

## Increased Urinary Levels of Tissue Polypeptide Specific Antigen (TPS) in Alcoholics

PAULA BARROS<sup>1</sup>, ARTURO GONZALEZ-QUINTEL<sup>1</sup>,  
CARMEN MELLA<sup>1</sup> and LUIS-FERNANDO PEREZ<sup>2</sup>

*Departments of <sup>1</sup>Internal Medicine and <sup>2</sup>Biochemistry,  
Complejo Hospitalario Universitario de Santiago, Santiago de Compostela 15706, Spain*

**Abstract.** *Background:* Urinary levels of tissue polypeptide specific antigen (TPS, cytokeratin-18) have been proposed as a marker of urothelial malignancies. Previous studies have shown that serum TPS levels are elevated in alcoholics. This study was designed to determine whether alcoholics had elevated urinary TPS levels as well. *Patients and Methods:* Serum and urinary TPS levels were determined in 24 alcoholics and 15 healthy controls by means of a commercial chemiluminescent immunoassay. *Results:* Serum TPS levels were higher in alcoholics than in controls (median 332 U/L, range 51-21241 U/L versus median 17 U/L, range 15-65 U/L, respectively,  $p<0.001$ ). Urinary TPS levels were also higher in alcoholics than in controls (median 244 U/L, range 22-1267 U/L versus median 66.5 U/L, range 15-600 U/L, respectively,  $p=0.001$ ). Urinary TPS levels were correlated with serum TPS levels in alcoholics. *Conclusion:* Urinary TPS levels are elevated in alcoholics. Consequently, the specificity of urinary TPS as a tumor marker may be limited in alcoholics.

The tissue polypeptide specific antigen (TPS) corresponds to a cytokeratin-18 fragment (1-3). Serum TPS levels are routinely employed as a marker for the diagnosis, prognosis and follow-up of a variety of epithelial neoplasms (4-7). Serum TPS levels may be found increased in patients with non-malignant liver disease (8-11), particularly alcoholic hepatitis (12). Serum TPS levels are markedly increased in alcoholic patients admitted to the hospital (12), with values over 10 times the upper reference limit in some cases (12). These levels decrease shortly after alcohol abstinence (12). Among alcoholics, serum TPS levels are higher in cases of alcoholic hepatitis than in cases of fatty liver (12). The serum TPS levels

in patients with alcoholic liver disease are correlated with biochemical evidence of liver necro-inflammatory activity and with the presence of Mallory bodies in the hepatocytes (12). Of note, cytokeratin-18, a normal constituent of the hepatocyte cytoskeleton (13), is the main component of Mallory bodies (14), a hallmark of alcoholic hepatitis (15). Moreover, serum TPS levels are correlated with hepatocyte cytokeratin-18 expression in alcoholic liver disease (16). Consequently, serum TPS levels may have diagnostic value as markers of alcoholic hepatitis (12). Conversely, the value of serum TPS as a tumor marker could be limited in alcoholics.

Urinary TPS has been proposed as a marker for the diagnosis and follow-up of urothelial and other urological malignancies (17-21). To our knowledge, no studies have been published that evaluate the possible alteration of urinary TPS in this patient subset. This study was designed to investigate urinary TPS levels in alcoholics. Both serum and urinary TPS levels were determined in a group of alcoholics and a group of healthy controls.

### Patients and Methods

#### *Subjects*

*a) Alcoholic patients.* A total of 24 alcoholics (17 males and 7 females) with a median age of 49 years (range 24-81 years) was studied. The median chronic alcohol consumption in these patients, as evaluated by the system of standard drinking units (22), was 100 g/day (range 40-240 g/day). All patients were admitted to the Internal Medicine Department from a University Hospital between March and May, 2005, because of alcohol-related disease. The diagnoses were: alcohol withdrawal syndrome (13 cases), complications of advanced alcoholic liver disease (7 cases), acute alcoholic hepatitis (3 cases) and dilated cardiomyopathy (1 case). Patients with a known cause for urinary TPS elevation (17, 18, 20), namely (a) malignant neoplasm; (b) recent urinary manipulation, including bladder catheterization; or (c) recent or current urinary tract infection, were excluded from the study.

*b) Controls.* A group of 15 healthy volunteers (8 males and 7 females) was included as a control. The median age was 40 years (range 27-56 years). The daily alcohol intake was lower than 10 g in these subjects. The same exclusion criteria (see above) were applied to this group.

