A Diagnostic-Prognostic Test for Bladder Cancer Using a Monoclonal Antibody-based Enzyme-linked Immunoassay for Detection of Urinary Fibrin(ogen) Degradation Products

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ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody was developed to detect the clinical value of urinary fibrinogen/fibrin degradation product levels for the identification and management of patients with bladder cancer. Assays were performed on 286 serial urine specimens from 56 bladder carcinoma patients. Specimens were grouped according to whether the patient had an evident tumor at the time of specimen collection (134 specimens, 41 patients) or was clinically disease-free following treatment (152 specimens, 38 patients). Many patients contributed specimens to both groups as determined by their clinical status at the time of collection. In addition, 45 specimens from 33 patients with inflammation of the urogenital tract and 81 specimens from 19 patients with renal or prostatic cancer were assayed for urinary fibrin degradation products. The ELISA, using a high-sensitivity procedure, identified 83% of the specimens from bladder cancer-positive patients with an overall accuracy with all specimens of 78% and a false-negative rate of 5% for all specimens tested. The high-sensitivity ELISA appeared most appropriate for monitoring bladder cancer patients for recurrence of tumor after surgery. The ELISA using a high-specificity procedure appeared most appropriate for screening. The high-specificity ELISA accurately identified 96% of urine specimens from non-bladder cancer patients with a false-positive rate of only 5%. These results demonstrate that the ELISA is an efficient, reliable, quantitative, and noninvasive immunoassay that can be useful both for the identification of bladder cancer patients and for monitoring the course of the disease.

INTRODUCTION

The number of bladder cancer patients in the United States has increased steadily in the last decade. The estimated number of cases for 1983 was 40,000 with a concurrent death toll of approximately 10,000. In the past, the disease was associated with particular industrial occupations; however, the present increase in frequency would indicate that the disease may be etiologically associated with a broader range of potential carcinogens in the environment.

There have been many attempts to develop an efficient, reliable, quantitative, noninvasive, diagnostic/prognostic procedure for bladder cancer. Many investigators have sought to identify bladder carcinoma patients by evaluating amounts of specific urinary proteins, such as carcinoembryonic antigen (5, 9, 11), tissue polypeptide antigens (15), putative bladder-tumor-associated antigens (6, 10, 22, 24), urinary cholesterol (14), and urinary excretion of various serum proteins (13). However, because of nonspecific proteinuria during inflammatory responses in the bladder, limited access to assay reagents and/or insufficient clinical evidence of efficacy, none of these procedures has been accepted as a practical aid in the identification of bladder cancer patients or in the management of the disease.

The assessment of fibrinogen/FDP3 in the urine has been suggested as a reliable indicator of bladder cancer (1, 23), especially in conjunction with urinary cytology (28, 31, 32). Although most patients with disseminated cancer present some evidence of disseminated intravascular coagulation, many never develop overt clinical manifestations. However, a net pathophysiological manifestation of various levels of disseminated intravascular coagulation is activation of prothrombin and plasminogen creating fibrin and the X-, Y-, D-, and E-degradation fragments detectable in serum and urine (2). Wajsman et al. (32) found that an HAI immunoassay for FDP that had a 32.2% sensitivity, combined with urinary cytological examination, increased the accuracy of the positive test results to 80%. The results suggested that a more rapid and accurate FDP test could better supplement urinary cytological examination in the early detection of bladder cancer.

Using a monoclonal antibody to FDP, we developed a specific and reproducible immunoassay procedure. In this paper, we describe the monoclonal antibody-based ELISA for fibrinogen and FDP and evaluate its use with a large panel of 286 urine specimens from 56 patients with bladder carcinoma and 126 specimens from 52 patients with cancer of the prostate or kidney, or nonmalignant diseases of the urinary tract. Our results show that the overall sensitivity, specificity, and accuracy of the FDP ELISA alone approximates the previous results (28, 31, 32) using a combination urinary FDP assay and urinary cytological examination.

