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# Intravesical Seeding of Upper Urinary Tract Urothelial Carcinoma Cells During Nephroureterectomy: An Exploratory Analysis from the THPMG Trial

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**Objective:** The Pirarubicin Monotherapy Study Group trial was a randomized Phase II study that evaluated the efficacy of intravesical instillation of pirarubicin in the prevention of bladder recurrence after nephroureterectomy for upper urinary tract urothelial carcinoma. This study conducted further analysis of the Pirarubicin Monotherapy Study Group cohort, focusing on intravesical seeding of cancer cells.
**Methods:** Using the data from the Pirarubicin Monotherapy Study Group trial, bladder recurrence-free survival rates and factors associated with bladder recurrence in the control group were analyzed.
**Results:** Of 36 patients in the control group, 14 with positive urine cytology had more frequent recurrence when compared with the 22 patients with negative cytology ($P = 0.004$). Based on the multivariate analysis in the control group, voided urine cytology was an independent predictive factor of bladder recurrence (hazard ratio, 5.54; 95% confidence interval 1.12–27.5; $P = 0.036$). Of 72 patients in the Pirarubicin Monotherapy Study Group trial, 31 had positive urine cytology. Among the 31 patients, 17 patients who received pirarubicin instillation had fewer recurrences when compared with 14 patients who received control treatment ($P = 0.0001$). On multivariate analysis, pirarubicin instillation was an independent predictor of better recurrence-free survival rates in the patients with positive urine cytology (hazard ratio, 0.02; 95% confidence interval, 0.00–0.53; $P = 0.018$). Of 21 patients with bladder recurrence, 17 had recurrent tumor around cystotomy or in the bladder neck compromised by the urethral catheter, supporting the notion that tumor cells seeded in the injured urothelium.
**Conclusions:** Intravesical instillation of pirarubicin immediately after nephroureterectomy significantly reduced the bladder recurrence rate in patients with positive voided urine cytology. The results suggest that intravesical seeding of upper urinary tract urothelial carcinoma occurs during nephroureterectomy.

*Key words: upper urinary tract urothelial carcinoma – intravesical seeding – voided urine cytology*

## INTRODUCTION

Following radical nephroureterectomy for upper urinary tract urothelial carcinoma (UUT-UC), ~20–50% of patients experience bladder recurrence (1–5). Two hypotheses have been proposed to explain subsequent bladder recurrence after nephroureterectomy: intraluminal seeding and implantation of cancer cells (6,7) and field cancerization (8,9). Some molecular studies of tumor cells from upper urinary tract and subsequent bladder tumors in patients with multifocal disease suggested that seeding or intraepithelial spread is a major mechanism for the multifocal development of urothelial cancer (10,11).

The Pirarubicin (THP) Monotherapy Study Group (THPMG) trial was a randomized phase II study that evaluated the efficacy of intravesical instillation of THP in the prevention of bladder recurrence after nephroureterectomy (12). The trial reported that a single instillation of THP within 48 h of nephroureterectomy was independently associated with a significantly reduced rate of bladder recurrence, and that the antitumor effect of THP instillation prevented intraluminal seeding and implantation of cancer cells from UUT-UC.

A number of factors, including tumor multiplicity, location, stage, grade, operative modality, gender and other factors, influence bladder recurrence in these patients (1,2,4,13–17). Recent reports indicated that positive preoperative urine cytology is a predictor of bladder recurrence following nephroureterectomy (16,17). Specifically, positive urine cytology (i.e. cancer cells from UUT-UC in the bladder) in patients who ultimately experience bladder recurrence supports the intraluminal seeding hypothesis of bladder recurrence (16).

The present study conducted an exploratory analysis of the THPMG trial cohort, focusing on intravesical seeding of cancer cells from UUT-UC.

## PATIENTS AND METHODS

The THPMG trial was a randomized phase II trial, in which patients clinically diagnosed with UUT-UC without a history or presence of bladder cancer were preoperatively randomly assigned to receive or not receive a single instillation of THP (30 mg in 30 ml of saline) into the bladder within 48 h after nephroureterectomy. Cystoscopy and urinary cytology were repeated every 3 months for 2 years or until the occurrence of first bladder recurrence. The details of trial eligibility, methods, objectives and results have been published previously (12). In the case of bladder recurrence, the location of recurrent tumor in the bladder was recorded. The study protocol was approved by the ethics committee of the Tohoku University Graduate School of Medicine and by the ethics committee or institutional review board of each participating institution. A written informed consent was obtained from all patients prior to participation in the study. The study protocol was registered and is available online at the University Hospital Medical Information Network Clinical Trials Registry website (www.umin.ac.jp/ctr/index.htm). The registration number of this trial is UMIN000004039.

