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Sensitivity and Specificity Analysis of Urine NMP22, Cytokeratin-18, CA 19-9 and Cytology, for Diagnosing Bladder Malignancy

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ABSTRACT

Introduction: *Bladder malignancy is a common urologic malignancy. Pathologic examination obtained by biopsy through cystoscopy is the gold standard for diagnosing, but this is invasive. The use of NMP22, cytokeratin-18 and CA 19-9 and as a marker in urine for bladder malignancy had been studied and had high sensitivity but expensive, otherwise, cytology for marker bladder malignancy is noninvasive, cheaper but had less sensitivity. This study to determine the sensitivity and specificity of the four current alternatives to diagnosis bladder malignancy.*

Methods: *Evaluation of the four current alternatives to bladder malignancy diagnosis was conducted from two urology centers in East Java, Soetomo Hospital Surabaya, and Saiful Anwar Hospital Malang. We evaluated 392 voided urinary specimens of patients with suspicion of bladder malignancy (patients with painless intermittent gross haematuria). All voided urine samples were evaluated by the NMP22, cytokeratin-18, CA 19-9 and cytology. The diagnostic value (sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the four examination methods were evaluated according to correlation with cystoscopic findings and histological findings.*

Results: *In total, 203 patients had histologically proven transitional cell carcinoma of the bladder. There is no significant difference in the stadium. Sensitivity, specificity, PPV and NPV for NMP22 were 90, 6 %, 77, 4%, 78%, and 90%. Sensitivity, specificity, PPV and NPV for cytokeratin-18 were 94%, 75%, 68%, and 95%. Sensitivity, specificity, PPV and NPV for CA 19-9 were 85%, 77, 8%, 81%, and 82% Sensitivity, specificity, PPV and NPV for cytology were 63%, 88%, 83% and 72%*

Conclusion: *Urine cytokeratin-18 had highest sensitivity for diagnosing bladder malignancy, but urine cytology had highest specificity.*

Keywords

Bladder Malignancy, CA19-9, Cytokeratin-18, NMP22, Urine Cytology.

Introduction

Bladder malignancy is one of the most world's health problems and often leads to death. It is estimated that around 275.000 people are diagnosed with bladder malignancy each year and 108.000 patients die because of this [1]. In the United States, bladder cancer is ranked fourth of all the most common malignancy in men, after malignancy of prostate, lung and colorectal. The disease is often found in men than women but the prognosis is worse in women. The American Cancer Society estimates the occurrence of new cases of bladder cancer in the US as many as 70.530 cases in the year 2010 and approximately 14.680 patients will die from this disease [2,3]. Malignant bladder disease often leads to recurrence and the progressivity is very quickly, so screening test is very important for early detection of bladder malignancy [4].

The gold standard for detecting bladder malignancy is a biopsy and pathologic examination, which biopsy is done through cystoscopy, but this examination is invasive and expensive. In addition, the

ability to diagnose with these methods can be reduced if there are early-stage and plat urothelial lesion, so making it difficult to distinguish between carcinoma in situ (CIS) with normal bladder tissue [5,6].

Some noninvasive examination method has been discovered, traditionally urine cytology through an examination of biomarkers for early detection and monitoring of bladder malignancy. There are 30 urine biomarkers have been reported for diagnosing bladder malignancy [7-10]. Potential biomarkers of disease progression and prognosis include nuclear matrix protein (NMP-22), matrix metalloprotease 2 and 9, fibrin/fibrinogen degradation product (FDP), bladder tumor antigen (BTA), telomerase, bladder cancer marker (BCLA-4, fibronectin and cytokeratin 8 and 18, urothelial carcinoma-associated 1 (UCA1), CA 19-9 and many more. Their sensitivities and specificities for diagnosing bladder malignancy are varied from 54-100% and 61-100% [9,10]. But the problem for use urine biomarkers that only a few are commercially available in Indonesia and the prize more expensive, especially for Indonesia where not all people covered by insurance.

In East Java urine biomarkers that available for diagnosing bladder malignancy are urine NMP22, urine cytokeratin 18 and urine CA 19-9. On the other side, urine cytology is a traditional tool for diagnosing bladder malignancy, this is a noninvasive method, cheaper but had lower sensitivity and depends on pathologic ability. This study was aimed to determine sensitivity and specificity of the four current alternatives to bladder malignancy diagnosis in East Java Indonesia.

