Urinary pH and the risk of recurrence in patients with non-muscle invasive bladder cancer

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Introduction: *To evaluate the effect of urine pH on tumor recurrence rates in patients undergoing surveillance after initial diagnosis of non-muscle invasive bladder cancer (NMIBC).*

Materials and methods: *All patients diagnosed with NMIBC at a tertiary referral center from January 2004 to March 2015 were reviewed. Our primary outcome was time to first recurrence after transurethral resection of bladder tumor (TURBT). Patients were analyzed according to the average urine pH of all urinalysis data over the surveillance period from TURBT to first recurrence. Kaplan-Meier survival analysis was used to determine differences in median time to recurrence. Cox proportional hazards regression was used to assess independent predictors of cancer recurrence.*

Results: *A total of 252 patients were included, of which 155 patients had average pH ≤ 6 (median pH 5.5) and 97 patients had average pH > 6 (median pH 6.8), p < 0.001. There was no significant difference in median time to recurrence between low/acidic pH (≤ 6) and high/basic pH (> 6) groups (28 months versus 17 months, respectively, p = 0.3444). Similarly, urine pH did not affect the risk of recurrence in a subgroup analysis stratified by smoking status. On multivariable Cox regression analysis, there was no association between average pH and recurrence among high grade tumors (HR = 1.33, 95% CI = 0.76 to 2.34, p = 0.3186), or low grade tumors (HR = 1.013, 95% CI = 1.01 to 1.58, p = 0.96).*

Conclusions: *There was no association between urine pH and risk of tumor recurrence, regardless of smoking status. These findings suggest that modification of urine pH is unlikely to decrease the frequency of tumor recurrence in patients with NMIBC.*

Key Words: bladder cancer, local neoplasm recurrence, urinary pH

Introduction

In 2018, it is estimated that approximately 81,190 people will be diagnosed with bladder cancer,[1] a majority of which will be diagnosed with non-invasive disease.[2] Recurrence rates for non-muscle invasive bladder cancer (NMIBC) are 50%-70%, and approximately 45% of those patients will recur in the first year after transurethral resection of bladder tumor (TURBT) alone.[3,4] Due to the high rate of recurrence and need for lifelong monitoring and treatment, the cost per patient of bladder cancer from diagnosis to death is the highest of all malignancies.[5] The cost of NMIBC is even more significant than that of higher-stage disease due to long-term surveillance protocols.[6] Therefore, identifying novel interventions that have the potential to reduce the risk of recurrence would be of particular value for both healthcare spending and patient outcomes.

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More specifically, bladder cancer presents a unique opportunity to investigate the effect of an acidic environment on carcinogenesis, as the bladder urothelium is chronically exposed to urine, where the pH may serve as a regulator of the tumor microenvironment. Chronic exposure to an acidic environment has been demonstrated to facilitate tumor cell invasiveness, metastatic potential, and resistance to chemotherapeutic agents,[7–10] thus the utilization of pH-related cancer therapeutics has been suggested as a novel approach to cancer therapy.[11] Furthermore, urine is a readily modifiable pH environment, and this application has shown efficacy in treating and preventing kidney stones.[12]

Limited evidence exists on the role of urine pH as it pertains to bladder cancer.[13] While some studies have suggested a potential role of acidic pH in modifying the risk associated with cigarette smoking and other carcinogenic compounds,[14,15] others have found no association between acidic urine and bladder cancer risk, even among smokers.[16,17] However, to our knowledge there are currently no studies primarily evaluating the role of urine pH on tumor recurrence in patients diagnosed with bladder cancer. We aim to evaluate the effect of urine pH on tumor recurrence rates in patients with high grade and low grade papillary urothelial carcinoma, undergoing surveillance after initial diagnosis of NMIBC.