*Correspondence to:* A. Gonzalez-Quintela, Department of Internal Medicine, Hospital Clínico Universitario, 15706 Santiago de Compostela, Spain. Tel: +34-981-951279, Fax: +34-981-950501, e-mail: mearturo@usc.es

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**Main determinations**

*a) Serum and urinary TPS.* Fasting blood samples were obtained for serum TPS assays. Simultaneous samples were obtained for the urinary TPS assay. In both samples, the TPS level was measured by means of a commercial chemiluminescent immunoassay (Immulite-TSP, DPC, Los Angeles, USA). The test employs the M3 monoclonal antibody specific for residues 322-340 in cytokeratin-18 (1, 2). The results obtained with Immulite-TPS are quite similar to those obtained with standard immunoradiometric assays (23). The upper normal serum TPS level with this technique is considered to be 80 U/L. No standard reference levels exist for urinary TPS. Previous studies that focused on the diagnosis of urothelial malignancy employed cut-off levels of 42 U/L (19), 100 U/L (20) and 279 U/L (18). In order to avoid spurious variation due to urine concentration, urinary TPS values were determined in relation to urinary creatinine (Advia 1650 analyzer, Bayer Diagnostics, Leverkusen, Germany) (17).

*b) Routine liver tests.* The serum levels of AST, ALT, GGT, bilirubin and albumin were assayed in the Advia 1650 analyzer. The prothrombin index was calculated by means of the Quick's method. The results of the routine liver tests in alcoholics and controls are represented in Table I.

*Statistical analyses.* The Mann-Whitney *U*-test was employed to compare quantitative variables between groups. The Fisher exact test was used for categorical variables and the Spearman test to assess correlation. All tests were two-tailed. Linear regression was used to further investigate the relationship between urinary TPS (as the dependent variable) and serum TPS. For that purpose, levels of both urinary and serum TPS were log<sub>10</sub>-transformed in order to normalize their distribution.

*Ethical issues.* The study conformed to the Helsinki declaration and was reviewed and approved by the regional (*Xunta de Galicia*) Ethics Committee. Written informed consent was obtained from all participants.

## Results

*Serum TPS levels in alcoholics and healthy controls.* Serum TPS levels were higher in alcoholics than in controls (median 332 U/L, range 51-21241 U/L versus median 17 U/L, range 15-65 U/L, respectively, *p*<0.001) (Figure 1A). A total of 19 alcoholics (79%) versus none of controls showed increased (>80 U/L) serum TPS levels (*p*<0.001). Five alcoholics (21%) showed serum TPS levels higher than 1000 U/L.

*Urinary TPS levels in alcoholics and healthy controls.* Urinary TPS levels were also higher in alcoholics than in controls (median 244 U/L, range 22-1267 U/L versus median 66.50 U/L, range 15-600 U/L, respectively, *p*=0.001) (Figure 1B). Among alcoholics, 23 individuals (96%) had urinary TPS values higher than 42 U/L, 22 (92%) had levels higher than 100 U/L and 11 (46%) had levels higher than 279 U/L.

The ratio of urinary TPS to urinary creatinine was also higher in alcoholics than in controls (median 4.63, range 0.73-24.99 versus median 0.50, range 0.05-5.01, respectively, *p*<0.001) (Figure 1C).

Table I. Routine laboratory tests in alcoholics and healthy controls.

Test	Alcoholic patients (n=24)	Healthy controls (n=15)
Serum AST (U/L)	52 (28-79)	12 (10-14)
Serum ALT (U/L)	32 (18-42)	13 (9-19)
Serum GGT (U/L)	195 (81-512)	8 (4-21)
Serum bilirubin (mg/dL)	1.5 (0.9-3.2)	0.5 (0.3-0.8)
Quick's index (%)	84 (60-93)	97 (92-100)
Serum albumin (g/dL)	3.6 (3-3.9)	4.4 (4.2-4.5)
Red blood cell mean corpuscular volume (fL)	103 (96-107)	90 (85-93)

Figures are medians and interquartile ranges (within parentheses). Upper normal serum levels for AST, ALT and GGT are 26 U/L, 29 U/L and 38 U/L, respectively. For all comparisons between alcoholics and healthy controls, *p*<0.005.

