

Relevance of Urine Telomerase in the Diagnosis of Bladder Cancer

Maria Aurora Sanchini, MSc

Roberta Gunelli, MD

Oriana Nanni, MSc

Sara Bravaccini, BSc

Carla Fabbri, MSc

Alice Sermasi, BSc

Eduard Bercovich, MD

Alberto Ravaioli, MD

Dino Amadori, MD

Daniele Calistri, PhD

THE INCIDENCE OF HUMAN BLADDER cancer has greatly increased over the last few decades, with more than 60 000 new cases diagnosed each year in the United States alone,¹ and now represents the 4th most common malignancy in men and the 10th most common in women.^{1,2} According to the latest reports from the National Cancer Institute,³ the incidence of this pathology is higher in industrialized than in developing countries.

Bladder cancer is 3 times more common among men than women, and the incidence increases with age. Approximately 80% of newly diagnosed individuals are aged 60 years or older.¹ At present, about 20% of patients die each year, but when the disease is diagnosed and treated in the early stage, the chances of survival are good, thus highlighting the importance of a timely and accurate diagnosis.

More than 90% of newly diagnosed bladder cancers are transitional-cell carcinomas. Approximately 75% of patients present with superficial cancer, 20% with invasive disease, and the remaining 5% with metastatic disease at first diagnosis.^{4,5}

Context The identification of new molecular markers is one of the most challenging goals for the early detection of bladder cancer because available noninvasive methods have neither sufficient sensitivity nor specificity to be acceptable for routine use.

Objective To develop a relatively simple, inexpensive, and accurate test that measures telomerase activity in voided urine to apply to large-scale screening programs for bladder cancer detection.

Design, Setting, and Participants Case-control study conducted in 218 men (84 healthy individuals and 134 patients at first diagnosis of histologically confirmed bladder cancer), frequency matched by age and recruited between March 2003 and November 2004 in Italy. Urine telomerase activity was determined using a highly sensitive telomeric repeat amplification protocol (TRAP) assay. Urine samples were processed for cytological diagnosis and TRAP assay. The diagnosis of bladder cancer was based on bioptic and cystoscopic examinations. The performance of the TRAP assay to detect urine telomerase activity was compared with urine cytology as an aid to early cancer detection. Quantification of urine telomerase activity was conducted in a blinded manner.

Main Outcome Measure Sensitivity and specificity of TRAP to detect bladder cancer.

Results Using a 50 arbitrary enzymatic unit cutoff value, we validated the results obtained in the pilot study. In the overall series, sensitivity was 90% (95% confidence interval [CI], 83%-94%) and specificity was 88% (95% CI, 79%-93%). Specificity increased to 94% (95% CI, 85%-98%) for individuals aged 75 years or younger. The same predictive capacity of telomerase activity levels was observed for patients with low-grade tumors or with negative cytology results.

Conclusions The present validation study demonstrated the ability of urine telomerase activity levels to accurately detect the presence of bladder tumors in men. This test represents a potentially useful noninvasive diagnostic innovation for bladder cancer detection in high-risk groups such as habitual smokers or in symptomatic patients.

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Established approaches for detecting bladder cancer include urine cytology and cystoscopy, used singly or in sequence. However, the invasiveness and relatively high cost of cystoscopic examination and the limited sensitivity of urinary cytology, especially for low-grade superficial lesions, make it of the utmost importance to develop a noninvasive, reliable, and simple test to increase the rate of detection of bladder cancer. Among the markers investigated for this purpose, an important role has been played by telomerase activity in voided urine or bladder washings,⁶⁻¹⁰ determined by the telomeric repeat amplification protocol

(TRAP) assay.¹¹ Initially, studies dealt with qualitative determinations. To obtain a more accurate and reliable estimate of telomerase activity levels, a quantitative TRAP assay was developed, based on the exponential amplification of the

Author Affiliations: Division of Oncology and Diagnostics (Mss Sanchini and Bravaccini and Dr Calistri) and Department of Urology (Drs Gunelli and Bercovich), Morgagni-Pierantoni Hospital, Forlì, Italy; Istituto Oncologico Romagnolo, Forlì, Italy (Ms Nanni); Department of Oncology, Infermi Hospital, Rimini, Italy (Mss Fabbri and Sermasi and Dr Ravaioli); and Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Forlì-Meldola, Italy (Dr Amadori).

