CORRESPONDENCE

Urinary Levels of Urokinase-Type Plasminogen Activator and Its Receptor in the Detection of Bladder Carcinoma

We read with great interest the report by Casella et al.[1] regarding the levels of urokinase (uPA) and its receptor (uPAR) in urine from patients with bladder carcinoma. The authors concluded that enhanced levels of uPA and uPAR are present in patients with transitional cell carcinomas of the bladder but that only uPA levels are risk-related. To our knowledge, we were the first to report that suPAR, the soluble form of uPAR, is present in urine and that the measurement of suPAR in urine from patients with malignant disease may have clinical significance.[2] In that study, we demonstrated that in contrast to serum levels, urine levels of suPAR fluctuate considerably across multiple samplings. In fact, fivefold disparities in these levels between samples obtained from the same individual within 6 hours of each other were not uncommon. We also demonstrated that the ratio of urine suPAR concentration to urine creatinine concentration was considerably more stable, even over extended periods (i.e., on the order of 1 month). These findings were confirmed in subsequent studies.[3] Therefore, we would like to express our concern regarding the validity of the suPAR concentration data used in the study performed by Casella and colleagues.

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Author Reply

We thank Dr. Sier and colleagues for their candid letter regarding our study.[1]

We chose not to control for kidney function; this decision was based on the assumption that for a marker to be clinically useful and to add predictive information to the information yielded by current tests, the marker should have a robust performance in all patients regardless of kidney function. We found that increased urine levels of urokinase (uPA) were associated with an increased risk of bladder carcinoma, whereas urine levels of the urokinase receptor (uPAR) were not. Urine levels of uPA have not been shown to fluctuate from sampling to sampling. Therefore, we concluded that urine levels of uPA represent a potentially valuable marker for bladder carcinoma detection. We found no association between urine levels of uPAR and the presence of bladder carcinoma or pathologic features. In our opinion, this finding, together with the dependence of urine levels of uPAR on sampling time (as Dr. Sier and colleagues appropriately have noted), supports the idea that urine levels of uPAR possess limited clinical utility in the diagnosis, staging, and management of bladder carcinoma.

To address the specific issue raised by Dr. Sier and colleagues, we retrieved the majority of the samples used in the original study and repeated the analysis with adjustments for creatinine levels. Using a system manufactured by Olympus America (Melville, NY), we measured creatinine levels for 170 of the original 229 patients in the voided urine samples used for measurement of uPAR and uPA levels. The mean urine creatinine concentration was standard deviation (SD) 73.1 ± 46.4 mg/dL (median, 65.2 mg/dL; range, 9.8–236.1 mg/dL). All samples were run in duplicate, with the mean value used for data analysis. Differences between duplicate measurements were minimal, as indicated by the intraassay precision coefficient of variation, which was equal to SD 5.8 ± 8.7%. Levels of uPA and uPAR (both in ng/mL) were divided by urine levels of creatinine (in mg/dL) to adjust for the effect of kidney function.

We found that both the uPA/creatinine ratio ($P < 0.001$) and the uPAR/creatinine ratio ($P = 0.010$) were greater in patients with bladder tumors compared with control patients. However, whereas there were significant differences between case patients and control patients in terms of the quartile distributions of the uPA/creatinine ratio ($P = 0.002$), there were no such differences with respect to the uPAR/creatinine ratio. In addition, uPA/creatinine ratios were higher among patients with abnormal urine cytology findings ($P = 0.038$), whereas uPAR/creatinine ratios were not associated with any clinicopathologic characteristics. The uPAR/creatinine ratio was significantly (but moderately) correlated with urine uPAR levels (correlation coefficient [CC], 0.496; $P < 0.001$), and the uPA/creatinine ratio was strongly correlated with urine uPA levels (CC, 0.904; $P < 0.001$). In univariate logistic regression analyses, uPA/creatinine ratio ($P < 0.001$), age ($P < 0.001$), and positive urinary cytology ($P = 0.006$), but not uPAR/creatinine ratio, were found to be associated with an increased risk of transitional cell carcinoma of the bladder. Using a multivariate logistic regression model, with adjustments made for the effects of urine uPAR levels and age, only increased uPA/creatinine ratio ($P = 0.003$) and positive cytology ($P < 0.001$) were found to be associated with the presence of bladder carcinoma. In conclusion, we confirmed that increased urine levels of uPA are associated with the presence of bladder carcinoma, regardless of kidney function, and that urine levels of uPAR are not associated with bladder carcinoma, even after adjustments for urine levels of creatinine are made.