Material and methods

Study population

Patients diagnosed with NMIBC at a tertiary referral center from January 2004 to March 2015 were retrospectively reviewed. Patients with muscle invasive disease, non-urothelial histology, or carcinoma in situ (CIS) were excluded. We excluded patients with any component of CIS on histologic analysis of their initial TURBT specimen because CIS represents a biologically distinct disease process from Ta and T1 urothelial carcinoma, with differing treatment paradigms and patterns of recurrence and progression.[18] We collected data on patients receiving intravesical bacillus calmette-guerin (BCG); patients receiving mitomycin C after TURBT were excluded. Standard surveillance protocol included cystoscopy every 3 months for the first year after TURBT, every 6 months for the subsequent year, and annual cystoscopy thereafter if no evidence of disease recurrence. Urine cytology was obtained at each follow up visit. Cystoscopy was performed more frequently for tumor recurrence at the discretion of the surgeon.

Primary outcomes

Our primary outcome of interest was time to first recurrence after TURBT. Recurrence was defined by visual confirmation on cystoscopy or biopsy.

Assessment

We collected data on patient demographics, smoking status, tumor grade on initial TURBT, presence of CIS, and other treatment including intravesical BCG. Histopathology was reported according to the WHO 2004 classification[19] after assessment by a dedicated genitourinary pathologist. For assessment of urine pH, we utilized average pH of all urinalysis data during the surveillance period from TURBT to first recurrence.

Statistical analysis

Follow up time was calculated from initial TURBT to the date of first recurrence as determined by direct visualization or biopsy. Patients that were lost to follow up or had no evidence of recurrence during the study period were right censored. Patients' pH values were calculated by taking the average of available pH values following each patient's decisive tumor resection. Descriptive statistics (including median, interquartile range (IQR), frequency, and percent) for patient characteristics and clinical variables were produced.

Kaplan-Meier survival analysis and the log-rank test was used to determine the difference in median time to recurrence between low/high pH levels. We compared patients with average pH ≤ 6 (low/acidic) versus > 6 (high/basic), consist with previously used pH cutoff values.[14] The median time to recurrence and associated 95% confidence interval were estimated for both groups. Previous studies have suggested a heterogeneous effect of urine pH on bladder cancer risk in smokers versus non-smokers,[14,15] and we further performed a subgroup Kaplan-Meier survival analysis to assess median time to recurrence between low/high pH levels in smokers and in non-smokers.

Cox proportional hazards regression was used to estimate the independent effect of predictors on cancer recurrence. Hazard ratios and associated 95% confidence intervals were estimated. The proportional hazards assumption was tested by visual inspection of the Kaplan-Meier plot as well as by including a time-dependent co-variate in the Cox model. In the multivariable Cox regression model, we adjusted for smoking status and BCG. Due to the clinical relationship between BCG and tumor grade, we stratified patients on tumor grade (high and low). We assessed the interaction between smoking status and pH level. All p values are two-sided. All statistical analyses were performed with SAS Version 9.4 (SAS Institute, Cary, NC, USA).

TABLE 1. **Patient characteristics - total cohort and stratified by average pH**

Variables	Total cohort (n = 252)	pH status pH ≤ 6 (n = 155)	pH > 6 (n = 97)	p value
Mean age at presentation (SD)	70.4 (9.9)	70.2 (10.2)	70.8 (9.5)	0.6519
Mean BMI (SD)	26.2 (4.6)	26.8 (5.0)	25.3 (3.80)	0.0148*
Smokers - no. (%)	180 (71.4)	114 (63.3)	66 (36.7)	0.3464
Median pack-years (IQR)	34.5 (15-54)	32.5 (15-60)	34.5 (22-50)	0.8178
BCG - no. (%)	110 (43.7)	64 (58.2)	46 (41.8)	0.3399
High tumor grade - no. (%)	134 (53.2)	80 (59.7)	54 (40.3)	0.5300
Median follow up (years) (IQR)	9 (3-23)	9.5 (3-26)	9 (3-20)	0.9736
Median average pH (IQR)	6 (5.5-6.5)	5.5 (5.5-6.0)	6.8 (6.5-7.13)	< .0001*