Corresponding Author: Daniele Calistri, PhD, Division of Oncology and Diagnostics, Morgagni-Pierantoni Hospital, Via Forlanini 34, 47100 Forlì, Italy (biomolec@ausl.flo.it).

primer-telomeric repeats generated in the telomerase reaction.¹²⁻¹⁵ Using this assay, telomerase activity has been detected in almost all superficial urothelial cell carcinomas, but not in healthy urothelia.¹⁶ We used the TRAP assay with the internal standard developed by Wright et al¹⁷ and added a reference curve to obtain more accurate and reproducible results.¹⁸

The promising results from our pilot study¹⁸ prompted us to carry out a case-control study, prospectively planned and performed blindly on urine from male individuals to validate the 50 arbitrary enzymatic units (AEUs) that emerged as the best cutoff and to define the diagnostic accuracy of different telomerase activity cutoff values in terms of sensitivity and specificity.

METHODS

Case Series

The study was conducted in 218 men (FIGURE 1), of whom 84 were healthy individuals and 134 were patients at first diagnosis of bladder cancer, frequency matched by age (≤ 75 years and >75 years). Median age was 62.4 years (range, 22-98 years) in healthy individuals and 69.8 years (range, 33-88 years) in patients.

Healthy individuals were recruited from hospital laboratory staff and geriatric wards, and none had been previously clinically diagnosed with any type

of cancer or with inflammatory pathologies of the urogenital tract.

Patients were prospectively enrolled from the Urology Departments of Pierantoni-Morgagni Hospital (Forlì) and Infermi Hospital (Rimini) between March 2003 and November 2004. All patients underwent cystoscopy as a reference standard for bladder cancer detection, and all tumors or suspicious lesions were resected. Patients who had undergone previous treatment were excluded.

The final diagnosis of bladder cancer was based on histologic examination. Histologic type and tumor cell differentiation were determined according to World Health Organization criteria. Fifteen (11%) tumors were well differentiated (G1), 55 (41%) were moderately differentiated (G2), and 57 (42%) were poorly differentiated (G3). There was 1 carcinoma in situ. Grading was not available for 6 patients.

Demographic data and medical history were collected at study entry. The local ethics committee reviewed and approved the study protocol for each center, and all participants provided written informed consent.

Urine Collection

Urine samples from both healthy individuals and patients were processed for cytological diagnosis and TRAP assay.

Each patient evaluated for bladder cancer provided a voided urine sample immediately before cystoscopy.