The present analyses were based on the data obtained from the THPMG trial (THP group, $n = 36$; control group, $n = 36$). Preoperative voided urine cytology was performed on diagnosis of urothelial carcinoma. The number of voided urine collection for cytological examination ranged from one to three depending on the physician's discretion. Urine cytology was judged as 'positive' based on at least one positive finding among multiple examinations. In order to focus on intravesical seeding of cancer cells from UUT-UC, factors associated with bladder recurrence were analyzed in the control group. Bladder recurrence-free survival rates after nephroureterectomy were estimated using the Kaplan–Meier method. The log-rank test was used to compare recurrence-free survival rates between the two groups. Covariates included in the analyses were gender, age, tumor side, operation method (laparoscopic or open surgery), urinary cytology, histological type, pathological T stage, tumor grade, tumor location and adjuvant chemotherapy. Multivariate analyses using Cox proportional hazards were performed to identify independent predictors of bladder recurrence after nephroureterectomy. All reported $P$ values were two-sided, and the statistical significance was set at 0.05. Statistical analyses were performed with SAS Release 8.2 software (SAS Institute, Inc., Cary, NC, USA).

## RESULTS

The characteristics of all 72 patients are listed in Table 1. In a previous report of the THPMG trial (12), urinary cytology had included voided urine cytology and retrograde ureteral catheter cytology. In that paper, preoperative diagnosis of UUT-UC was mainly performed with voided urine cytology and computed tomography or retrograde pyelography, and diagnostic ureteroscopy was performed when UUT-UC was hard to diagnose by these examinations. Therefore, the term 'urinary cytology' was used to indicate cytology from voided urine or ureteral catheter for the purposes of describing the rate at which urinary cytology was used for diagnosis. In the present study, there was no statistically significant difference in the rate of positive voided urine cytology when comparing the THP and control groups ($P = 0.810$).

Figure 1 shows the bladder recurrence-free survival rates for patients with negative voided urine cytology and positive voided urine cytology in the control group. Of 36 patients in the control group, 14 patients with positive voided urine cytology had more frequent bladder recurrence when compared with the 22 patients with negative urine cytology (57.1% at 1 and 74.3% at 2 years vs. 14.4% at 1 and 20.1% at 2 years; log-rank $P = 0.004$). The median follow-up period was 8.4 months (range, 3.0–30.1 months) in patients with positive urine cytology and was 24.5 months (range, 2.8–32.2 months) in patients with negative cytology.

To elucidate the mechanism of intravesical seeding from UUT-UC, the factors associated with bladder recurrence in the control group were analyzed. Cox multivariate analyses of factors associated with bladder recurrence in the control group are described in Table 2. Based on the multivariate analysis in

