Dysregulated expression of inhibitors of apoptosis is thought to contribute to cancer by abnormally extending cell viability, favoring the accumulation of mutations, and promoting resistance to therapy. A novel modulator of the cell death/viability balance in cancer was recently identified as survivin, a member of the inhibitor of apoptosis gene family. Undetectable in most normal adult tissues, survivin becomes the top fourth transcript expressed in common human cancers, in which it correlates with unfavorable disease and abbreviated overall survival.

Urothelial (transitional cell) carcinoma of the bladder is the fourth most common cancer in men and the eighth most common cancer in women in the United States, accounting for more than 54000 new cases and 11200 deaths every year. Recurrences of bladder cancer occur in up to 80% of patients and constitute a formidable obstacle to long-lasting remissions, frequently anticipating muscle invasion, and disseminated disease. Despite considerable efforts to develop safe, reliable, noninvasive screening strategies for bladder cancer, the identification of a single predictive/prognostic marker of the disease has remained elusive. Consistent with a proposed role of deregulated apoptosis in urothelial cancer, survivin was found in 78% of bladder cancers, but not in normal urothelium, and its expression correlated with accelerated recurrences. Because of its expression in cancer but not in normal tissues, we investigated the potential suitability of survivin as a new molecular marker for detection of bladder cancer.

**Methods**

**Urine Specimens**

One hundred fifty-eight urine specimens were collected at the urology clinics at Yale-New Haven Hospital and at the Veterans Affairs, New England Health Care Systems, West Haven, Connecticut, Division. Random clean-catch or straight catheter urine samples were obtained from individuals who were categorized into 5 different groups: group 1 included healthy volunteers (n=17) who were not taking any medication; group 2 patients had a mean (SD) age of 47.6 (20.8) years who were not taking any medication (n=17); group 2 patients had a mean (SD) age of 60.0 (18.1) years with diagnosis of non-neoplastic urinary tract disease, genitourinary cancer (n=30), new-onset or recurrent bladder cancer (n=46), or treated bladder cancer (n=35), recruited from 2 New England urology clinics.

**Main Outcome Measures**

Detectable survivin levels, analyzed by a novel detection system and confirmed by Western blot and reverse transcriptase polymerase chain reaction (RT-PCR), in urine samples of the 5 participant groups.

**Results**

Survivin was detected in the urine samples of all 46 patients with new or recurrent bladder cancer using a novel detection system (31 of 31) and RT-PCR (15 of 15) methods. Survivin was not detected in the urine samples of 32 of 35 patients treated for bladder cancer and having negative cystoscopy results. None of the healthy volunteers or patients with prostate, kidney, vaginal, or cervical cancer had detectable survivin in urine samples. Of the 30 patients with nonneoplastic urinary tract disease, survivin was detected in 3 patients who had bladder abnormalities noted using cystoscopy and in 1 patient with an increased prostate-specific antigen level. Patients with low-grade bladder cancer had significantly lower urine survivin levels than patients with carcinoma in situ (P=.002).

**Conclusions**

Highly sensitive and specific determination of urine survivin appears to provide a simple, noninvasive diagnostic test to identify patients with new or recurrent bladder cancer.

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neoplastic urinary tract disease or hematuria (n = 30); group 3 patients had a mean (SD) age of 71.5 (9.9) years with diagnosis of genitourinary cancer, excluding bladder cancer (n = 30); group 4 patients had a mean (SD) age of 69.7 (8.7) years with diagnosis of new-onset or recurrent bladder cancer (n = 46); and group 5 patients had a mean (SD) age of 76.1 (8.9) years and were undergoing treatment or had already received treatment for bladder cancer and had negative cystoscopic findings on the day of urine collection (n = 35). Treatment measures in group 5 included intravesical bacillus Calmette-Guerin, thiotepa, transurethral resection, partial cystectomy, and radiation. Group 4 included patients who, after urine collection, underwent similar treatment measures and/or salvage cystectomy or radical cystectomy.