MATERIALS AND METHODS

Patients. All 56 bladder tumor patients had prior (BT) or concurrent (BT') transitional cell carcinoma of the bladder documented by micro-

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1 Supported by grants from Litton Industries.
2 To whom requests for reprints should be addressed.

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scopic examination of tissue sections. The mean age of patients with bladder cancer was 67; 44 were male and 12 were female. Patients with superficial bladder tumors were monitored by means of cystoscopc examination at 3-month intervals, and urinary cytological examination and random bladder biopsy were performed as clinically indicated. Cystectomy patients were monitored by means of periodic X-rays, urograms, and liver function tests. Urinary fibrinogen/FDP was determined in 152 specimens from bladder tumor-negative patients specimens and 134 specimens from BT* patients, and the results were compared with those obtained with urine specimens from 52 patients with carcinoma of the prostate (58 specimens), carcinoma of the kidney (23 specimens), or nonneoplastic diseases of the urinary tract (45 specimens). The latter group included specimens from 33 patients with urogenital tract inflammatory disease.

Urine Samples. Fresh urine samples were collected between 8 a.m. and 4 p.m. All urine samples were adjusted to pH 6.5 to 7.0 with 1 N NaOH solution within a few h of collection, and the samples were divided into aliquots for storage at −70°C. Urine samples were centrifuged at 1700 rpm for 5 min immediately before assay.

Monoclonal Antibody Production. Splenic lymphocytes from BALB/c mice immunized at 3-week intervals with 2 s.c. (45 μg) and 1 i.p. (70 μg) injection of urine from a patient with bladder carcinoma were fused at 5:1 ratio with mouse plasmacytoma cells (12). Fusions were performed 3 days after the last injection. Hybridomas selected in hypoxanthine/aminopterin/thymidine medium were screened for production of antibody to fibrinogen. One hybridoma (LIAB-6), an IgM with a high apparent affinity for fibrinogen, was cultivated as an ascites tumor in BALB/c mice, to fibrinogen. An aliquot (25 μl) of the digested fibrinogen supernatant was sequentially distributed to ensure the blind nature of the assay. Similarly, specimens from bladder cancer patients and from nonmalignant or nonbladder carcinoma patients were intermixed. The bead-blocking solution, washing solution, diluent, 2.2'-azino-di3-ethyl-benzthiazoline sulphonic acid), H2O2, and NaF stop solution were obtained from Liton Bionetics, Inc., Laboratory Products Division, Charleston, SC.

To test for fibrinogen/FDP in urine, beads were allowed to react with purified LIAB-6 monoclonal antibody at 11.5 μg/ml, diluted in PBS. Beads were coated in batches of 15 beads/ml of antibody solution for 2 hr, at room temperature, and unreacted areas of the bead were blocked with a solution of normal goat serum used in PBS. Beads were dried and stored for up to several weeks at 4°C or were used immediately to assay fibrinogen/FDP in urine.

Each urine sample was tested in duplicate at a dilution of 1/2.5 (high-sensitivity procedure) or 1/5 (high-specificity procedure) in PBS. The LIAB-6 monoclonal antibody-coated bead was allowed to react with 150 μl of diluted urine for 90 min at 37°C on a rocking platform. Beads were washed free of unbound material and were added to 150 μl of HRP-conjugated goat antibody to human fibrinogen (Cappel Laboratories, West Chester, PA) diluted 1/375 (high-sensitivity procedure) or 1/500 (high-specificity procedure). After another 90-min incubation at 37°C and washing to remove unbound conjugate, the beads were allowed to react with 200 μl of 0.03% 2,2'-azino-di3-ethyl-benzthiazoline sulphonic acid) solution containing 0.03% H2O2 for 10 min at room temperature. The reaction was stopped with 25 μl of 0.8% NaF; the absorbance at 405 nm was determined with an automated 96-welled plate reader.