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Pure versus Follicular Variant of Papillary Thyroid Carcinoma

Clinical Features, Prognostic Factors, Treatment, and Survival

I read with interest the article regarding papillary thyroid carcinoma by Zidan et al.[1]

The premise that these two types of papillary thyroid carcinoma behave in similar fashion was fostered by the results of this study. Unfortunately, there are several flaws that call into question the conclusions reached by the authors.

First, there actually is no classic or usual papillary thyroid carcinoma that is "pure" papillary. Careful pathologic examination will always disclose some follicular pattern.

Second, the percentage of follicular variant tumors in this series is remarkable (approximately 40%). This either could reflect some unusual referral pattern or perhaps epidemiologic variations. However, I believe the problem may lie with the morphologic definition of follicular variant papillary thyroid carcinoma used by the authors. Their study defines follicular variant as a tumor with "pure follicular architecture occupying at least 80% of the neoplasm…".[1] No reference is given for this definition. The initial modern description of the follicular variant of papillary thyroid carcinoma indicates that the entire tumor be follicular in pattern[2]; most expert thyroid pathologists adhere to this and allow only for the presence of occasional abortive papillae in such tumors. Hence, a tumor that was up to 20% nonfollicular (and I assume therefore papillary) is a papillary thyroid carcinoma of classic or usual type and not a follicular variant.

Third, the definition of papillary thyroid carcinoma used by the authors refers to nuclear features of the tumor cells. The authors state: "The nuclei of papillary carcinoma are enlarged and ovoid and contain thick nuclear membranes, small nuclei (*I assume the authors mean nucleoli*) that often are pressed against the nuclear membrane, intranuclear grooves, and intranuclear cytoplasmic inclusions. The nuclei frequently overlap each other." Here the authors reference the World Health Organization (WHO) classification.[3] However, the authors then define their follicular variant tumors as showing "…at least 2 nuclear features typical of papillary carcinoma." Again, no reference is cited.

Follicular tumors of the thyroid that have some but not all of the nuclear features of papillary carcinoma have engendered much debate and discussion.[4,5] In fact, one group has offered that tumors having only some of the nuclear features should be considered either benign or as follicular tumors of uncertain malignant potential[6]; they should not be included in a group of unequivocal papillary thyroid carcinomas.

Therefore, I want to express concern with regard to the conclusions reached by the authors of this article because the pathologic definitions are so nonconventional. If they have classified follicular tumors that are indeed adenomas as papillary carcinomas, obviously the clinical outcomes would be different than for true carcinoma. Similarly, if they categorize usual papillary thyroid carcinomas as follicular variants and then use this group of cases for comparison with "pure" papillary thyroid carcinoma outcomes, they are further confusing the issue.

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Author Reply

In response to Dr. LiVolsi's first comment concerning our article,[1] papillary thyroid carcinomas include both mixed papillary-follicular tumors and those that contain purely papillary carcinoma cells. This is true even when the mixed tumor is comprised almost entirely of follicular elements (i.e., when characteristic features such as ground glass nuclei and psammoma

bodies are present). This pathology definition was used by Simpson et al. in a study of 1578 patients with thyroid carcinoma.[2] In our study, the use of the term "pure" papillary carcinoma was interchangeable with "usual" papillary carcinoma that is not of the follicular variant.[1]

In response to Dr. LiVolsi's second comment, we would like to point out that in our study the percentage of follicular variant of papillary thyroid carcinoma (FVPTC) was 41%. A search of the English language literature determined the percentage of FVPTC to be between 13% to $>$ 80% of all papillary thyroid carcinoma cases.[3,4]

The definition of follicular variant as a tumor with pure follicular architecture occupying at least 80% of the neoplasms and with at least 2 nuclear features typical of papillary carcinoma (i.e., "ground glass" nucleoplasm, pseudoclear nuclei, overlapping nuclei, and grooved nuclei) was used by Carangiu et al.[5] In any case, according to most expert thyroid pathologists, the difference in defining FVPTC is negligible and could include the definition suggested by Dr. LiVolsi.