Urine Detection of Survivin

Urine specimens were filtered onto a nitrocellulose membrane using a microfiltration apparatus in a module providing a 48-wells-lot format. The blot was analyzed for the presence of survivin using a polyclonal antibody. The protocol for analysis involved a first amplification reaction was carried out with survivin primers 5'-CTGCTCTGACGCCCTTTCTCAAA-3' (forward) and 5'-AATAAAC-CCTGGAATGGTGCA-3' (reverse) with denaturation at 94°C for 15 seconds, annealing at 53°C for 15 seconds, and extension at 72°C for 1 minute for 20 cycles, followed by incubation at 72°C for 5 minutes. A 463-base pair fragment of the survivin cDNA was subjected to a second round of amplification with nested survivin primers 5'-CCGCATCTCTACATTCAAGAC-3' (forward) and 5'-CTTGCTCTTTCTCTGTCC-3' (reverse), with denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 45 seconds for 30 cycles, followed by incubation at 72°C for 5 minutes. The amplified survivin cDNA of 279 base pair was separated on a 2.0% solution of agarose gel and visualized by ethidium bromide staining. Control reactions were amplified using β-actin-specific primers 5'-AGCGGGAAATGCGTGTG-3' (forward) and 5'-CAGGTACATGTTGGTGC-3' (reverse) with generation of a 309-base pair fragment.

Statistical Analysis

The relationship between urine survivin and patients’ diagnosis was analyzed by a χ² test. Nonparametric statistical analysis was used to compare the weighted urine survivin score with the grading classification system performed at the Yale-New Haven Hospital. The calculation of predictive accuracy is not appropriate for this study since the diagnosis was known at the time of urine collection.

RESULTS

A representative experiment of detection of urine survivin using the BioDot test is shown in FIGURE I. Determination of urine survivin with the BioDot method was carried out in 138 of the 158 specimens collected for this study (TABLE I). Twenty additional urine samples were analyzed for survivin expression by reverse transcriptase polymerase chain reaction (RT-PCR) to independently evaluate the
specifity of the new method. Survivin was not detected in urine of the 16 volunteers, 6 patients with benign prostatic hyperplasia, 2 with interstitial cystitis, 3 with renal calculi, 6 with urinary tract infection, or 6 with other nonneoplastic urinary tract disease (Table 1). Urine survivin was detected in 3 of 5 patients with cryptogenic hematuria (weighted survivin score, 2), who presented with a history of retention and dysuria post-transurethral prostate resection, and revealed a trabeculated, irregularly thickened bladder, by cystoscopy (see “Comment” section). One patient with increased prostate-specific antigen levels but without diagnosis of prostate cancer was positive for urine survivin (Table 1). This patient also had a trabeculated, thickened bladder, by cystoscopy. Survivin was not detected in urine specimens of 19 patients with prostate, 8 with renal, 1 with vaginal, or 1 with cervical cancer (Table 1). In contrast, urine survivin was detected in all 31 patients with new-onset or recurrent bladder cancer (Table 1). Histopathologic grading (grades I through IV) of the 31 patients in group 4 analyzed for urine survivin by the novel method included 13 patients with grade II, 7 patients with grade III, and 5 patients with grade IV tumors. Carcinoma in situ was found in association with the papillary and invasive carcinomas of 5 patients and in association with high-grade urothelial cancer of the ureter in 1 patient. Thirty of 33 patients in group 5 analyzed by the novel system had no detectable urine survivin (Table 1). Five of these 30 patients were receiving bacillus Calmette-Guerin and had completed 3 to 5 treatments, the other 25 were status posttreatment with negative cystoscopy findings. Three patients in group 5 with initial diagnosis of grade II noninvasive bladder cancer had positive test results for urine survivin after undergoing negative cystoscopic examination. One of the 3 patients had urine cytology positive for bladder cancer. Two of the 3 patients were treated with transurethral resection of the bladder tumor and 1 was treated with fulguration.