Every plate contained fibrinogen standards and urine from a single patient diluted 1:2.5, 1:40, and 1:320 as internal controls to ensure reproducibility of the assay from day to day and plate to plate. The absorbance value at 1/320 dilution was used as the lower limit of a positive assay for each plate. Each sample was assayed in duplicate; multiple specimens collected at various times from the same patient were randomly distributed throughout the entire assay panel rather than sequentially distributed to ensure the blind nature of the assay. Similarly, specimens from bladder cancer patients and from nonmalignant or nonbladder carcinoma patients were intermixed. The bead-blocking solution, washing solution, diluent, 2,2'-azino-di3-ethyl-benzthiazoline sulphonic acid), H2O2, and NaF stop solution were obtained from Liton Bionetics, Inc., Laboratory Products Division, Charleston, SC.

Cell Lines and Fluorescence Localization of Cell Surface Fibrinogen. Bladder carcinoma cell lines HT-1376, J82, RT-4, Scaber, and T-24, and colon carcinoma cell line HT-29 were obtained from the American Type Culture Collection, Rockville, MD, and maintained in the recommended medium supplemented with 10% fetal bovine serum, gentamicin (50 μg/ml), and nonessential amino acids as indicated. Surface localization of fibrinogen was studied with LIAB-6 antifibrinogen monoclonal antibody at 2.3 and 1.5 μg/ml or mouse IgG at 2.3 μg/ml at a ratio of
0.1 ml LIAB-6 or IgM/10^6 cells. Primary incubation at 25°C for 60 min was followed with multiple washes in 0.1% bovine serum albumin in Hanks' balanced salt solution and incubation in fluorescein-conjugated goat anti-mouse IgM antibody at 4°C for 30 min. Cells were washed 3 times with cold bovine serum albumin in Hanks’ balanced salt solution suspended in cold 50% glycerol in PBS and examined immediately for surface fluorescence using a phase-contrast/fluorescence microscope.

RESULTS

Monoclonal antibody LIAB-6, the product of the fusion of spleen cells from mice immunized with urine from a bladder cancer patient and mouse myeloma cells, is an IgM antibody specific for an epitope on native fibrinogen and on terminal degradation Product E which results from plasmin digestion of native fibrin and fibrinogen. Plasmin digestion of radiolabeled fibrinogen followed by gradient gel electrophoresis resulted in essentially all the radiolabel being associated with a fragment of molecular size characteristic of degradation fragment E (Chart 1A). No radiolabel was detected in the higher molecular weight region characteristic of fragment D, the other terminal plasmin digestion fragment of fibrinogen. These results were confirmed with specific rabbit antiserum to fragments D and E. Anti-fragment D did not precipitate any labeled proteins and did not remove or reduce the size of the single digestion peak (Chart 1B). Anti-fragment E, however, immunoprecipitated the labeled fragment and completely removed it from the digestion mixture (Chart 1C). LIAB-6 also immunoprecipitated the radiolabeled fragment and greatly reduced its concentration in solution demonstrating that monoclonal antibody LIAB-6 was specific for an epitope on the E plasmin digestion fragment (Chart 1D).

Western immunoblots were used to further establish LIAB-6 specificity. Immunodetection of plasmin-digested fibrinogen with the rabbit antiserum to fragment D revealed bands corresponding to native fibrinogen, intermediate digestion fragments X and Y, and the terminal digestion fragment D (Chart 1F). Likewise, rabbit antiserum to fragment E recognized native fibrinogen, fragments X, Y, and E (Chart 1G). LIAB-6 recognized fragment E (Chart 1H), but showed no reactivity with native fibrinogen or fragments X, Y, or D. However, ELISA assays (Table 1) have demonstrated LIAB-6 reactivity with native fibrinogen and reactivity with intermediate digestion fragments X and Y, which contain fragment E, may be inferred from this evidence. The finding that LIAB-6 recognized only fragment E in the Western blots may indicate a higher avidity of the monoclonal for the small fragment.