**Table 1.** Patient characteristics

| | THP group $n = 36$ (%) | Control $n = 36$ (%) |
|---|---|---|
| Gender | | |
| M | 22 (61.1) | 21 (58.3) |
| F | 14 (38.9) | 15 (41.7) |
| Age (years) | | |
| <69 | 18 (50) | 19 (52.8) |
| ≥69 | 18 (50) | 17 (47.2) |
| Tumor side | | |
| Rt | 17 (47.2) | 21 (58.3) |
| Lt | 19 (52.8) | 15 (41.7) |
| Tumor site | | |
| Calix or pelvis | 21 (58.3) | 19 (55.9) |
| Pelvis and ureter | 2 (5.6) | 1 (2.8) |
| Ureter | 13 (36.1) | 16 (44.4) |
| Operation | | |
| Laparoscopic | 16 (44.4) | 17 (47.2) |
| Open | 20 (55.6) | 19 (52.8) |
| Voided urine cytology | | |
| Positive | 17 (47.2) | 14 (38.9) |
| Negative | 19 (52.8) | 22 (61.1) |
| Histology Type | | |
| UC | 33 (91.7) | 32 (88.9) |
| UC and SCC | 3 (8.3) | 2 (5.6) |
| UC and AC | 0 | 2 (5.6) |
| Grade | | |
| Low | 24 (66.7) | 15 (41.7) |
| High | 12 (33.3) | 21 (58.3) |
| Tumor stage | | |
| pTa | 10 (27.7) | 6 (16.7) |
| pT1 | 9 (25) | 14 (38.9) |
| pT2 | 6 (16.7) | 2 (5.6)2 |
| pT3 | 11 (30.6) | 14 (38.9) |
| Nodal status | | |
| pN0 | 20 (55.6) | 19(55.9) |
| pN1 | 0 | 1 (2.8) |
| pNx | 16 (44.4) | 16 (44.4) |
| Concomitant CIS | | |
| No | 32 (88.9) | 36 (100) |
| Yes | 4 (11.1) | 0 (0) |
| Adjuvant chemotherapy | | |
| Yes | 7 (19.4) | 7 (19.4) |
| No | 29 (80.6) | 29 (80.6) |

THP, Pirarubicin; M, male; F, female; UC, urothelial carcinoma; AC, adenocarcinoma; SCC, squamous cell carcinoma.

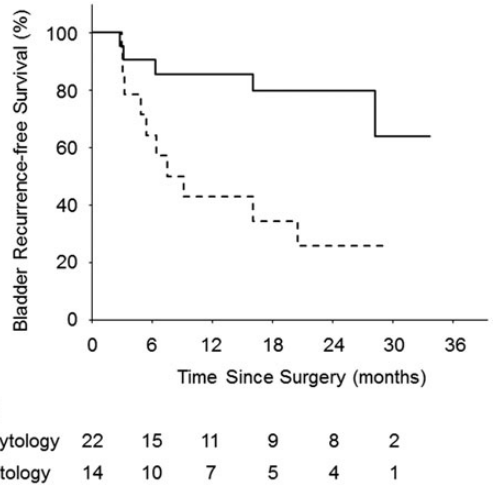



**Figure 1.** Bladder recurrence-free survival rates after nephroureterectomy in control patients, as estimated using the Kaplan–Meier method. The log-rank test was used to compare recurrence-free survival rates between patients with negative urine cytology and those with positive urine cytology. The solid line represents the patients with negative voided urine cytology and the dotted line represents the patients with positive voided urine cytology. Log-rank $P = 0.004$.

**Table 2.** Multivariate analysis of factors associated with bladder recurrence in the control group of the THPMG trial ($n = 36$)

| | HR | 95% CI | *P* value |
|---|---|---|---|
| Gender (M, F) | 1.65 | 0.37–7.36 | 0.512 |
| Age (years) | 1.03 | 0.95–1.11 | 0.533 |
| Tumor side (R, L) | 1.46 | 0.31–6.87 | 0.636 |
| Presence of ureter tumor | 4.57 | 0.87–24.1 | 0.074 |
| Open or laparoscopic | 0.53 | 0.14–2.05 | 0.359 |
| pT-stage | 0.27 | 0.05–1.40 | 0.12 |
| Voided urine cytology | 5.54 | 1.12–27.5 | 0.036 |
| Tumor grade | 1.66 | 0.34–8.11 | 0.529 |
| Histology type | 0.09 | 0.01–1.32 | 0.079 |
| Adjuvant chemotherapy | 1.16 | 0.18–7.37 | 0.877 |

HR, hazard ratio; CI, confidence interval.

the control group, voided urine cytology was an independent predictive factor for bladder recurrence [hazard ratio (HR), 5.54; 95% confidence interval (CI), 1.12–27.5; $P = 0.036$]. Of the 21 patients who had subsequent bladder recurrence following nephroureterectomy, 12 (57.2%) had recurrent tumors around the wall of the cystotomy (including the trigon and the orifice of resected ureter), and five (23.8%) had recurrent tumors at the bladder neck (Fig. 3).