When normalized for a weighted mean (SD) survivin score, patients with carcinoma in situ had considerably higher survivin score (2.5 [0.5]; n=6) than patients with grade II bladder cancer (1.3 [0.6]; n=13). The correlation between weighted survivin score and histopathology or grading of the various bladder cancer cases is shown in Table 2 and Table 3, respectively. By Western blot, a single survivin band of 16.5 kd was detected in the urine cell pellet from a patient with bladder can-

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**Table 1. Survivin Detection in 138 Urine Specimens Using a Novel Detection Method**

<table>
<thead>
<tr>
<th>Urine Specimens</th>
<th>Total No. of Patients</th>
<th>No. of Patients Survivin-Negative</th>
<th>No. of Patients Survivin-Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (control healthy volunteers)</td>
<td>16</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Group 2 (nonneoplastic urinary tract diseases)</td>
<td>29</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Hermaturia</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Benign prostatic hyperplasia</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Increased prostate specific antigen</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Intestinal cystitis</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Renal calculi</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Other*</td>
<td>6</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Group 3 (genitourinary cancers except bladder)</td>
<td>29</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Prostate</td>
<td>19</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Renal</td>
<td>8</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Vaginal</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Cervical</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Group 4 (new or recurrent bladder cancer)†</td>
<td>31</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>Group 5 (treated bladder cancer)‡</td>
<td>33</td>
<td>30</td>
<td>3§</td>
</tr>
</tbody>
</table>

*Includes 1 patient with papillary necrosis, 2 with prostatitis, 1 with vesicoureteral reflux, and 2 with renal transplant with elevated creatinine.
†Includes 1 patient with urothelial cancer of the ureter.
‡Patients had normal cystoscopy results.
§Two of these patients were treated with transurethral resection of the bladder tumor, and 1 with fulguration. One of these patients had urine cytology positive for bladder cancer.

---

**Figure 1. Urine Detection of Survivin**

- Survivin, µg/mL
- Patient Group
- 0
- RCC
- TCC
- TCC/R
- TCC/R
- Control
- 0.001
- PC
- TCC
- TCC/R
- TCC/R
- Control
- 0.05
- PC
- TCC
- TCC/R
- TCC/R
- Control
- 0.01
- PC
- TCC
- TCC/R
- TCC/R
- Control
- 0.05
- PC
- TCC
- TCC/R
- TCC/R
- Control
- 0.25
- PSA
- TCC/R
- TCC/R
- TCC/R
- Control
- 1
- BPH
- TCC/R
- TCC/R
- TCC/R
- Control

Increasing concentrations of recombinant survivin or urine specimens from the indicated patient groups were applied to a slot-blot apparatus. The membrane was incubated with an antibody to survivin followed by horseradish peroxidase-conjugated goat antirabbit IgG. Bands were visualized by chemiluminescence and quantitated by densitometry.
optosis inhibitor survivin in urine of 
patients with bladder cancer. Survivin 
was found in urine samples of all 46 pa-
tients with new or recurrent bladder 
cancer, but not in any of the 17 healthy 
volunteers, or in any of the 30 pa-
tients with other urologic cancers, and 
only in 4 of 30 patients with nonneo-
plastic genitourinary disorders. Import-
antly, of the 3 patients with hematu-
ria who tested positive for urine 
survivin, 1 had a positive cytology re-
sult for bladder cancer and another was 
diagnosed with bladder cancer within 
6 months of survivin detection. More-
over, 32 of 35 patients treated for blad-
der cancer and achieving cystoscopic 
remission had negative test results for 
survivin urine. There is a positive cor-
relation between a weighted urine sur-
vivin score and more advanced histo-
pathologic tumor grading.