A solid-phase, capture-type ELISA was developed with monoclonal antibody LIAB-6 on polycarbonate-coated steel beads to capture urinary fibrinogen/FDP. Polyclonal goat anti-human fibrinogen conjugated with HRP was used to detect the captured fibrinogen/FDP. The assay was designed to detect native fibrinogen as well as plasmin-digested fibrinogen because experiments, in which thrombin was added to patient urine to clot intact fibrinogen, had shown that urinary fibrinogen was present in both native and degraded forms (Table 1).

The sensitivity of the ELISA was assessed by extinction titration of bladder cancer urine and a standard preparation of human fibrinogen obtained from a commercial HAI method FDP assay kit. For reference purposes, the sensitivity of the ELISA was compared to that of the HAI method. Both tests indicated the same ratio of native fibrinogen to FDP in tumor patient urine

FIBRINOGEN-FDP ANALYSIS FOR BLADDER CANCER

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Table 1

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Titer or sensitivity</th>
<th>Without thrombin</th>
<th>With thrombin</th>
<th>Fibrinogen standard (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High positive bladder tumor patient urine</td>
<td>HAI method</td>
<td>1:16</td>
<td>1:8</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>1:54</td>
<td>1:32</td>
<td>39</td>
</tr>
</tbody>
</table>

(Table 1). The ELISA assay was 4 times more sensitive than the HAI method in the urine assay and 32 times more sensitive than the HAI procedure in the fibrinogen standard assay (Table 1).

Urine specimens from 7 patients were assayed in triplicate in 3 consecutive ELISA to determine the reliability of a single determination. S.D.s ranged from 0.012 to 0.031 A. The variance observed among specimens collected from the same patient was also determined. Chart 2 shows the results of 14 collections.

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FIBRINOGEN-FDP ANALYSIS FOR BLADDER CANCER

Table 2
Analysis of fibrinogen/FDP in urine of patients with carcinoma of the bladder.
1. High-sensitivity assay

The sensitivity of the assay was calculated by:

\[
\text{Sensitivity} = \frac{\text{Number of true positive (TP) specimens}}{\text{Total number of specimens from BT* patients}} \times 100
\]

and was 83% (92/111) including uncertain values and 85% (90/106) excluding uncertain values. Values within 0.015 A of the cutoff value were defined as uncertain (see "Results"). The accuracy of the assay was calculated by:

\[
\text{Accuracy} = \frac{\text{Number of true positive (TP) specimens} + \text{true negative (TN) specimens}}{\text{Total number of specimens}} \times 100
\]

and was 74% (179/242) including uncertain values and 75% (172/229) excluding uncertain values.

<table>
<thead>
<tr>
<th>Source of specimens (no. of patients)</th>
<th>Assay result</th>
<th>No. of urine specimens (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT* patients (37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (TP)*</td>
<td>92 (83)</td>
<td>90 (85)</td>
</tr>
<tr>
<td>Negative (FN)*</td>
<td>19 (8)</td>
<td>16 (7)</td>
</tr>
<tr>
<td>Uncertain</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>BT- patients (34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (FP)</td>
<td>44 (18)</td>
<td>41 (18)</td>
</tr>
<tr>
<td>Negative (TN)</td>
<td>87</td>
<td>82</td>
</tr>
<tr>
<td>Uncertain</td>
<td></td>
<td>5</td>
</tr>
</tbody>
</table>

* The total number of patients was 71. Each specimen was classified as BT* or BT- depending upon whether the patient had clinically evident disease at the time of collection.