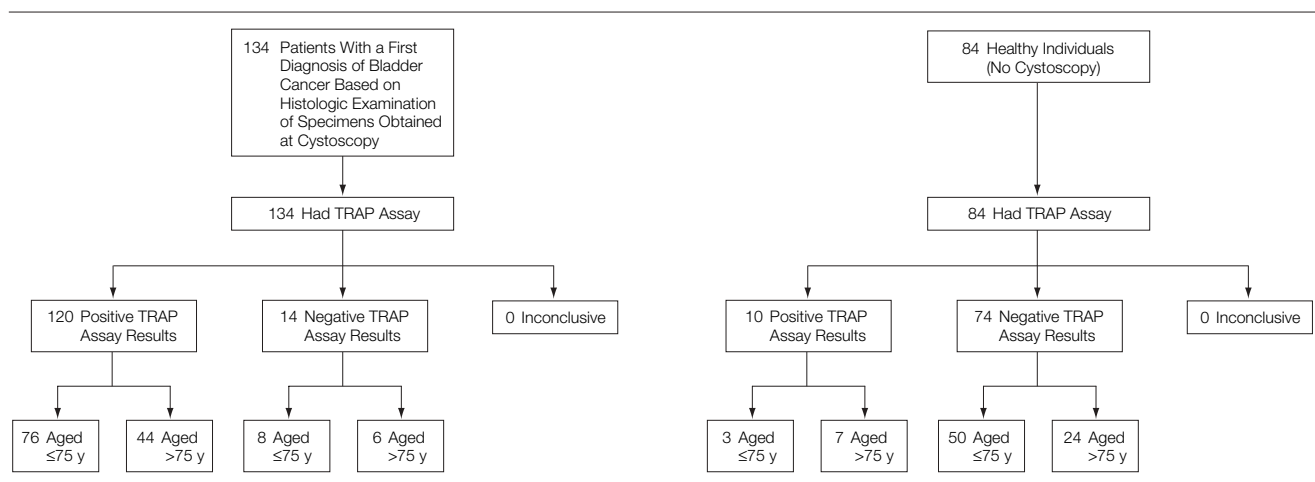
Cytology

Cytological examination was performed in all the urine samples from healthy individuals ($n=84$) and in 103 of the 134 bladder cancer patients analyzed with TRAP assay. Forty-eight (46.6%) patients had positive cytology, 40 (38.8%) had negative cytology, 8 (7.8%) patients with suspicious cytology findings had evidence of bladder cancer at histologic examination, and 7 (6.8%) had nonassessable cytology because of a lack of exfoliated cells. The cytological examination was unavailable for 31 patients because they bypassed this preliminary urine evaluation and directly underwent cystoscopy.

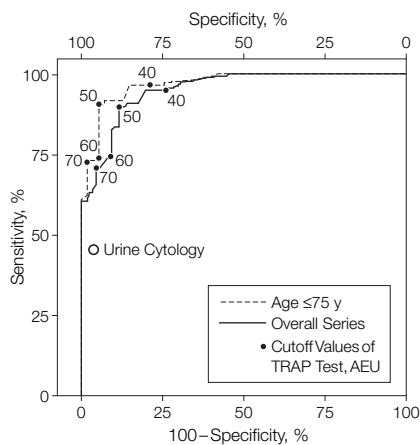
TRAP Assay

Cell extract preparation and TRAP assay were carried out as previously described.^{18,19} Cells were pelleted by centrifugation (850g for 10 minutes at 4°C) within 1 to 3 hours of urine sample collection, washed once in phosphate-buffered saline, resedimented by centrifugation (2300g for 5 minutes at 4°C), and stored at -80°C until use (a maximum of 12 months). The pelleted cells were resuspended in 200 μL of lysis reagent¹⁹ and left on ice for 30 minutes.

Figure 1. Study Flow Diagram



TRAP indicates telomeric repeat amplification protocol. The target condition in this study was bladder cancer.

Figure 2. Receiver Operating Characteristic Curve of Telomerase Activity

For the overall series, the area under curve is 0.951 (95% confidence interval [CI], 0.925-0.976) and for individuals aged ≤ 75 years, 0.968 (95% CI, 0.942-0.993). Points are marked to demonstrate the sensitivity and 1-specificity of urine cytology and of the telomeric repeat amplification protocol (TRAP) test at cutoff points of 40, 50, 60, and 70 arbitrary enzymatic units (AEUs).

Cell lysates were centrifuged (10 000g for 20 minutes at 4°C), and the supernatant extracts were stored at -80°C. Aliquots of each urine sample containing 1 μ g of protein lysate were used for the TRAP assay. Telomerase products were evaluated on fluorescence electropherograms, and the area underlying the different peaks was calculated. To obtain semiquantitative levels of telomerase activity, an internal telomerase assay standard (ITAS; 25 attograms¹⁷), amplified by the same 2 primers used for the telomerase activity assay, was included in the TRAP buffer. Protein concentrations corresponding to 10, 30, 100, 300, 1000, and 3000 cells of a human bladder cancer line (MCR)¹⁸ were analyzed in each assay and used as the reference curve. To obtain quantitative evaluations of telomerase activity, the areas of each sample were also normalized to the 150-base pair ITAS peak. The relative telomerase activity per cell for each sample is presented as the percentage of the ratio of TRAP ladder/ITAS per cell vs the value of MCR and expressed in AEU. All experiments were performed in duplicate, and when varia-

tions were greater than 15%, observed in about 10% of cases, a third analysis was performed. Telomerase activity was expressed as a continuous variable in all analyses.

Statistical Analysis

The population size was defined on the basis of results from the previous pilot study¹⁸ in which we obtained 93% sensitivity and 90% specificity using the 50-AEU cutoff value for the subgroup of male individuals. In fact, for the 84 healthy individuals and 134 bladder cancer patients of the present study, we predicted the 95% confidence interval (CI) to be $\pm 5\%$ with respect to the single estimated value for sensitivity and specificity. To avoid bias in the clinical utility of the TRAP assay, we analyzed all samples prospectively, without previous knowledge of the patient's clinicopathologic status.