BMI = body mass index; BCG = bacillus calmette-guerin

Results

Mean age was 70.4 years old (SD 9.9). A total of 155 (62%) patients had average pH ≤ 6 (median pH 5.5, IQR 5.5-6.0) and 97 (38%) patients had average pH > 6 (median pH 6.8, IQR 6.5-7.1), p < 0.001. Once stratified by average pH, the groups were similar, as there was no significant difference in key patient and tumor variables such as smoking history, tumor grade, or BCG use (all p values > 0.05, Table 1). Patients in the acidic pH group did have a slightly greater body mass index (BMI) (26.8 versus 25.3, p = 0.015), though this difference was likely clinically insignificant.

On multivariable Cox regression analysis, we observed no association between average pH and bladder cancer recurrence among patients with high grade tumors (HR = 1.33, 95% CI = 0.76 to 2.34, p = 0.3186), adjusting for BCG use and smoking, Table 2. Similarly, pH was not associated with recurrence of bladder cancer among patients with low grade tumors (HR = 1.013,

TABLE 2. **Multivariable Cox regression analysis evaluating the effect of pH as a continuous variable on recurrence, adjusted for BCG and smoking – among HIGH tumor grade cohort (n = 134)**

Parameter	Hazard ratio	95% hazard ratio	Confidence limits	p value
Average pH	1.078	0.730	1.592	0.7042
BCG use	1.235	0.688	2.217	0.4788
Smoking	1.435	0.794	2.595	0.2321

BCG = bacillus calmette-guerin

TABLE 3. **Multivariable Cox regression analysis evaluating the effect of pH as a continuous variable on recurrence, adjusted for BCG and smoking – among LOW tumor grade cohort (n = 118)**

Parameter	Hazard ratio	95% hazard ratio	Confidence limits	p value
Average pH	0.977	0.660	1.447	0.9090
BCG use	2.424	1.401	4.193	0.0015*
Smoking	0.685	0.364	1.289	0.2407

BCG = bacillus calmette-guerin

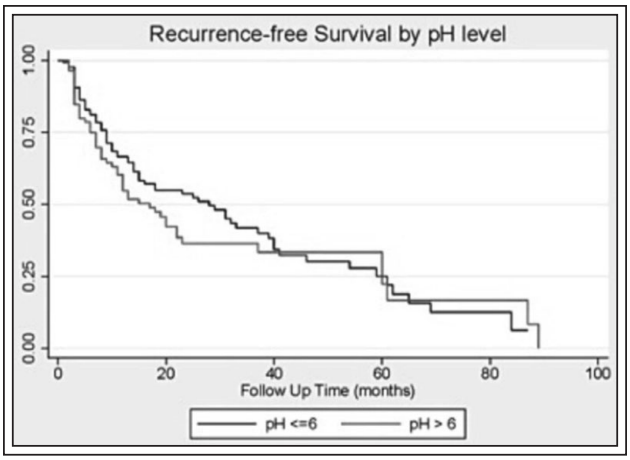

Figure 1. Kaplan-Meier analysis of recurrence-free survival in patients with average pH ≤ 6 (black) vs. > 6 (gray).