<table>
<thead>
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The occurrence of fibrinogen and FDP in the urine of bladder carcinoma patients and the potential value of these determinations in the detection and management of this disease was assessed with a coded panel of 412 randomly arranged specimens from 108 patients. The results are shown in Chart 3. In Tables 2 and 3, the results are broken down in patient categories for evaluation purposes. The value of the assay is apparent in Chart 3 which shows that most specimens from BT* patients gave higher assay results than those from patients in the other groups. In view of the S.D.s for individual specimens of 0.012 to 0.031 A and as an aid in identifying positive and negative values, an uncertain zone within the assay extending 0.015 A above and below the cutoff value was defined. Table 2 includes results presented and calculated both with and without inclusion of the uncertain values. However, exclusion of uncertain values had little impact on the overall evaluation, since the most specimens (229/242) did not fall within the uncertain zone. The high-sensitivity ELISA was 83 to 85% sensitive in detecting those patients with a clinically detectable tumor and 74 to 75% accurate in determining the current status of bladder carcinoma patients. The difference in overall accuracy compared to the sensitivity in detecting bladder cancer is a reflection of a 18% rate of false-negative values compared to 7 to 8% rate of false-negative from one bladder carcinoma patient obtained over a period of 1 week and assayed together in a single test. The mean value was 0.705 ± 0.193. The large S.D. reflects a single low value (0.198) obtained on the second collection day. A repeat analysis of the same specimens confirmed the validity of the assay result and the aberrant single sample. At both Day 1 and Day 2, the lowest values were associated with the first voided specimen. Except for the first specimen collection on Day 2, the other 13 collections were in agreement.

The occurrence of fibrinogen and FDP in the urine of bladder carcinoma patients and the potential value of these determinations in the detection and management of this disease was assessed with a coded panel of 412 randomly arranged specimens from 108 patients. The results are shown in Chart 3. In Tables 2 and 3, the results are broken down in patient categories for evaluation purposes. The value of the assay is apparent in Chart 3 which shows that most specimens from BT* patients gave higher assay results than those from patients in the other groups. In view of the S.D.s for individual specimens of 0.012 to 0.031 A and as an aid in identifying positive and negative values, an uncertain zone within the assay extending 0.015 A above and below the cutoff value was defined. Table 2 includes results presented and calculated both with and without inclusion of the uncertain values. However, exclusion of uncertain values had little impact on the overall evaluation, since the most specimens (229/242) did not fall within the uncertain zone. The high-sensitivity ELISA was 83 to 85% sensitive in detecting those patients with a clinically detectable tumor and 74 to 75% accurate in determining the current status of bladder carcinoma patients. The difference in overall accuracy compared to the sensitivity in detecting bladder cancer is a reflection of a 18% rate of false-negative values compared to 7 to 8% rate of false-negative
values. In the analysis of specimens from patients with nonmalignant disease or tumors other than bladder carcinoma, the high-sensitivity ELISA showed 86% accuracy or specificity (Table 3). It is important to emphasize that this group included specimens from 29 patients with urinary tract infections.

When designing the assay procedure, we selected sample volumes and reagent concentrations for maximum sensitivity with the expectation that some values assigned as false-positive on the basis of a negative tumor status might in actuality be true-positive responses to a clinically undetected tumor. Whereas a false-positive value might subsequently be clinically proven to be a true-positive, a false-negative value would be unlikely to change. However, this maximum level of sensitivity is not desirable in every application. Chart 4 and Tables 4 and 5 show the results obtained on the same panel with the assay redesigned to give a lower degree of sensitivity but higher specificity for bladder cancer. In the patients with bladder cancer, the false-positive rate fell to 6% of the total (Table 4), and in the patients with nonmalignant urogenital disease, the false-positive rate fell to 4% (Table 5). Table 6 compares the 2 assay procedures. The high-sensitivity procedure appears better for predicting tumor status in patients with bladder cancer (83%); however, its overall specificity for bladder carcinoma (76%) is not as high as that of the high-sensitivity procedure (92%). Clearly, the choice of procedure will be dictated by the intended use for the assay. For screening, specificity is very important, and a lower level of sensitivity may be acceptable; for monitoring of tumor status in patients known to have bladder cancer, higher sensitivity to the recurrence of tumor is more important, and a lower level of specificity may be acceptable.