The threshold value for optimal sensitivity and specificity was determined using a receiver operating characteristic (ROC) curve,²⁰ constructed by calculating the true-positive (sensitivity) and false-positive (1-specificity) rates at several cutoff values. Sensitivity, specificity, and relative 95% CIs were calculated for the most discriminant cutoff values. The relationship between urine telomerase activity and histological grading was analyzed using the median test. For all tests, a 2-sided $P = .05$ was regarded as significant. Data analyses were performed with SAS release 8.0 (SAS Institute Inc, Cary, NC).

All statistical analyses were performed at the Unit of Biostatistics and Clinical Trials of Istituto Oncologico Romagnolo, Forlì, Italy.

RESULTS

The median telomerase activity value in urine was 27 AEU (range, 0-88) in healthy individuals and 112 AEU (range, 30-382) in patients. We did not observe any patients with a telomerase activity value lower than 30 AEU or any healthy individuals with a telomerase activity value higher than 90 AEU. Moreover, in patients with nega-

tive or positive cytology, the median telomerase activity values in urine were 99 (range, 38-265) and 134 (range, 37-253) AEU, respectively.

As primary end point, we validated the results obtained in the pilot study using a 50-AEU cutoff value. In the overall series, 90% (95% CI, 83%-94%) sensitivity and 88% (95% CI, 79%-93%) specificity were observed.

As secondary end point, the diagnostic relevance of urine telomerase activity was analyzed for the overall series and for the subgroups of individuals 75 years or younger and older than 75 years. The ROC curve analysis provides a graphic demonstration of the sensitivity and specificity of telomerase activity in the overall series and the even higher specificity in the subgroup of individuals 75 years or younger (FIGURE 2).

In particular, sensitivity in the overall series ranged from 61% to 100% and specificity from 54% to 100% according to the different AEU cutoff values (TABLE 1). As shown in Figure 2, a similar sensitivity and an even higher specificity (94%) (95% CI, 85%-98%) was obtained in the subgroup of individuals 75 years or younger.

Although an increase in urine telomerase activity levels was observed from histologic grades 1 to 3, it did not reach statistical significance (TABLE 2).

The sensitivity of urine telomerase activity in detecting bladder tumors was similar in the subgroups of patients with different tumor grades at all AEU cutoff values. In particular, at 50 AEU the sensitivity was 93%, 87%, and 89% for grades 1, 2, and 3, respectively (TABLE 3).

COMMENT

Telomerase has been investigated as a potentially useful biomarker for early cancer detection^{8,21-23} and prognosis²⁴ and for monitoring residual disease.²¹ Elevated levels of telomerase expression, in particular of the human telomerase reverse transcriptase catalytic subunit, have been observed in almost all human tumor histotypes, including bladder cancer. In contrast, telomer-

ase activity has not been detected in most of the somatic cells.²⁵⁻³⁰

We confirmed the high sensitivity and specificity of urine telomerase levels, in particular of the 50-AEU cutoff observed in our pilot study, in detecting bladder cancer.¹⁸

Moreover, we observed that sensitivity is not age dependent, whereas specificity is higher in individuals younger than 75 years.

The test we developed requires a small amount of urine; is noninvasive, inexpensive, and easy to perform; and permits a quantitative evaluation of telomerase activity in urine. Furthermore, it is objective, reproducible, and specific and is not reliant on the expertise of the cytopathologist.⁹ Another important advantage of this test is its ability to also identify low-grade tumors, which often escape detection during cytologic examination.

However, notwithstanding the validated optimal diagnostic accuracy of the test, it is not recommended for use in routine screening programs because of the low incidence of bladder cancer, and should be aimed at high-risk subgroups. Specifically, smokers have about a 3-fold increased risk of developing bladder cancer compared with nonsmokers. It might be even more advantageous in terms of cost/benefit to use the TRAP assay in selected individuals who present with hematuria. For this subgroup, the incidence of bladder cancer is about 10% to 15% and the sensitivity of urinary cytology is only 30% to 50%.³¹⁻³³ Although cystoscopy is the gold standard for the diagnosis of bladder cancer because of its 90% sensitivity, the invasiveness and low specificity of the procedure in symptomatic patients³⁴ make it important to identify a manageable and more accurate diagnostic tool.