*Relationship between serum and urinary TPS levels.* Among alcoholics, there was a positive correlation between serum TPS and urinary TPS (*Rho*=0.392; *p*=0.05). No such correlation was observed among controls (*Rho*=-0.167; *p*=0.55). Similarly, linear regression analysis showed a trend of increasing urinary TPS levels along with serum TPS levels among alcoholics (Figure 2A). This phenomenon was not observed among controls (Figure 2B). The ratio of urinary TPS (U/L) to serum TPS (U/L) was higher in controls than in alcoholics (median 2.24, range 0.37-22.27 versus median 0.90, range 0.03-9.15, respectively, *p*=0.007).

## Discussion

The results of this study confirmed previous reports indicating that serum TPS levels are markedly elevated in alcoholics (12, 16). Here, the vast majority of alcoholic patients showed high (>80 U/L) and one-fifth showed extremely high (>1000 U/L) serum TPS levels. The median TPS values of alcoholics were 20 times higher than those of healthy controls.

To our knowledge, this is the first study to show that urinary TPS is elevated in alcoholics in comparison with healthy controls. The increase in serum TPS levels was present both in absolute terms and as a ratio to urinary creatinine. Thus, urinary TPS elevation in alcoholics was not an artifact induced by different urinary concentrations in alcoholic patients. Subjects with evidence or suspicion of urinary tract infection, malignant neoplasm or urinary manipulations were excluded from the study. Thus, the urinary TPS elevation in alcoholics did not seem to be due to an uncontrolled confounding factor. Median urinary TPS values in alcoholics were 4 to 9 times higher than those observed in controls. Furthermore, the magnitude of the elevation of urinary TPS in alcoholics exceeded, in many

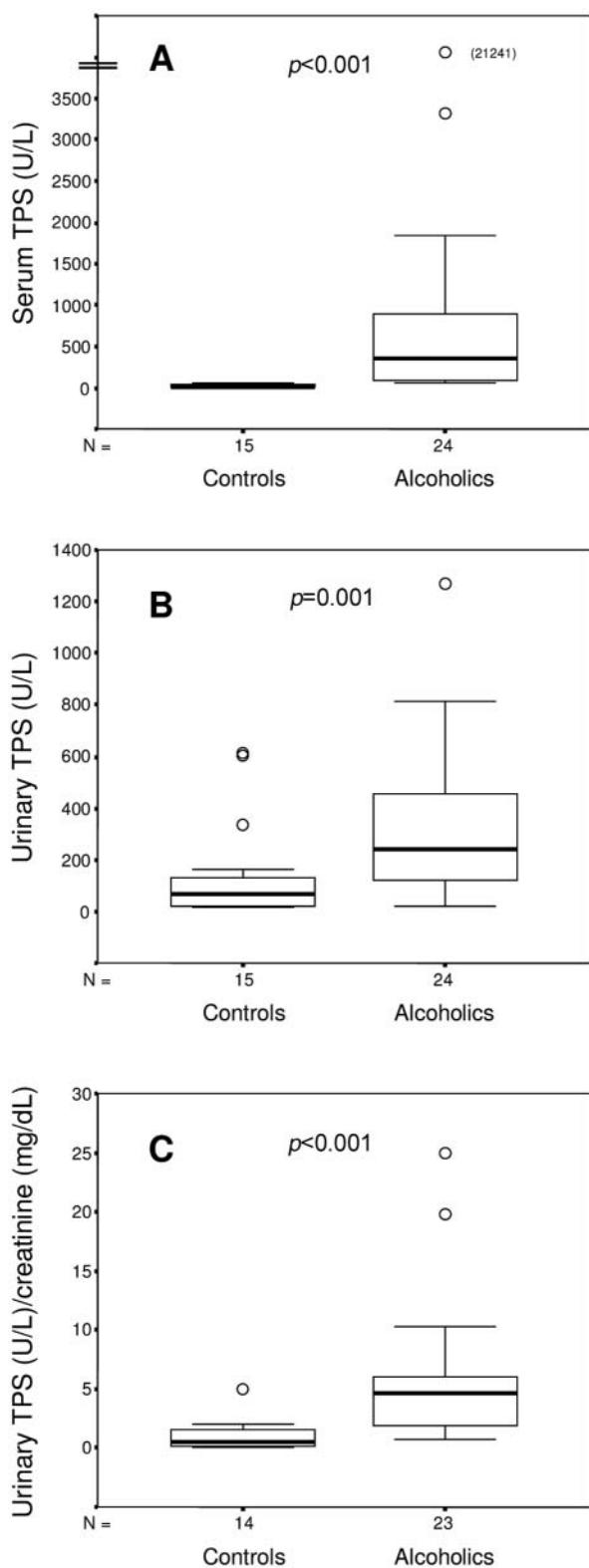


Figure 1. Boxplot of TPS levels in alcoholics and healthy controls: (A) serum TPS, (B) urinary TPS, (C) ratio of urinary TPS to urinary creatinine (note that urinary creatinine level was not available in one alcoholic and one control subject).