The monitoring of treatment efficacy requires frequent specimen analysis. The more frequent the analysis, the less significant are false-positive or false-negative results, since the pattern of assay values rather than individual results form the basis of interpretation. Any marked change in assay results would be readily confirmed in a subsequent assay. Chart 5 illustrates this point with data from several patients from whom urine specimens were collected over a period of several weeks. The data in Chart 5 (top) are typical of most patients. Assay results were consistently within the positive or negative range, although wide fluctuations were apparent, and a drop to the negative range or rise to a positive value on a single specimen collection was not indicative of a change in tumor status. In addition, the ELISA was sensitive to changes in tumor burden as reflected in Chart 5 (bottom); 2 patients with no detectable diseases after surgery produced assay values that fell after treatment and remained in the negative range on subsequent assays. Decreasing assay values after tumor removal were expected. In a few such cases, the assay value did not become negative immediately after surgery but was persistently and decreasingly positive for several weeks, eventually becoming and remaining negative.

Table 3
Analysis of fibrinogen/FDP in urine of patients with nonmalignant urogenital disease or tumors at sites other than the bladder. I. High-sensitivity assay

<table>
<thead>
<tr>
<th>Source of specimens (no. of patients)</th>
<th>Assay result</th>
<th>No. of negative specimens/total specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with nonmalignant urogenital disease (32)</td>
<td>Negative (TN)</td>
<td>38/42 (90)</td>
</tr>
<tr>
<td>Patients with prostatic carcinoma (14)</td>
<td>Negative (TN)</td>
<td>43/48 (90)</td>
</tr>
<tr>
<td>Patients with renal carcinoma (4)</td>
<td>Negative (TN)</td>
<td>14/20 (70)</td>
</tr>
</tbody>
</table>

* TN, true negative.

Chart 4. High-specificity ELISA. Scattergraph of individual specimen results (A600 nm) grouped according to whether, at the time of specimen collection, the patients had clinically evident bladder carcinoma (BT*), recent history of bladder carcinoma but no current evidence of disease (BT-), or urogenital tract inflammation or current or prior evidence of prostatic or renal carcinoma (urogenital disease, bladder tumor-negative). Data are the mean of duplicate determinations. Values below the solid line were scored as negative; those above, as positive.

Table 4
Analysis of fibrinogen/FDP of patients with carcinoma of the bladder. II. High-specificity assay

<table>
<thead>
<tr>
<th>Source of specimens (no. of patients)</th>
<th>Assay result</th>
<th>No. of specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT* patients (38)</td>
<td>Positive (TP)</td>
<td>120 (60)</td>
</tr>
<tr>
<td></td>
<td>Negative (FN)</td>
<td>60 (30)</td>
</tr>
<tr>
<td>BT- patients (38)</td>
<td>Negative (TN)</td>
<td>139 (70)</td>
</tr>
<tr>
<td></td>
<td>Positive (FP)</td>
<td>16 (8)</td>
</tr>
</tbody>
</table>

* Total number of patients was 74. Specimens from individual patients were classified as either BT* or BT- according to the presence of clinically evident disease at the time of collection.
* TP, true positive; FN, false negative; TN, true negative; FP, false positive.
* The rate of false-negative results was 23% (60/259) as calculated by the formula described in Table 2.
* The rate of false-positive results was 8% (16/259) as calculated by the formula described in Table 2.
The presence of urinary FDP undoubtedly related to the presence of tumor but did not appear to be a product of the tumor cells. Attempts to demonstrate fibrinogen production by several lines of bladder carcinoma cells using monoclonal antibody LIAB-6 or polyvalent rabbit anti-human fibrinogen in fluorescence assays were unsuccessful.

It was important to consider whether the analysis of urinary fibrinogen or FDP was equivalent to, or superior to, a simple protein assay in predicting tumor status. When the results of the high-sensitivity assay were compared with the protein concentrations of each sample, many discrepancies between protein content and assay result were apparent, and no overall correlation of assay value with protein concentration was found (data not shown).