In addition to telomerase, several new, alternative laboratory tests based on the detection of different substances (eg, BTA tests, NMP22, fibrinogen degradation products, hyaluronic acid, multicolor fluorescence in situ hybridization assay),³⁵⁻⁴³ as well as novel research procedures (microsatellite

Table 1. Sensitivity and Specificity of Urine Telomerase Activity in the Overall Series and in Individuals Aged ≤ 75 Years

Cutoff, AEU	% (95% CI)			
	Overall Series (N = 218)		≤ 75 y (n = 137)	
	Sensitivity	Specificity	Sensitivity	Specificity
30	100	54 (43-64)	100	58 (45-71)
40	96 (91-98)	73 (62-81)	96 (90-99)	77 (65-87)
50	90 (83-94)	88 (79-93)	90 (82-95)	94 (85-98)
60	76 (68-83)	90 (82-95)	75 (65-83)	94 (85-98)
70	69 (61-76)	95 (88-98)	70 (60-79)	98 (90-100)
80	63 (54-70)	98 (92-99)	62 (51-72)	98 (90-100)
90	61 (53-69)	100	61 (50-70)	100

Abbreviations: AEU, arbitrary enzymatic unit; CI, confidence interval.

Table 2. Relationship Between Telomerase Activity and Histologic Grade*

	Histologic Grade		
	1	2	3
Patients, No.	15	55	57
AEUs, median (range)	88 (38-382)	100 (30-265)	122 (35-344)

Abbreviation: AEU, arbitrary enzymatic units.

*Median test, $\chi^2 = 0.76$, $P = .68$.

Table 3. Sensitivity of Urine Telomerase Activity in Patients With Different Tumor Grades

Cutoff, AEUs	% (95% CI)		
	Grade 1	Grade 2	Grade 3
30	100	100	100
40	93 (70-99)	96 (88-99)	95 (86-98)
50	93 (70-99)	87 (76-94)	89 (79-85)
60	73 (48-89)	71 (58-81)	79 (67-88)
70	60 (36-90)	65 (52-77)	75 (63-85)
80	53 (30-75)	60 (47-72)	68 (56-79)
90	47 (25-70)	58 (45-70)	68 (56-79)

Abbreviations: AEU, arbitrary enzymatic units; CI, confidence interval.

analysis, DNA methylation, RNA expression, real-time polymerase chain reaction analysis),⁴⁴⁻⁴⁶ have become available in an attempt to improve the sensitivity of cytology for the diagnosis of bladder cancer. However, many problems, such as low sensitivity, unsatisfactory specificity levels, or technical difficulties for the application of these tests in large population studies, have limited their clinical utility.

In conclusion, we believe that our telomerase activity urine assay, with the reliability verified in pilot and confirmatory studies, represents a promising and potentially important contribution to the early diagnosis of bladder carcinoma, in particular for high-risk

subgroups. Further prospective studies on larger patient populations are needed to assess the diagnostic role of urinary telomerase, to define the ability of this assay to detect low-grade tumors, and to forecast clinical relapse.

Author Contributions: Ms Sanchini had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Sanchini, Nanni.

Acquisition of data: Sanchini, Gunelli, Fabbri, Sermasi, Bercovich, Ravaoli, Amadori, Calistri.

Analysis and interpretation of data: Sanchini, Bravaccini, Calistri.

Drafting of the manuscript: Sanchini, Gunelli, Bercovich, Ravaoli, Calistri.

Critical revision of the manuscript for important intellectual content: Sanchini, Nanni, Bravaccini, Fabbri, Sermasi, Amadori, Calistri.

Statistical analysis: Sanchini, Nanni.

Obtained funding: Sanchini, Amadori, Calistri.

Administrative, technical, or material support: Sanchini, Gunelli, Bravaccini, Fabbri, Sermasi, Bercovich, Ravaioli, Amadori, Calistri.

Study supervision: Sanchini, Calistri.