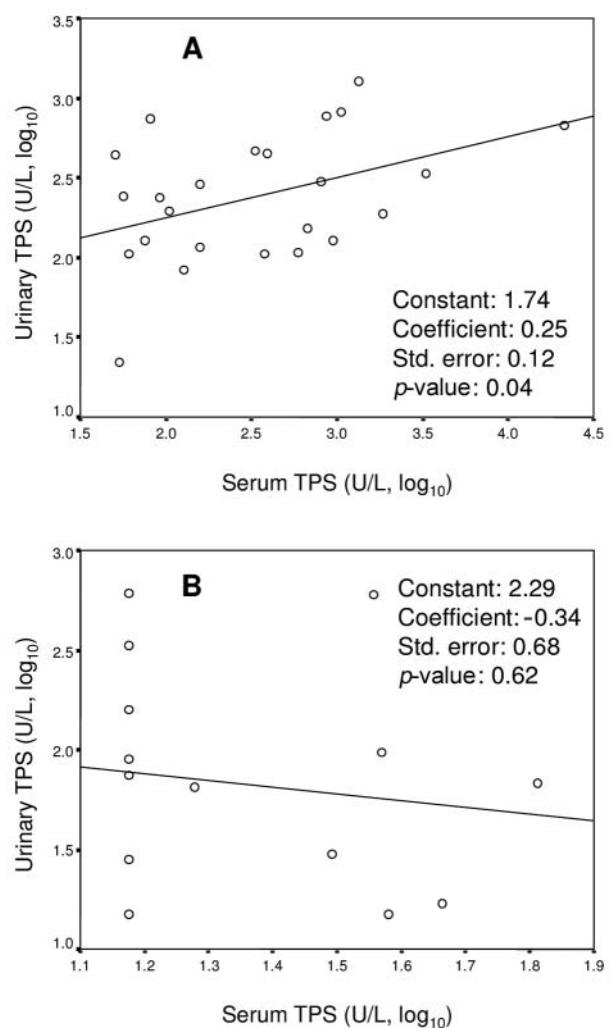


Figure 2. Scatterplot and linear regression of urinary TPS levels in relation to serum TPS levels in alcoholic patients (A) and healthy controls (B). Note that the TPS values were  $\log_{10}$ -transformed.

cases, the cut-off levels established in previous studies investigating the diagnostic value of urinary TPS in urothelial malignancies (18-20).

The mechanisms of urinary TPS elevation in alcoholics cannot be inferred from the results of the present study. Theoretically, urinary TPS may originate from cytokeratin-18 release from urothelial cells or from glomerular filtration of cytokeratin-18 fragments. No arguments support a direct effect of alcohol on urothelial cells that favors cytokeratin release. Glomerular filtration of cytokeratin-18 fragments seems the more likely. The molecular size of cytokeratin-18 possibly precludes glomerular filtration, but it should be noted that the TPS assay detects a specific antigenic portion of the molecule (2). Fragmentation and release of cytokeratin-18 occurred during apoptosis (24, 25).

Moreover, apoptosis was involved in alcoholic liver disease (26). Along this line, in the present study, serum TPS levels were correlated with urinary TPS levels in alcoholics but not in controls, and the ratio of urinary TPS to serum TPS was higher in controls than in alcoholics. Taken together, these results suggest that, under normal conditions, most urinary TPS originates from the urinary tract but, under pathological conditions (such as alcoholism) that increase serum levels of cytokeratin-18 or its fragments, some urinary TPS could originate from glomerular filtration. This hypothesis, however, is untested and warrants further study.

Alcohol abuse is relatively common in some populations and, importantly, alcohol consumption may be linked to smoking, the main risk factor for urothelial malignancies. In summary, our results suggest that alcoholism could be a limitation to the specificity of urinary TPS as a tumor marker.

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