**DISCUSSION**

This paper describes a monoclonal-antibody-based ELISA for determining the presence of fibrinogen and FDP in urine. The assay was evaluated: (a) as a specific method for identifying patients with bladder carcinoma and distinguishing these patients from those with inflammatory urogenital disease and carcinomas at sites other than the bladder; and (b) as a highly sensitive monitor of bladder tumor patients after treatment. The high-specificity procedure correctly identified as a negative 92% of the urine specimens from patients with conditions other than clinically detected bladder carcinoma and exhibited a very low rate of false-positive results (5%), a level of accuracy that is important in the screening of individuals at increased occupational or environmental risk of developing bladder cancer. In addition to causing needless concern to the patient, falsely elevated assays necessitate further clinical examination, probably involving cytoscopy, which is the mainstay of the diagnosis of bladder cancer, but which is neither innocuous nor inexpensive.

Microscopic examination of cells in urine is also an important aid in the diagnosis and management of bladder cancer. However, urinary cytology is not useful in patients with well-differentiated carcinomas, the accuracy is less than 30%, since 74% will have false-negative examinations (8). Moreover, microscopic examination of cells is not an objective measurement, and the accuracy of such observations varies considerably from hospital to hospital. In contrast, the accuracy of urinary ELISA did not appear to be limited to high-grade lesions. In 6 serial urine specimens from patients with grade I transitional cell carcinoma, only one false-negative was observed. Three true-positives and 2 true-negatives occurred, for an overall accuracy of 83%.

The ELISA procedure also offers important technical and practical advantages over cytological examination. It is a semiautomated procedure that can be used by a relatively unskilled technician to screen 200 to 300 urine specimens in a few hr. Thus, it is much less expensive and time-consuming than microscopic examination of urine. Furthermore, internal controls and standards included with each 96-well assay plate provide quick and accurate means of evaluating the reliability of the assay and of scoring assay results. This evaluation procedure contrasts sharply with the more subjective screening procedure which requires hours of evaluation by highly skilled personnel. The

**Table 5**

<table>
<thead>
<tr>
<th>Source of specimens (no. of patients)</th>
<th>Assay result</th>
<th>No. of negative specimens/total no. of specimens (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with nonmalignant urogenital disease (33)</td>
<td>Negative (TN)*</td>
<td>41/42 (98)</td>
</tr>
<tr>
<td>Patients with prostatic carcinoma (15)</td>
<td>Negative (TN)</td>
<td>47/49 (96)</td>
</tr>
<tr>
<td>Patients with renal carcinoma (4)</td>
<td>Negative (TN)</td>
<td>21/22 (95)</td>
</tr>
</tbody>
</table>

* TN, true negative.

**Table 6**

<table>
<thead>
<tr>
<th>Evaluation parameter</th>
<th>Formula</th>
<th>% of high-sensitivity assay</th>
<th>% of high-specificity assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>No. of TP specimens</td>
<td>83 (92/111)</td>
<td>50 (60/120)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Total no. of specimens</td>
<td>78 (274/352)</td>
<td>78 (292/372)</td>
</tr>
<tr>
<td>Specificity</td>
<td>No. of TN specimens (excluding specimens from BT* patients)</td>
<td>86 (95/110)</td>
<td>96 (109/113)</td>
</tr>
<tr>
<td>Overall specificity</td>
<td>No. of TN specimens</td>
<td>78 (182/241)</td>
<td>92 (232/252)</td>
</tr>
<tr>
<td>False negative</td>
<td>No. of FN specimens</td>
<td>5 (19/352)</td>
<td>16 (60/372)</td>
</tr>
<tr>
<td>False positive</td>
<td>No. of FP specimens</td>
<td>17 (59/352)</td>
<td>5 (20/372)</td>
</tr>
</tbody>
</table>

* TP, true positive; TN, true negative; FN, false negative; FP, false positive.
ELISA determination of fibrinogen/FDP can provide needed objectivity, sensitivity, and specificity in the detection of well-differentiated as well as the more aggressive carcinomas of the bladder.

