African Journal of Urology (2018) **24**, 264–269

African Journal of Urology

Official journal of the Pan African Urological Surgeon's Association
web page of the journal

www.ees.elsevier.com/afju
www.sciencedirect.com

Uro-oncology
Original article

Sensitivity and specificity of 47 kDa polyclonal antibody for detection of bladder cancer cells in urine of hematuria patients

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Received 8 May 2018; received in revised form 13 November 2018; accepted 29 November 2018; Available online 13 December 2018

KEYWORDS
Bladder;
Polyclonal antibody;
Hematuria;
Proteomic;
Diagnostic performance

Abstract

Objective: To investigate the performance analysis of immunocytochemistry using 47 kDa polyclonal antibody compared with cytology in urine of patients hematuria symptom.

Patients and methods: Patients with a suspicion of bladder tumor based on workup of hematuria were prospectively collected. The voided urine was performed for cytology and test with 47 kDa polyclonal antibody.

Results: This study involved 39 patients consisting of 35 (89.74%) male and 4 (10.25%) female patients. For male patients, they showed 6 (17.14%) of positive cytology cases and 25 (71.42%) of positive immunocytochemistry cases. For female patients, they showed 0 (0.00%) of positive cytology cases and 2 (50.00%) of positive immunocytochemistry cases. The sensitivity and specificity value for immunocytochemistry using polyclonal antibody to the 47 kDa were 100.00% (95% CI = 54.07%–100.00%) and 36.36% (95% CI = 20.40%–54.88%), respectively. The positive and negative predictive value for immunocytochemistry of polyclonal antibody to the 47 kDa were 22.22% (95% CI = 8.62%–42.26%) and 100.00%

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Peer review under responsibility of Pan African Urological Surgeons' Association.

https://doi.org/10.1016/j.afju.2018.11.008

(95% CI = 73.54%–100.00%), respectively. In conclusion, urine detection with 47 kDa polyclonal antibodies has higher sensitivity value and negative predictive value (100.00%) than urine cytology.
Conclusion: This technique becomes an alternative early detection of bladder cancer for high-risk populations which can be easily and specifically carried out in primary care in Indonesia.

Introduction

Bladder cancer is a malignancy of the urinary tract [1]. In the United States, nearly 75,000 new cases and 16,000 deaths occur each year as a result of bladder cancer [2]. It is estimated that there were 118,000 new cases of bladder cancer and 52,000 deaths from bladder cancer in Europe in 2012 [3]. In Indonesia, according to the 10-year study by Umbas between January 1995 until December 2004 at the Cipto Mangunkusumo Hospital and Dharmais Cancer Hospital, there were 340 patients with a 15% increase every year at the average age of 54 years [4]. In addition, transitional cell carcinoma was the largest number (80.66%) with 60% of patients diagnosed as advanced local and advanced stage (locally advanced and advanced). Study at the Doctor Soetomo Hospital also found a similar tendency, that bladder cancer was often diagnosed at an advanced stage [5].

The main risk factors for the development of bladder cancer were smoking and exposure to specific carcinogens [6]. Until now, two diagnostic and surveillance measures for bladder cancer are cystoscopic examination and bladder biopsy for histologic confirmation. Invasive bladder cancer sometimes spreads to the outside the bladder and affects other organs. However, when the disease is diagnosed and treated in its early stages, the survival rate will be high. The preliminary diagnosis is extremely important in this disease, and various facts underline the necessity of developing a non-invasive, real, and simple examination to increase the rate of detection of bladder cancer [7].

Urine is liquid derived from plasma and filtrated through the renal glomerulus. Urine becomes one of the clinical samples used for biomarker findings. It is estimated that 70% of urine protein is derived from the kidneys and urinary tract [7–9]. Various plasma proteins are also abundant in high concentrations in the urine specimen, thus making it more difficult for the detection of potential biomarkers found in low concentrations [10–12]. Although cystoscopic examination becomes the golden standard for diagnosing and monitoring tumor progression, as well as its recurrence, the method is invasive and associated with high cost and patient discomfort. In contrast with cystoscopy, urine cytology is non-invasive, but has a low sensitivity in low-grade tumors, which grow slowly and are less aggressive [13,14]. Our previous studies proved that the existence of protein with molecular weight of 47 kDa is specific to bladder cancer. Furthermore, the polyclonal antibodies can be made from this protein. The preliminary researches prove that this polyclonal antibody test is positive for bladder cancer, but negative for prostate cancer, rectal cancer, and breast cancer [15]. In addition, some studies were also conducted to identify noninvasive urine bioassay for the detection, surveillance, prediction of recurrence, and progression of bladder cancer. Nonetheless, there are no prospective studies to analyze the performance of 47 kDa polyclonal antibodies compared with cytology test as diagnosis or early detection of bladder cancer patients.

