade 5, 20 in grade 6, 44 in grade 7 (3+4), 11 in grade 7 (4+3), 2 in grade 8 and 5 in grade 9. No patient received neoadjuvant treatment. All patients were followed-postoperatively with serial PSA measurements and clinical examinations done at regular intervals.

Laboratory tests. Blood samples were drawn from patients and serum was collected and centrifuged at room temperature for 10 minutes at 1500 ×*g*. Serums samples were immediately frozen at –80°C in different aliquots until retrieved for measurement of IL-6 and sIL-6R. IL-6 serum levels were measured with a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) using specific monoclonal antibodies for IL-6 (Invitrogen, Camarillo, CA, USA). Streptavidin peroxidase was used as the substrate. The intensity of colouration obtained in the reaction was proportional to the IL-6 concentration in the sample. The minimum detectable level of this assay is 0.104 pg/ml. Every sample was assayed in duplicate and the mean of two results was used. Serum sIL-6R levels were measured with a solid-phase sandwich ELISA (Immunotech, Marseille, France). This assay uses specific monoclonal antibodies against sIL-6-R and

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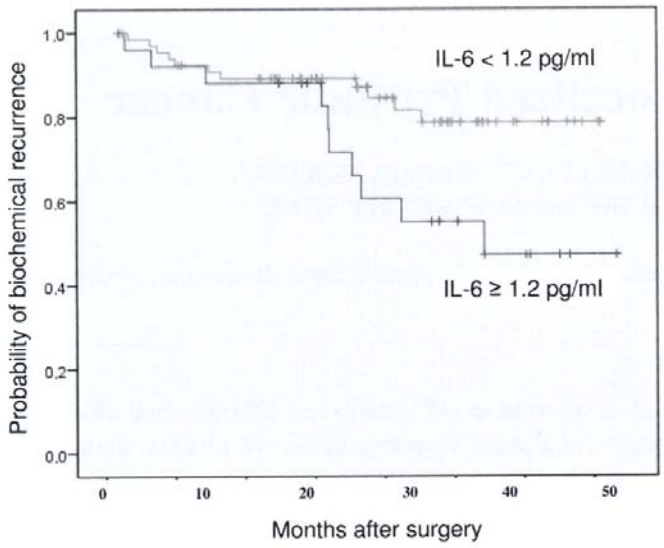

Figure 1. *Probability of biochemical recurrence in relation to IL-6 serum levels.*

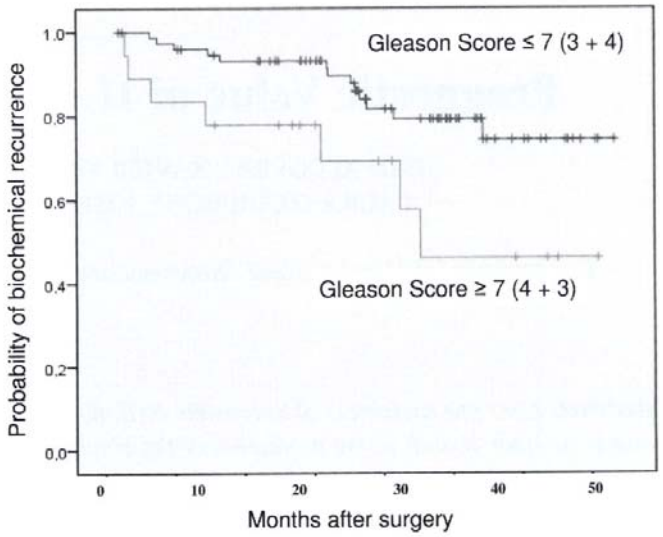

Figure 2. *Probability of biochemical recurrence in relation to Gleason score.*

streptavidin peroxidase was used as the chromogenic substrate. The intensity of the colour obtained in the assay was proportional to the concentration of sIL-6R in the sample. The lower detection limit was 4 pg/ml. Every sample was assayed in duplicate and the mean value from two results was used. PSA and free PSA serum levels were measured with an electrochemiluminiscent assay (Cobas e 411, Roche Diagnostics, Mannheim, Germany). The percentage free PSA and total PSA (%fPSA) was calculated for all patients.

Definitions. Postoperative biochemical recurrence was defined as a PSA elevation higher than 0.4 ng/ml. Biochemical recurrence-free survival was defined as the period of time that elapsed between radical prostatectomy and either the date of PSA failure or the date of the last PSA measurement.

Statistical analysis. Statistical analysis was performed with SPSS software, version 16.0 (SPSS Inc, Chicago, IL, USA). The parametric *t*-test was used for quantitative variables, with the Mann-Whitney *U*-test being applied for nonparametric variables. Categorical variables (success rates at the last evaluation) were compared using the chi-square test. Actuarial estimates for survival were calculated with the use of life table methods. The log-rank test was used to compare the curves based on Kaplan-Meier models. A multivariate Cox proportional hazards regression model was used to estimate the prognostic relevance of IL-6, sIL-6R, PSA, %free PSA, TNM classification and Gleason score. All p-values <0.05 reflected statistically significant differences.