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REFERENCES

- American Cancer Society. *Cancer Facts and Figures 2004*. Atlanta, Ga: American Cancer Society; 2004.
- Johansson SL, Cohen SM. Epidemiology and etiology of bladder cancer. *Semin Surg Oncol*. 1997;13:291-298.
- National Cancer Institute Web site. General information about bladder cancer. Available at: <http://seer.cancer.gov/publications/ethnicity/bladder.pdf>. Accessed September 2, 2005.
- Messing EM, Young TB, Hunt VB, et al. Comparison of bladder cancer outcome in men undergoing hematuria home screening versus those with standard clinical presentations. *Urology*. 1995;45:387-396.
- Herr HW, Shipley WU, Bajorin DF. Cancer of the bladder. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds. *Cancer: Principles and Practice of Oncology*. 6th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001:1396-1418.
- Kinoshita H, Ogawa O, Takechi Y, et al. Detection of telomerase activity in exfoliated cells in urine from patients with bladder cancer. *J Natl Cancer Inst*. 1997;89:724-730.
- Yoshida K, Sugino T, Tahara H, et al. Telomerase activity in bladder carcinoma and its implication for noninvasive diagnosis by detection of exfoliated cancer cells in urine. *Cancer*. 1997;79:362-369.
- Muller M, Krause H, Heicappell R, Tischendorf J, Shay JW, Miller K. Comparison of human telomerase RNA and telomerase activity in urine for diagnosis of bladder cancer. *Clin Cancer Res*. 1998;4:1949-1954.
- Dalbagni G, Han W, Zhang ZF, et al. Evaluation of the telomeric repeat amplification protocol (TRAP) assay for telomerase as a diagnostic modality in recurrent bladder cancer. *Clin Cancer Res*. 1997;3:1593-1598.
- Ramakumar S, Bhuiyan J, Besse JA, et al. Comparison of screening methods in the detection of bladder cancer. *J Urol*. 1999;161:388-394.
- Kim NW, Piatyszek MA, Prowse KR, et al. Specific association of human telomerase activity with immortal cells and cancer. *Science*. 1994;266:2011-2015.
- Hirose M, Abe-Hashimoto J, Ogura K, et al. A rapid, useful and quantitative method to measure telomerase activity by hybridization protection assay connected with a telomeric repeat amplification protocol. *J Cancer Res Clin Oncol*. 1997;123:337-344.
- Kim NW, Wu F. Advances in quantification and characterization of telomerase activity by the telomeric repeat amplification protocol (TRAP). *Nucleic Acids Res*. 1997;25:2595-2597.
- Gelmini S, Caldini A, Becherini L, et al. Rapid, quantitative nonisotopic assay for telomerase activity in human tumors. *Clin Chem*. 1998;44:2133-2138.
- Yahata N, Ohyashiki K, Ohyashiki JH, et al. Telomerase activity in lung cancer cells obtained from bronchial washings. *J Natl Cancer Inst*. 1998;90:684-690.
- Lin Y, Miyamoto H, Fujinami K, et al. Telomerase activity in human bladder cancer. *Clin Cancer Res*. 1996;2:929-932.
- Wright WE, Shay JW, Piatyszek MA. Modifications of a telomeric repeat amplification protocol (TRAP) result in increased reliability, linearity and sensitivity. *Nucleic Acids Res*. 1995;23:3794-3795.
- Sanchini MA, Bravaccini S, Medri L, et al. Urine telomerase: an important marker in the diagnosis of bladder cancer. *Neoplasia*. 2004;6:234-239.
- Fedriga R, Gunelli R, Nanni O, Bacci F, Amadori D, Calistri D. Telomerase activity detected by quantitative assay in bladder carcinoma and exfoliated cells in urine. *Neoplasia*. 2001;3:446-450.
- Deeks JJ. Systematic reviews in health care: systematic reviews of evaluations of diagnostic and screening tests. *BMJ*. 2001;323:157-162.
- Hiyama E, Gollahon L, Kataoka T, et al. Telomerase activity in human breast tumors. *J Natl Cancer Inst*. 1996;88:116-122.