Methods

This is retrospective with cross-sectional study design. We collected data from medical record bladder malignancy patients (proven by pathologic examination) who underwent urine NMP22 examination, urine cytokeratin-18 examination, urine CA 19-9 and urine cytology examination in two urology centers at East Java, Soetomo Hospital Surabaya and Saiful Anwar Hospital Malang.

As for control group, we collected data from no bladder malignancy (patients with suspected bladder malignancy but pathologic examination show there is no bladder malignancy or have other urologic abnormality) who underwent urine NMP22 examination, urine cytokeratin 18 examination, urine CA 19-9 and urine cytology examination. We notice age, sex, stage of malignancy and calculate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each examination method.

Results

A total of 86 patients were collected from April 2010 - March 2013 with bladder malignancy was tested with urine NMP22, 18 patients were tested with urine cytokeratin, 20 patients were tested with urine CA 19-9 and 79 patients were tested with urine cytology. From control patients, 93 patients tested with urine NMP22, 32 patients tested with urine cytokeratin 18, 20 patients tested with urine CA 19-9 and 104 patients tested with urine cytology.

From Soetomo Hospital Surabaya, the examination did simultaneously in 20 patients bladder malignancy and 20 patients control (urine NMP22, urine CA 19-9 and urine cytology tested simultaneously). From Saiful Anwar Hospital Malang, examination done separately for each marker with 66 bladder malignancy patients tested with urine NMP22, 18 patients with urine cytokeratin-18 and 59 patients with urine cytology with 169 control patients.

From data characteristic there is no difference between age and distribution of stage patients with bladder malignancy tested with four examination type and also no difference in control group (Table 1).

Sensitivity, specificity, PPV and NPV for NMP22 were 90, 6 %, 77, 4%, 78%, and 90%. Sensitivity, specificity, PPV and NPV for urine cytokeratin-18 were 94%, 75%, 68%, and 95%. Sensitivity, specificity, PPV and NPV for urine CA 19-9 were 85%, 77,8%, 81%, and 82% Sensitivity, specificity, PPV and NPV for urine cytology were 63%, 88%, 83% and 72% (Tables 2-5).

No	Characteristic	Bladder Malignancy Patients				P
		NMP22 (n 86)	Cytokeratin18 (n 18)	CA 19-9 (n 20)	Cytology (n 79)	
Age		56 ± 4,5 yo	55 ± 5,5 yo	56 ± 7,5 yo	57 ± 8,5 yo	0,254
Sex	Man	66	13	17	60	0,156
	Women	20	5	3	19	0,065
Stage	Locally	40	7	10	41	
	Locally advanced	24	7	5	23	0,055
	Metastatic disease	22	5	5	15	0,053

Table 1: Data Characteristic.

	Bladder malignancy (+)	Non bladder malignancy (-)	Total
Positive test urine NMP22	78	21	99
Negative test urine NMP22	8	72	80
Total	86	93	179

Table 2: Urine NMP22 test result.

	Bladder malignancy (+)	Non bladder malignancy (-)	Total
Positive test urine cytokeratin	17	8	25
Negative test urine cytokeratin	1	24	25
Total	18	32	50

Table 3: Urine cytokeratin-18 test result.

	Bladder malignancy (+)	Non bladder malignancy (-)	Total
Positive test urine cytology	15	4	19
Negative test urine cytology	5	16	21
Total	20	20	40

Table 4: Urine CA 19-9 test result.

	Bladder malignancy (+)	Non bladder malignancy (-)	Total
Positive test urine cytology	50	10	60
Negative test urine cytology	29	74	103
Total	79	84	163

Table 5: Urine cytology test result.

Discussion

Bladder malignancy is one of the most world's health problems. The incidence rate of bladder cancer in the United States in 2005, 63,210. It's very progressive and rate of recurrence is high, so screening test is very important for early detection of bladder malignancy [4]. The gold standard for detecting bladder malignancy is biopsy through cystoscopy, but this is invasive and expensive [5].

Nowadays, there are 30 urine biomarkers have been reported for diagnosing bladder malignancy [9,10]. This is a noninvasive method but only few are commercially available in Indonesia and the price more expensive. In Indonesia, urine biomarkers that available for diagnosing bladder malignancy are urine NMP22 urine CA 19-9 and urine cytokeratin.

Nuclear matrix Protein 22 (NMP22), a protein matrix of nucleus cells that responsible for chromatid regulation and cell separation during cell division process. Nuclear matrix Protein 22 (NMP22) is removed from the nucleus cell tumor during cell death. Nuclear matrix Protein 22 (NMP22), expression significantly higher in malignant bladder tissue than normal bladder [8]. Based on the result of previous studies, suggest that the result of NMP22 examination can be increased up to 80-fold in tumor cells contained in the urinary tract [11].