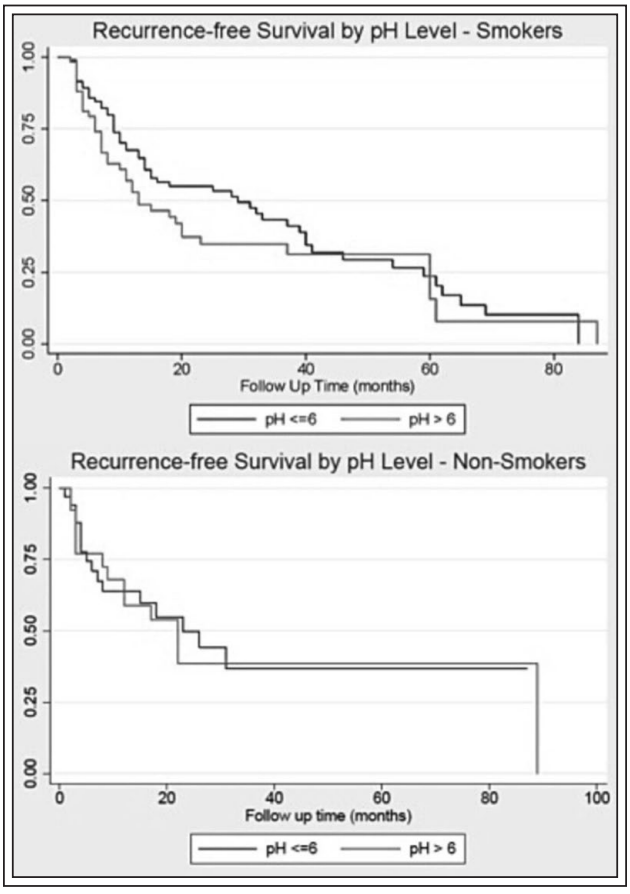

Figure 2. Subgroup Kaplan-Meier analysis of recurrence-free survival in patients with average pH ≤ 6 (black) vs. > 6 (gray) in smokers **(A)** and non-smokers **(B)**.

95% CI = 1.01 to 1.58, p = 0.96, Table 3). There was no significant evidence of interaction between pH and smoking in the multivariable Cox regression model.

Kaplan-Meier survival analysis demonstrated no significant difference in median time to recurrence between acidic and basic pH groups (28 months versus 17 months, respectively; p = 0.34), Figure 1. A subgroup analysis was then performed to assess for differences in time to recurrence between pH groups after patients were stratified by smoking status. Among smokers (n = 180), we found no significant difference in median time to recurrence between acidic and basic pH groups (29 months versus 13 months, respectively; p = 0.23), Figure 2a. Similarly, among non-smokers (n = 72), no difference in median time to recurrence was seen between acidic or basic pH groups (23 months versus 22 months, respectively; p = 0.88), Figure 2b.

Discussion

While the relationship between urine pH and bladder cancer incidence has been previously investigated, our study is the first to primarily evaluate the role of urine pH on recurrence rates in bladder cancer. In patients with non-muscle invasive urothelial carcinoma of the bladder, we did not find an association between urine pH and risk of tumor recurrence. Similarly, we found no interaction between smoking status and urine pH, and further found that urine pH did not affect the risk of recurrence in a subgroup analysis by smoking status.

The pH of the cellular microenvironment has been identified as an important feature in carcinogenesis across cancer subtypes, and has further delineated a potential role of pH modification in cancer therapy. The difference in pH between normal cells and cancer cells is thought to represent the most dramatic disparity yet discovered between normal physiology and cancer pathophysiology.[10] pH derangements are a common feature of most types of cancer, and it is hypothesized that the acidic tumor microenvironment generated through relative ischemia and high metabolic activity selects for tumor cells with adaptations, such as expression and activation of proton exchangers, which confers them a survival advantage.[20] Furthermore, acidic extracellular pH has been shown to facilitate cellular proliferation, apoptosis evasion, tumor invasion, and metastatic potential in melanoma and other malignancies.[7-9] Proton pump inhibitors (PPIs) have been utilized to modify the pH of the tumor microenvironment; PPIs are protective against carcinogenesis in Barrett's esophagus[21] and confer a survival advantage in head and neck squamous cell carcinoma.[22] They have also been shown to inhibit

cellular proliferation and induce apoptosis in vitro in gastric cancer,[23] hepatoblastoma,[24] multiple myeloma,[25] and triple-negative breast cancer.[26]

Despite the oncologic influence pH may have on cancer pathophysiology, the relationship of urine pH and bladder cancer remains largely unknown and widely under-investigated. Limited evidence exists on the role of urine pH and risk of developing bladder cancer, with conflicting results. Alguacil and colleagues investigated the role of urinary pH and smoking in association with bladder cancer risk, finding that consistently acidic urine (defined as multiple pH readings ≤ 6.0 on home collected urine dipsticks) increased the risk of bladder cancer [odds radio (OR) = 1.5, 95% confidence interval (CI): 1.2-1.9]. They also found a significant interaction between smoking and urine pH, with acidic pH increasing the odds of bladder cancer among smokers,[14] thereby citing experimental evidence that acidic conditions can influence the presence of carcinogenic compounds (free aromatic amines) derived from tobacco smoke[15] as a potential explanation for their observations.