- Breslow RA, Shay JW, Gazdar AF, Srivastava S. Telomerase and early detection of cancer. *J Natl Cancer Inst*. 1997;89:618-623.
- Shay JW, Gazdar AF. Telomerase in the early detection of cancer. *J Clin Pathol*. 1997;50:106-109.
- Tatsumoto N, Hiyama E, Murakami Y, et al. High telomerase activity is an independent prognostic indicator of poor outcome in colorectal cancer. *Clin Cancer Res*. 2000;6:2696-2701.
- Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer*. 1997;33:787-791.
- Califano J, Ahrendt SA, Meisinger G, Westra WH, Koch WM, Sidransky D. Detection of telomerase activity in oral rinses from head and neck squamous cell carcinoma patients. *Cancer Res*. 1996;56:5720-5722.
- Kannan S, Tahara H, Yokozaki H, et al. Telomerase activity in premalignant and malignant lesions of human oral mucosa. *Cancer Epidemiol Biomarkers Prev*. 1997;6:413-420.
- Mutirangura A, Supiyaphun P, Trirekanan S, et al. Telomerase activity in oral leukoplakia and head and neck squamous cell carcinoma. *Cancer Res*. 1996;56:3530-3533.
- Miyoshi Y, Tsukinoki K, Imaizumi T, et al. Telomerase activity in oral cancer. *Oral Oncol*. 1999;35:283-289.
- Holt SE, Shay JW. Role of telomerase in cellular proliferation and cancer. *J Cell Physiol*. 1999;180:10-18.
- Keese SK, Briggman JV, Thill G, Wu YJ. Utilization of nuclear matrix protein for cancer diagnosis. *Crit Rev Eukaryot Gene Expr*. 1996;6:189-214.
- Getzenberg RH, Konety BR, Oeler TA, et al. Bladder cancer-associated nuclear matrix proteins. *Cancer Res*. 1996;56:1690-1694.
- Sarosdy MF, Hudson MA, Ellis WJ, et al. Improved detection of recurrent bladder cancer using the Bard BTA stat test. *Urology*. 1997;50:349-353.
- Sharma S, Zippe CD, Pandrangi L, Nelson D, Agarwal A. Exclusion criteria enhance the specificity and positive predictive value of NMP22 and BTA stat. *J Urol*. 1999;162:53-57.
- Konety BR, Getzenberg RH. Urine based markers of urological malignancy. *J Urol*. 2001;165:600-611.
- Kausch I, Böhle A. Bladder cancer, II: molecular aspects and diagnosis. *Eur Urol*. 2001;39:498-506.
- Lokeshwar VB, Soloway MS. Current bladder tumor tests: does their projected utility fulfill clinical necessity? *J Urol*. 2001;165:1067-1077.
- Glas AS, Roos D, Deutekom M, et al. Tumor markers in the diagnosis of primary bladder cancer: a systematic review. *J Urol*. 2003;169:1975-1982.
- Grossman HB, Messing E, Soloway M, et al. Detection of bladder cancer using a point-of-care proteomic assay. *JAMA*. 2005;293:810-816.
- Halling KC, King W, Sokolova IA, et al. A comparison of cytology and fluorescence in situ hybridization (FISH) for the detection of urothelial carcinoma. *J Urol*. 2000;164:1768-1775.
- Halling KC, King W, Sokolova IA, et al. A comparison of BTA stat, hemoglobin dipstick, telomerase and Vysis Urovysion assays for the detection of urothelial carcinoma in urine. *J Urol*. 2002;167:2001-2006.
- Sarosdy MF, Schellhammer P, Bokinsky G, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. *J Urol*. 2002;168:1950-1954.
- Placer J, Espinet B, Salido M, Solé F, Gelabert-Mas A. Clinical utility of a multiprobe FISH assay in voided urine specimens for the detection of bladder cancer and its recurrences, compared to urinary cytology. *Eur Urol*. 2002;42:547-552.
- Sourvinos G, Kazanis I, Delakas D, Cranidis A, Spandidos DA. Genetic detection of bladder cancer by microsatellite analysis of p16, RB1 and p53 tumor suppressor genes. *J Urol*. 2001;165:249-252.
- Dulaimi E, Uzzo RG, Greenberg RE, Al-Saleem T, Cairns P. Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. *Clin Cancer Res*. 2004;10:1887-1893.
- Smith SD, Wheeler MA, Plescia J, Colberg JW, Weiss RM, Altieri DC. Urine detection of survivin and diagnosis of bladder cancer. *JAMA*. 2001;285:324-328.