To assess the efficacy of intravesical instillation of THP immediately after surgery for the patients with positive urine cytology, the bladder recurrence-free survival rate of the THP group was compared with that of the control group according

to the status of voided urine cytology (Fig. 2). Among 72 patients in either the THP group or the control group, 31 had positive voided urinary cytology. Of these 31 patients, 17 who received THP treatment had significantly fewer recurrences when compared with the 14 patients who received control treatment (6.2% at 1 year and 6.2% at 2 years vs. 57.1% at 1 year and 74.3% at 2 years; log-rank $P = 0.0001$). Based on the multivariate analysis, THP instillation was an independent predictor of better recurrence-free survival rates in the patients with positive urine cytology (HR, 0.02; 95% CI, 0.00–0.53; $P = 0.018$) (Table 3).

## DISCUSSION

Pirarubicin, or (2″R)-4′-*O*-tetrahydropyranyl-doxorubicin (THP), is an anthracycline derivative that is absorbed into tumor cells with greater speed when compared with doxorubicin (18,19). Among bladder cancer patients, a single early instillation of THP reduces the post-transurethral resection of bladder tumor bladder recurrence rate (20). The THPMG trial demonstrated that intravesical instillation of THP immediately after radical nephroureterectomy for UUT-UC significantly reduced the rate of bladder recurrence (12). Two hypotheses have been proposed to explain bladder recurrence following nephroureterectomy: intraluminal seeding and implantation of cancer cells (6,7) and field cancerization (8,9). The antitumor effect of instillation of THP might prevent intraluminal seeding and implantation of cancer cells from UUT-UC (12).

In the current study, patients with positive voided urine cytology had more frequent recurrence when compared with the patients with negative urine cytology. Based on the multivariate analysis in the control group, voided urine cytology was an independent predictor of bladder recurrence (HR, 5.54; 95% CI, 1.12–27.5; $P = 0.036$). Previous studies have reported that positive preoperative urine cytology was a predictor of bladder recurrence following nephroureterectomy (16,17). Kobayashi et al. (16) suggested that preoperative positive urine cytology was a prognostic factor for bladder recurrence after nephroureterectomy because the bladder had been continuously exposed to cancer cells dropping from UUT-UC in the preoperative period. However, in present study, intravesical instillation of THP immediately after surgery significantly reduced the bladder recurrence rate of patients with positive voided urine cytology. This observation suggests that the intravesical seeding and implantation of cancer cells from UUT-UC occur during surgery rather than before surgery. Therefore, intravesical instillation of THP immediately after surgery resulted in an anti-tumor effect against cells disseminated from the upper urinary tract and might be a reasonable strategy to prevent bladder recurrence.

In the case of positive voided urine cytology in the present study, the cancer cells disseminated from UUT-UC and floating in the bladder formed a bladder tumor postoperatively rather than preoperatively. During radical nephroureterectomy, the ureteral orifice is excised through a cystotomy following a tight suture of the bladder wall, and a urethral catheter is left in place for several days after surgery. Surgical manipulations and the presence of a urethral catheter can induce changes in the epithelial lining of the bladder (21,22). The intact urothelium had the ability to resist tumor cell adherence, while the injured urothelium can serve as a site for adherence (23,24). Physical or chemical injury to the bladder is associated with a marked increase in the adherence of bacteria, tumor cells or crystals to the urothelium (23). Therefore, the cancer cells from the UUT-UC that are floating in the bladder might adhere to the injured urothelium and form a bladder tumor following radical nephroureterectomy. In the current study, 81% of the patients who had bladder recurrence had recurrent tumor in the areas around the wall of cystotomy

**Table 3.** Multivariate analysis of factors associated with bladder recurrence in patients with positive urine cytology ($n = 31$)

| | HR | 95% CI | *P* value |
|---|---|---|---|
| THP instillation | 0.02 | 0.00–0.53 | 0.018 |
| Gender (M, F) | 0.61 | 0.09–4.11 | 0.607 |
| Age | 0.98 | 0.87–1.12 | 0.808 |
| Tumor side (R, L) | 5.37 | 0.43–67.2 | 0.192 |
| Presence of ureter tumor | 1.07 | 0.15–7.54 | 0.943 |
| Open or laparoscopic | 0.14 | 0.01–1.74 | 0.126 |
| pT-stage | 0.22 | 0.02–2.13 | 0.192 |
| Tumor grade | 0.79 | 0.07–9.27 | 0.850 |
| Histology type | 0.22 | 0.00–11.8 | 0.460 |
| Adjuvant chemotherapy | 6.45 | 0.50–83.2 | 0.153 |