Results

Table I shows the results obtained with the tests evaluated in the present study in relationship to pathological stage and Gleason score obtained in the prostatectomy specimens. No statistical differences were evident for any of the tests.

Overall, only 21 (22%) out of 96 patients presented a biochemical recurrence at a mean follow-up time of 63.8 months (SD: 30.7 months). Using the log-rank test, we found that patients with preoperative IL-6 serum levels higher than 1.2 pg/ml had a significantly increased probability of biochemical recurrence (p=0.031; Figure 1). We also observed that patients with a Gleason score equal to or higher than 7 (4+3) had a significantly increased probability of biochemical recurrence (p=0.033; Figure 2). However, there was no evidence of a relation between biochemical recurrence and TNM classification (p=0.083), PSA (p=0.105), % free PSA (p=0.473) or sIL-6R (p=0.241). In the multivariate Cox proportional hazard model, the preoperative serum levels of IL-6 were a significant predictor of biochemical recurrence after radical prostatectomy (p=0.040). For the other tests evaluated in this study, no statistical differences were evident in this multivariate evaluation (Table II).

Discussion

Recently, different groups have focused their interest on the role of inflammation in the pathogenesis of prostate cancer. Epidemiological studies suggest the correlation of prostatitis and sexually transmitted infections with increased prostate cancer risk (16-17). On the other hand, De Marzo *et al* (18) described the development of chronic inflammation related to infectious and non-infectious agents, called proliferative inflammatory atrophy (PIA), as precursor lesions of prostate cancer. In PIA, epithelial cells are in an atrophic status and different stress signalling pathways are activated in relation to an increase in inflammation (19).

In addition, genetic studies have shown a higher risk of prostate cancer in subjects with several polymorphic variant alleles of genes encoding inflammatory or anti-inflammatory cytokines, including TNF-α, IL-6, IL-8, IL-10 or IL-1-RA

Table I. *Preoperative serum levels of IL-6, sIL-6R, PSA and %fPSA according to pathological stage and Gleason score.*

	IL-6	sIL-6R	PSA	%fPSA
Pathological stage				
T2	0.99±0.86	93.22±25.72	7.38±3.77	14.38±7.42
T3	1.33±1.45	91.00±30.72	8.38±3.37	11.14±5.17
p-Value	0.186	0.740	0.267	0.095
Gleason score				
≤7 (3+4)	0.87±0.65	91.25±22.34	7.62±4.40	15.09±7.64
≥7 (4+3)	1.16±1.17	91.65±27.35	7.63±3.32	12.53±6.74
p-Value	0.192	0.944	0.995	0.135

Results as mean±SD.

Table II. *Relationship between the studied parameters and biochemical recurrence on multivariate analysis.*

	p-Value
IL-6≥1.2 pg/ml	0.040
sIL-6R≥105 pg/ml	0.467
PSA≥10 ng/ml	0.235
%fPSA<10%	0.669
Gleason score≥7 (4+3)	0.508
Pathological stage (T2 *vs.* T3)	0.314

(12, 20-23). Among these, IL-6 plays a key role in the pathogenesis of prostate cancer (9-10). Few studies have examined the role of IL-6 and its soluble receptor in the prognosis of prostate cancer. Nakashima *et al.* (24) reported that IL-6 is independently associated with survival in a series of 74 patients with prostate cancer. On the other hand, Shariat *et al* (25), in a cohort of 120 patients treated with radical prostatectomy, reported that the preoperative IL-6 and sIL-6R predicted biochemical progression after surgery, suggesting an association with occult metastatic disease present at the time of radical prostatectomy. More recently, Shariat *et al* (26) purposed a nomogram that includes IL-6 and sIL-6R to predict the risk of biochemical recurrence following radical prostatectomy. However, in a later study, this group proposed a predictive model that included only sIL-6R (27).

Our results are in agreement with data published by Shariat's group and we also observed that IL-6 predicts biochemical recurrence in patients treated with radical prostatectomy. Discrepancy with other authors in reference to the value of sIL-6R can be explained by differences in the methods used for its measurement due to the insufficient standardization of these assays. The clinical data presented here for IL-6 support the notion of a biological role for this cytokine in prostate cancer, including its capacity in prostate cancer growth through activation of the androgen receptor or the regulation of tumor angiogenesis (9-10, 28). These clinical and biological data confirm the role of the proinflammatory cytokine IL-6 in prostate cancer and support the inclusion of IL-6 as a tumor marker in patients with prostate cancer (29). Larger prospective studies and the standardization of the assays for the measurement of IL-6 and sIL-6R are required to confirm or refute the role of these cytokines as tumor markers in prostate cancer.

Acknowledgements

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