From our study, we found that urine NMP22 examination for bladder malignancy had high sensitivity (90, 6%), moderate specificity (77, 4%), 78% positive predictive value and 90% negative predictive value. This resembles a previous study that was conducted by Poulakis, et al. which shows urine NMP22 sensitivity was quite high (85%), whereas specificity was lower 68% [9]. In another study urine NMP22 showed that sensitivity was high (91, 3%) whereas specificity was lower (87, 5%) [10].

Cytokeratin is intermediate filaments; their main function is to enable cells to withstand mechanical stress. In humans, 20 different cytokeratin isotypes have been identified. Cytokeratin 8, 18, 19, and 20 have been associated with bladder cancer [11-14]. The Urinary Bladder Cancer (UBC) test detects cytokeratin 8 and 18 fragments in the urine. The sensitivity of the UBC test varies from 35% to 79% and depends on tumor grade and stage [15,16]. However, UBC tests were inferior to voided cytology in test quality [17]. From our study, we found that urine cytokeratin examination for bladder malignancy had high sensitivity (94%), moderate specificity (75%), 68% positive predictive value and 95% negative predictive value.

Carbohydrate Antigen 19-9 (CA 19-9) is tumor linked glycoprotein antigen as a carbohydrate determinant. Carbohydrate Antigen 19-9 recognizes tumor by the sialyl-Lewis structure that ssynthesized by sialyltransferase and Lewis transferase. Carbohydrate Antigen 19-9 produced primarily at colon epithelial, ileum, gaster, pancreatic, liver, and small amount at urinary tract and lungs. Carbohydrate Antigen 19-9 not organ specific tumor marker, although had high sensitivity and specificity for pancreatic cancer [18,19]. Study review from Sharia et al, show that sensitivity and specificity NMP22 not much better than CA 19-9 for diagnosing bladder malignancy [19]. Source of CA 19-9 in urine not yet clearly explained, some study suggest luminal umbrella cell is source place that this antigen produced [20].

A study from Mahander, et al, show increasing CA 19-9 levels in urine patients with high-grade bladder malignancy compared with low-grade bladder malignancy [21]. A study from Pal et al. shows that in bladder malignancy patients urine CA 19-9 levels increase significantly than normal people and Vestergaard et al, show that urine CA 19-9 levels are high in dysplatic urothelial than patients with normal urothelial [18,20]. Pode et al, found that sensitivity and specificity urine CA 19-9 for diagnosing bladder malignancy are 80%-85% [22]. From our study, we found that urine CA 19-9 examination for bladder malignancy had high sensitivity (85%), moderate specificity (77, 8%), 81% positive predictive value and 82% negative predictive value.

Cytology of voided urine or bladder washes is the most established noninvasive method in the workup of hematuria (blood in the urine; the most common presentation of bladder cancer) and follow-up in patients with a history of bladder cancer and is used as an adjunct to cystoscopy. This involves microscopic identification of exfoliated tumor cells based on cytological criteria. Briefly, exfoliated tumor cells obtained as sediment after centrifugation of a midstream voided urine sample are fixed and stained using the Papanicolaou procedure [23].

The method has high specificity but relatively low sensitivity, particularly in well-differentiated bladder tumors [23]. A meta-analysis that included data on 18 published series with 1,255 patients reported a sensitivity of 34% and specificity of 99% (95% confidence interval 20%-53% and 83%-99.7%, respectively) [24]. Several factors contribute to this poor ability of urine cytology to detect cancer cells: only a small sample of urine can be processed and only a fraction of the sample can be used for final analysis which reduces the chance of capturing tumor cells. Background cells such as erythrocytes and leukocytes also confound the cytological technique [25]. From our study, we found that urine cytology examination for bladder malignancy had moderate sensitivity (63%), moderate specificity (88%), 83% positive predictive value and 72% negative predictive value.

This study shows that urine biomarkers (urine NMP22, urine cytokeratin-18 and urine CA 19-9) had higher sensitivity than urine cytology, but urine cytology had the highest specificity. This useful for development or poor country like Indonesia where not

all examination exists. But the choice of what examination will be done to the patient should be discussed between clinician and patients itself because maybe some patients want the best examination.

Conclusion

Urine cytokeratin-18 had highest sensitivity for diagnosing bladder malignancy, but urine cytology had highest specificity.

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