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PRELIMINARY REPORT

Serum Paraoxonase-1 Concentration as a Potential Predictor of Urinary Bladder Cancer Recurrence. A Five Year Follow-Up Study

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This study provides preliminary information on the usefulness of measuring serum paraoxonase-1 (PON1) concentration and activity (and other inflammatory markers) to predict tumor recurrence in patients with urinary bladder cancer. We studied a total of 39 hospitalized patients in whom the diagnosis of urinary bladder cancer was confirmed by transurethral resection. After five years of follow-up, 29 patients presented with tumor recurrence. As control subjects, we also studied 61 healthy subjects and a further 132 hospitalized patients who had a urinary catheter-related infection due to causes other than cancer. Results showed that urinary bladder patients had lower serum PON1 concentration and activity, and higher chemokine (C-C motif) ligand 2, C-reactive protein, and procalcitonin concentrations than the control individuals. Patients with tumor recurrence had significantly lower serum PON1 concentration than patients without tumor recurrence. The mean area under the curve of the receiver operating characteristics plot for serum PON1 concentration in discriminating patients with and those without tumor recurrence was 0.755 and the best combination of sensitivity and specificity was obtained at PON1 = 100 mg/L (0.72 and 0.80, respectively). Establishing this value as a cut-off, positive predictive value was = 0.91, and negative predictive value was = 0.50. These results suggest that the measurement of serum PON1 concentration may be a high-sensitivity marker of tumor recurrence in urinary bladder cancer patients. © 2018 IMSS. Published by Elsevier Inc.

Key Words: Biomarkers, Paraoxonase-1, Tumor recurrence, Urinary bladder cancer.

Introduction

Urinary bladder cancer is one of the most frequent types of neoplasia in men, with 21,093 new cases diagnosed in Spain in 2015 (1). Up to 70% of patients with non−muscle invasive bladder cancer have tumor recurrence, and about 10−15% progress to muscle-invasive disease. The mortality rate associated with invasive bladder cancer is high despite optimal treatment (2). Currently available histopathological classifications are limited by inter- and intra-observer variability; a problem that may have important implications for the diagnosis, treatment and follow-up of these patients. Hence, it is imperative to find biomarkers to diagnose bladder cancer early so as to implement treatment, monitor response and identify tumor recurrence.

Recent investigations suggest that paraoxonase-1 (PON1) plays a role in the molecular disorders associated with cancer (3). PON1 is an antioxidant enzyme found in the circulation bound to high-density lipoproteins (HDL)

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and, as well, in the cytoplasmic membrane and microsomal fractions of most cells (4,5). Alterations in the circulating levels of PON1 in various types of cancer have been reported (3), and a study in patients with breast cancer observed that decreased serum PON1 activities were associated with tumor recurrence and short-term death (6).

Recently we conducted a study on alterations in serum PON1 levels and other inflammation markers in urinary catheter-related infections in hospitalized patients (7). Some of these patients were hospitalized to confirm the diagnosis of urinary bladder neoplasia. After five years of routine clinical follow-up, we retrospectively collated their medical records to investigate the incidence of tumor recurrence and the relationships with the measured biochemical variables. The results described in the present article provide preliminary evidence indicating that the determination of circulating serum PON1 concentration would be a good predictor of tumor recurrence in patients with urinary bladder cancer.

Patients and Methods

Participants

Commencing in March 2011, we identified a total of 39 hospitalized patients in whom the diagnosis of urinary bladder cancer was confirmed by transurethral resection. Exclusion criteria were: severe alcohol abuse, psychiatric diseases, or liver impairment. The patients had routine clinical follow-up and, after five years, their clinical notes were collated for the purpose of the current study.

Results of the patients were compared with those of two control groups: Control Group 1 (CG1) consisted of 61 ostensibly healthy subjects participating in an epidemiological study conducted in our geographical area. Since our study patients with cancer had urinary infections, we also studied another Control Group (CG2) consisting of 132 hospitalized patients who had a urinary catheter-related infection due to causes other than cancer. These subjects had a similar age and gender distribution as the urinary bladder cancer patients, and had no clinical or biochemical evidence of renal insufficiency, liver disease, neoplasia or neurological disorders.

The study was approved by the Ethics Committee (Institutional Review Board) of the *Hospital Universitari de Sant Joan*. All the participants provided written informed consent to participation in the study on the understanding that anonymity of data was guaranteed.

Biochemical Analyses

The physiological substrates for PON1 have not, as yet, been identified. Since PON1 has esterase and lactonase activities (5), we measured the catalytic activity of PON1 using two different substrates: paraoxon (an ester), and thiobutyl butyrolactone (TBBL, a synthetic lactone), as previously described (8). Serum PON1 concentrations were determined by an in-house enzyme-linked immunosorbent assay (9,10). The serum concentration of C-reactive protein (CRP) was measured using a high sensitivity method (Horiba ABX, Montpellier, France). The concentration in serum of procalcitonin and the concentration of the chemokine (C-C motif) ligand 2 (CCL2) in EDTA-plasma were measured by enzyme-linked immunosorbent assay (Biovendor, Brno, Czech Republic, and Prepotech, London, UK, respectively).

Statistical Analyses

All calculations were performed with the SPSS 22.0 statistical package (SPSS Inc., Chicago, IL, USA). Differences between groups were assessed with the Mann-Whitney *U* test, since most of the studied variables had non-parametric distributions. Qualitative data were analyzed with the χ^2 test. Results are shown as medians and 95% confidence interval (CI). Biochemical variables' diagnostic accuracy were assessed using receiver operating characteristic (ROC) plot analysis (11).

Results

Twenty-nine of the 39 patients with urinary bladder cancer had at least one episode of tumor recurrence in the five year period of follow-up. Sixteen patients had 1 episode, five patients had two, four patients had three, two patients had four, one patient had five, and one patient had six. Compared to CG1, urinary bladder patients with tumor recurrences had lower serum PON1 concentrations and TBBLase activities, and higher CCL2, CRP, and procalcitonin concentrations. Compared to CG2, they had lower PON1 and procalcitonin concentrations and higher paraoxonase and TBBLase activities. Patients with tumor recurrence had significantly lower serum PON1 concentrations than patients without recurrence (Table 1). The parameter that presented the greatest differences between the patients with urinary cancer who presented recurrences and those that did not was the concentration of PON1. The mean area under the curve of the ROC plot for this parameter in discriminating patients with and those without tumor recurrence was 0.755 (Figure 1) and the best combination of sensitivity and specificity was obtained at PON1 = 100 mg/L (0.72 and 0.80, respectively). Using this value as a cut-off, the positive predictive value was = 0.91, and the negative predictive value was = 0.50.

Discussion

A quantitative genome-wide methylation analysis of non-muscle-invasive bladder tumors found increased methylation of the *PON1* gene which was associated with decreased expression (12). This would explain the reduced serum