Other investigators have also recognized the value of FDP determinations for the diagnosis and monitoring of bladder cancer patients (18, 23, 26-28, 30-32). Combined with urinary cytology, correct diagnosis was achieved in 80% of the patients in one study (32) and 86% of the patients in another study (28). In both of these studies, the FDP assay methods were not quantitative and were relatively insensitive (21). Future studies must focus on the use of the high-specificity ELISA as a screen for individuals at risk of developing bladder tumors to determine whether the ELISA can be used as an alternative to urinary cytological examination or whether cytological and cystoscopic examinations should be used in combination with frequently performed ELISA to enhance the accuracy of the ELISA without compromising the low rate of false-positive results.

The high-sensitivity ELISA correctly identified 83% of the patients with clinically detectable bladder carcinoma at some cost in specificity. False-positive results increased from 5% with the high-specificity procedure to 17% with this high-sensitivity method. The high-sensitivity procedure appeared most valuable as a monitor of changes in tumor status of patients under treatment for bladder cancer, a situation in which small but consistent increases in the fibrinogen/FDP titer may indicate possible recurrence and the need for an extensive clinical examination. In contrast, persistently low or decreasing levels may indicate a favorable situation and a good prognosis; 83% sensitivity far exceeds the results that can be expected of any other monitoring procedure (5, 6, 9, 11, 13). Furthermore, many other markers are nonspecifically elevated in patients with urinary tract infection or stones (9, 11). The ability to differentially diagnose bladder cancer in these patients would be very useful. The ELISA false-positive rate of 18% is low in comparison with that of other markers (6, 11). The ELISA false-positive results appear to be caused by such factors as residual FDP excretion after tumor removal, irreversible kidney damage, or clinically undetected tumor recurrence. False-negative values can result from non-representative urine collection or the presence of a tumor not associated with fibrinogen/FDP excretion. Long-term studies of individual patients with frequent sample collection will provide the pattern necessary to distinguish among these possibilities and lessen the adverse effects of false assay values.

Previous studies have reported elevated levels of urinary fibrinogen/FDP in upper but not lower urinary tract infections (17, 33). In our study, an elevated assay result was obtained with one patient with hematuria and one with a renal stone in the...
high-sensitivity procedure; however, only one of 29 specimens from patients with urinary tract infection had an elevated assay value. Immunotherapy with bacillus Calmette-Guérin, which produces a marked inflammatory response in the bladder, was not associated with elevated assay values. Nevertheless, an elevated FDP value in association with an upper urinary tract infection would be interpreted with reservation pending more extensive clinical studies to establish this relationship.

The ELISA described here has many advantages over other assays for FDP. It is more stable and convenient than radioimmunoassays. Compared to HAI assays, which are insufficiently sensitive for use as either a screen or a monitor, the ELISA is much easier to perform, needs no prior sample preparation or concentration, and can provide a quantitative analysis. The monoclonal antibody enhances the specificity and reproducibility of the assay procedure.

Finally, the fact that we could find no evidence of fibrinogen or FDP production by several lines of cultured bladder carcinoma cells indicates that the urinary fibrinogen/FDP probably arise from deposits of tumor-derived immune complexes (2, 7). However, our failure to find a correlation between our assay results and the results of protein assays on the same urine specimens is clear evidence that glomerular pathology is not the sole determinant of the urinary fibrinogen/FDP titer. Activation of plasminogen by the kidney-associated activator urokinase and subsequent fibrin/fibrinogen deposition may increase the filtration of low-molecular-weight digestion end-products recognized in the ELISA (7). A second source of urinary FDP is undoubtedly the tumor itself. Deposition of fibrin by tumor- and tissue-derived procoagulant and digestion of fibrin by plasminogen activators released as a component of the inflammatory response and by the proliferating tumor itself provide a source of low-molecular-weight digestion products which are not retained by the healthy kidney and which pass into the urine (for review, see Ref. 19). These factors are sufficient to explain the selective increase in urinary fibrinogen/FDP seen in bladder cancer.

REFERENCES

A Diagnostic-Prognostic Test for Bladder Cancer Using a Monoclonal Antibody-based Enzyme-linked Immunoassay for Detection of Urinary Fibrin(ogen) Degradation Products

Richard P. McCabe, Donald L. Lamm, Martin V. Haspel, et al.


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