In contrast, other studies have questioned the role of urine pH in bladder cancer. In a case-control study in Japan, Wada et al found no association between acidic urine and bladder cancer risk overall (OR = 0.87, 95% CI: 0.39-1.93) or even among smokers (OR = 0.74, 95% CI NR), though they only obtained a single measure of urine pH after cancer diagnosis.[16] Although they did not directly measure urine pH, Wright and colleagues looked at estimated net renal acid excretion as an indirect measure of urine pH, finding no overall association with bladder cancer risk among smokers from a population-based nutrition survey. They found only a weak association in the cohort with the most acidic urine and over 45 years of smoking history (OR = 1.72, 95% CI: 0.96-3.10). They concluded that although a certain subset of individuals may be at increased risk, urine pH is not a major risk factor for bladder cancer.[17]

While studies such as these have examined the relationship of urine pH and risk of bladder cancer, ours is the first to directly evaluate whether urine pH may play a role in recurrence after diagnosis of non-invasive disease. Urine pH is associated with a number of metabolic syndrome features (BMI ≥ 30 kg/m^2, waist circumference > 88 cm, etc.),[27] and obesity is additionally associated with disease recurrence, progression, and cancer-specific mortality in NMIBC.[28] Moreover, in the study by Maeda et al, which focused on Mitomycin C's intravesical efficacy as it relates to urinary pH, it was discovered that both low urine pH and tumor multifocality were independently associated with recurrence in patients with Ta/T1 bladder cancer.[13] However, it is unclear from this study whether urine pH during the entire period of follow up (i.e. not just during periods of intravesical mitomycin C instillation) influence tumor recurrence.

Ultimately, the strengths of our study revolve around our well-annotated clinical database which allowed us to capture, assess, and account for clinically meaningful disease parameters such as tumor grade, presence of CIS, and intravesical treatment. Furthermore, our urine pH values were determined by an average of multiple urine samples collected and processed by a central laboratory. Other studies have relied on patient reported urine pH values,[14] which are subject to reporting bias, or relied on questionnaire-based information to determine an indirect measure of urine pH.[17] In our study, all care was performed at a single center, which ensured a standardized surveillance protocol for detection of tumor recurrence.

The findings of our study must be interpreted within the context of the study design. Urine pH was determined by the average urine pH from urinalysis data over the surveillance period. While we feel this gives a more accurate representation of a patient's urine pH than a single or estimated value, it does not account for variability in exposure time to a given pH, although a temporal relationship between acidic pH and oncogenesis has yet to be established. Furthermore, we did not analyze for potential determinants of urine pH, such as body habitus, dietary intake, or medications[29] which although unproven, may potentially have independent effects on risk of tumor recurrence. Finally, this study represents a single-center series from a tertiary referral center, so it may not be truly representative of a larger, more heterogeneous patient population. Although a prospective study with a larger number of patients could address some of these limitations, these results still represent an important contribution to the limited literature regarding the association of urine pH and bladder cancer.

Conclusions

Our results show no association between urine pH and risk of tumor recurrence, regardless of smoking status. These findings suggest that based on our current knowledge, targeting urine pH as an intervention strategy is unlikely to decrease the frequency of tumor recurrence in patients already diagnosed with non-muscle invasive urothelial carcinoma of the bladder. Further study is needed to improve our understanding of the role of pH in bladder cancer oncogenesis as we continue to investigate strategies to improve cancer control. □

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