Third, our statement "The nuclei of papillary carcinoma are enlarged and ovoid and contain thick nuclear membranes, small nuclei…" does indeed contain a typographical error and should read "small nucleoli." The corrected text describes the World Health Organization classification,[6] which is widely accepted. The statement "at least 2 nuclear features typical of papillary carcinoma" referred to is a part of the previously mentioned definition of FVPTC.[5]

We disagree with Dr. LiVolsi's expressed concern with regard to our conclusions. It should be noted that although our study emphasized the clinical behavior of the disease, all slides were carefully read and analyzed by experts in thyroid pathology. All acceptable pathology criteria were utilized. It is widely accepted that to distinguish between follicular carcinoma and benign follicular adenoma, histologic examination must be performed and invasion through the tumor capsule or vascular invasion must be demonstrated. Accordingly, the false-positive and false-negative rates for nodules characterized as malignant and benign, respectively, are reported to be $<$ 5%.[7] This low percentage of error probability will not change our results significantly.

Our study clarifies and confirms the conclusions of several previous studies that have documented that mixed tumors (FVPTC) with any areas of papillary features have the same natural history and prognosis as papillary thyroid carcinoma without follicular features.[8]

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Echinococcus against Cancer: Why Not?

Hydatid disease caused by the tapeworm *Echinococcus granulosus* is a frequently encountered parasitic infection in endemic areas of the world, such as the Mediterranean countries. The prevalence rate is reported to be approximately 1–2% in Turkey.[1] Despite this high figure, we have noticed an exceedingly low rate of incidental diagnoses of hydatid disease among patients undergoing surgery for various solid tumors. Based on this initial observation, we screened the medical reports of patients who have been operated on for various solid tumors to determine the prevalence of hydatid disease.

A total of 2086 patients who underwent surgery for various solid tumors between 1990 and 2001 were evaluated to determine the prevalence of hydatid disease. Prior to surgery, each patient underwent a full medical examination, diagnostic imaging using chest X-ray, abdominal ultrasonography, computed abdominal and

thoracal tomography for clinical staging, laboratory investigations including complete blood count, and liver and kidney function tests, which should be sufficient for the evaluation of hydatid disease. In addition, 350 patients who were admitted for various trauma-associated conditions without any apparent health problem were evaluated for hydatid disease. All trauma patients also were examined using the same diagnostic criteria as the cancer patients. In an attempt to evaluate the presence of cancer at the time of diagnosis of *Echinococcus granulosus*, we screened the medical reports of a total of 1000 patients who were treated for hydatid disease between 1968 and 2001.

The coexistance of both conditions (cancer and hydatid disease) was observed in only 2 patients: a 52-year-old female who was diagnosed with primary gastric carcinoma who had no family history and a 54-year-old man with liver carcinoma who was not positive for any liver carcinoma risk factors. Both patients were found to have liver hydatidosis, and cystectomy was performed for pathologic verification of the diagnosis at the time of definitive cancer surgery. However, among the 350 trauma patients, 7 patients (2%) were found to be positive for hydatid disease. This frequency is similar to a previously reported population study.[1] These results indicate that the prevalence of hydatid disease was significantly lower than was expected in the cancer patients. Only 2 patients were found to be positive for hydatid disease, whereas at least 21 patients were expected to be positive.

In the group of patients diagnosed with hydatid disease, we identified only 1 patient who underwent surgery for esophageal carcinoma, but this occurred 8 years prior to the diagnosis of hydatid disease.

Suppression of neoplastic growth via infectious agents has been observed for bacterial (*Listeria monocytogenes*, *Corynebacterium parvum*) and protozoan (*Toxoplasma gondii*, *Besnoitia jellisoni*) pathogens. Stimulation of the immune response (such as nonspecific macrophage activation to kill tumor cells or systemic inhibition of angiogenesis) has been put forward to explain the biologic basis of these observations.[2] It is interesting to note that an antigenic similarity between various tumors and cysts of *Echinococcus granulosus* infection was reported[3,4] for cancer-associated O-glycosylated Tn antigen.[5] Tn antigen is a glycoprotein that is expressed during the early phases of various malignancies, including carcinomas of the breast, pancreas, lung, gastrointestinal tract, upper aerodigestive tract, and genitourinary tract, as well as melanoma, thymoma, various leukemia/lymphomas, sarcoma, and central nervous system tumors. Therapeutic vaccination of patients with advanced breast carcinoma using Tn autoantigen produced encouraging results, and $CD8^+$ T-cell–mediated cytotoxic immunity was proposed to play a role in the protection against cancer.[5] In our study, we were unable to determine the follow-up outcome for patients with hydatid disease; at the same time, initial studies for detecting hydatid disease were not adequate for detecting malignancy. Based on these observations, we propose that *Echinococcus granulosus* may elicit a protective effect against the development of cancer through common antigenic properties with cancerous cells including the Tn antigen. However, despite these interesting findings, we believe a well designed prospective study and experimental studies would be more suitable to determine this relation.

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