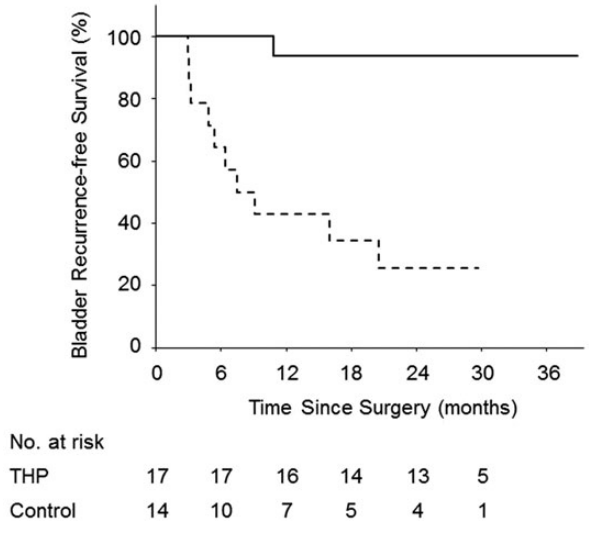
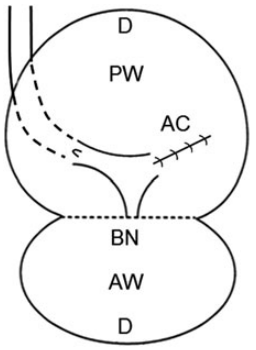



**Figure 2.** Bladder recurrence-free survival rates after nephroureterectomy in patients with positive voided urine cytology, as estimated using the Kaplan–Meier method. The log-rank test was used to compare the recurrence-free survival rates between the THP-treated group and the control group. The solid line represents the THP-treated group and the dotted line represents the control group. Log-rank $P = 0.0001$.




| Site of recurrence | |
|---|---|
| Around cystotomy | 12 |
| Bladder neck | 6 |
| Posterior wall | 5 |
| Anterior wall | 0 |
| Dome | 2 |
| Contralateral side | 1 |
| Total 26 sites ( 21 patients ) | |

**Figure 3.** Site of intravesical recurrent tumors of 21 patients. AC, around cystotomy; BN, bladder neck; PW, posterior wall; D, dome; AW, anterior wall.

(including the trigon and the orifice of the resected ureter) or the bladder neck, which could have been compromised by the urethral catheter, which supports the notion that tumor cells seeded in the injured urothelium.

The patient population in this study was relatively small, and a large-scale, multicenter, prospective study is necessary to validate the findings of this study. In the previous THPMG trial (12), an open surgery was associated with reduced risk of bladder recurrence. In the current study, however, on multivariate analysis of the control group, the open surgery was not an independent factor of bladder recurrence, and on univariate analysis, the difference missed statistical significance with a $P$ value of 0.052 (data not shown), probably due to small number of the control group ($n = 36$). Further, the status of voided urine cytology was not used in the randomization of the THPMG trial. Although there was no significant difference in the rate of positive voided urine cytology when comparing the THP and the control groups in this trial, the status of voided urine cytology should be included as a factor for randomization in future studies.

## CONCLUSIONS

Intravesical instillation of THP immediately after surgery significantly reduced the bladder recurrence rate of patients with positive voided urine cytology. These results suggest that intravesical seeding of UUT-UC occurs during radical nephroureterectomy. A Phase III, large-scale, multicenter study is needed to confirm the therapeutic efficacy of THP instillation and to clarify the mechanism of intravesical seeding from UUT-UC.

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## Conflict of interest statement

None declared.

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## Appendix

The following list represents contributors and institutions involved in this study: Dr Masataka Aizawa of Sendai Shakai Hoken Hospital, Dr Sadafumi Kawamura and Dr Hiroshi Aoki of Miyagi Cancer Center, Dr Atsushi Takeda and Dr Shunichi Namiki of Osaki Citizen Hospital, Dr Yoshihiro Ikeda of Tohoku Rosai Hospital, Dr Satoru Tokuyama of Iwaki Kyoritsu General Hospital, Kazuhiko Orikasa and Shigeto Ishidoya of Tohoku University Graduate School of Medicine.