For its overexpression in cancer but 
not in normal tissues, and its unfa-
vorable predictive and/or prognostic 
significance in various malignan-
cies, survivin may constitute a use-
ful molecular marker in cancer. This 
may be particularly relevant in blad-
der cancer, in which simple and 
noninvasive diagnostic means to moni-
tor response to therapy and simplify fol-
low-up protocols are urgently needed. 
Although regarded as the criterion stan-
ard, urine cytology has low sensi-
tivity (30%-40%) in bladder cancer, and 
fails to detect superficial, low-grade le-
sions. In this context, several urine 

**COMMENT**

In this study, we describe a simple, 
antibody-based test to identify the ap-

![Figure 2. Western Blot of Urine Survivin](https://jama.jamanetwork.com/)

Total RNA was extracted from urine cell pellets and reverse-transcribed by random priming. Amplification reactions were carried out with survivin-specific nested prim-
ers (279 bp) or β-actin-specific primers (309 bp). M indicates molecular weight markers in base pair; TCC, analysis of 5 representative patients with new or recurrent 
bladder cancer (group 4).

**Table 3. Correlation Between Weighted Urine Survivin Score and Bladder Cancer Grading**

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of Cases Tested</th>
<th>Mean (SD) Survivin Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>13</td>
<td>1.3 (0.6)</td>
</tr>
<tr>
<td>III</td>
<td>7</td>
<td>1.5 (0.8)</td>
</tr>
<tr>
<td>IV</td>
<td>5</td>
<td>2 (1)</td>
</tr>
<tr>
<td>IV†</td>
<td>1</td>
<td>3†</td>
</tr>
</tbody>
</table>

*The weighted survivin score was calculated using a stan-
dard curve with increasing concentrations of recombi-
nant survivin as follows: 0, not detectable; 1, 0.001 to 0.25 
µg/mL; 2, 0.25 to 1 µg/mL; and 3, more than 1 µg/mL. 
Histopathological analysis was carried out using the 
Broader cytologic grading system for the classification of 
papillary transitional cell tumors, grades I through IV. 
†One of the 6 patients with associated carcinoma in situ 
had urothelial cancer of the ureter (grade IV; survivin 
score, 3).
markers including bladder tumor antigen, nuclear matrix protein, telomerase activity, hyaluronic acid/hyaluronidase, and fibrin degradation products have been characterized for their potential diagnostic/predictive value in bladder cancer.10,20

In this patient series, the sensitivity of the urine survivin test for new or recurrent bladder cancer was 100%, and its specificity for other neoplastic and nonneoplastic genitourinary tract diseases was 95% (P<.02). However, the overall specificity of the test is likely to vary depending on which patient population is the focus of clinical interest. A screening test for group 1 individuals will have a false-positive rate of essentially zero, whereas patients with clinical symptoms in groups 2 and 3 will likely have a combined false-positive rate of 3% to 10%. However, similarly to the 2 patients with hematuria described above, these individuals should be closely followed up because they may subsequently develop bladder cancer. Because of its high specificity, the urine survivin test may be useful to complement cytology and/or other diagnostic markers10,20 to better monitor bladder cancer patients and identify early recurrences or de novo neoplasms. Other potential advantages of the urine survivin test include its simplicity, suitability as a point-of-service procedure, and its cost-effectiveness, using 1-step detection with a single antibody to survivin that has now become commercially available. Analysis of a larger patient series may establish the general suitability of urine survivin detection for monitoring response to therapy and follow-up protocols in bladder cancer.

Author Contributions: Dr Smith participated in study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, important intellectual content, and provided statistical expertise, obtained funding, and provided administrative, technical, or material support. Ms Wheeler participated in study concept and design, acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, important intellectual content, and provided administrative, technical, or material support. Ms Plescia participated in study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and provided administrative, technical, or material support. Dr Colberg participated in study concept and design and acquisition of data. Dr Weiss participated in study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and provided administrative, technical, or material support. Dr Altieri participated in study concept and design, analysis and interpretation of data, drafting of the manuscript, and obtained funding.

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