Patients and methods

Subjects

Patients suspected of having bladder cancer by hematuria will be prospectively included as subjects of the research for clinical trials of the institutions based on the approval of institutional ethics board. Patients with suspected bladder cancer are included as the subjects prior to transurethral resection of the bladder or radical cystectomy. Patients with a histological diagnosis different from bladder cancer or with a previous bladder cancer history were subsequently excluded. Collected variables included various patient demographics, date of urinary collection, type of specimen (voided, washing, or catheterized), surgical pathology, recurrence, and follow-up. This research was conducted in Urology Division, Department of Surgery and Department of Pathology, the Ulin Banjarmasin Hospital, Faculty of Medicine, University of Mangkurat, Banjarmasin, South Kalimantan, Indonesia. The total number of 35 samples in this study was determined based on the calculation of sample size for a diagnostic test [16].

Processing of samples and cytology

All 24 h-collected urine was prepared as ThinPrep slides and then stained with Papanicolaou stain. All slides were analyzed by one to four pathologists having cytopathology education. Specimens prepared and carried out by Papanicolau procedure were grouped based on two categories of cytology, including negative and positive [17].

Immunocytochemistry

First we performed retrieval antigen (DAKO, Denmark) on slice of bladder cancer. Furthermore, the tissue was incubated with primary antibodies specific to 47 kDA protein (1:6400), then stained with an avidin-streptavidin-horseradish peroxidase kit (BioGenex, Fremont, CA, USA). The observations were performed with a camera-equipped microscope (Image-Pro Plus 6.0 image analysis system — Media Cybernetics Corporation, Rockville, MD, USA). Observations were made on five randomly selected field of view (200× magnification). The optical density obtained from the five points will be rated on average [15].

Statistical analysis

The data obtained here were analyzed for the sensitivity, specificity, positive predictive, and negative values. Sensitivity is the proportion of subjects who positively identified by the gold standard as a positive by the measuring instrument. Sensitivity is described as the percentage of people without the disease who test negative. The positive predictive value is the proportion of patients who really positive (true positive) of the total patients showed positive results of confirmatory tests. Negative predictive value is the percentage of all patients were actually negative (healthy/true negative) among all patients showed negative test results. The analysis of sensitivity, specificity, positive predictive value, and negative predictive value were carried out with version 15.2.2 MedCalc expressed as a percentage. Besides, the Spearman correlation test was carried out with SPSS version 16.0 (IBM, New York, US).

Ethics

This research has been approved by research ethics committee Faculty of Medicine University of Brawijaya, Malang, Indonesia

Results

Clinical characteristics

This study involved 39 patients with complaints of hematuria. It consisted of 35 (89.74%) male patients and 4 (10.25%) female patients. The average age of male patients was 56.91 ± 15.71 years. While the average age of female patients was 48.75 ± 10.87 years. For male patients, they showed 6 (17.14%) of positive cytology cases and 25 (71.42%) of positive immunocytochemistry cases. For female patients, they showed 0 (0.00%) of positive cytology cases and 2 (50.00%) of positive immunocytochemistry cases (Table 1).

Table 1 Distribution of patients, criteria for cytology and pathology criteria.

	Frequency
Male patients	
- Total	35 (89.74%)
- Average age (year)	56.91 ± 15.71
- Positive cytology	6 (17.14%)
- Positive immunocytochemistry	25 (71.42%)
Female patients	
- Total	4 (10.25%)
- Average age (year)	48.75 ± 10.87
- Positive cytology	0 (0.00%)
- Positive immunocytochemistry	2 (50.00%)

Urine cytology

Urine cytology results were negative if malignant cells were not identified. If malignant cells were identified, the value was positive, as shown in Fig. 1. In this study, analysis of urine cytology identified positive values in 6 cases of 39 cases (15.38%). Meanwhile, using immunocytochemistry with 47 kDa polyclonal antibody techniques, the study showed positive values in 27 of 39 cases (69.23%). The negative values of immunocytochemistry with 47 kDa polyclonal antibody were characterized by the absence of dark brown color in the cell membrane. Positive values were characterized by dark brown cell membrane, as presented in Fig. 2.

Histology

Fig. 3 shows the histology results of the six patients who were positive for cytology. After transurethral resection of the bladder, we got histological results of bladder transitional cell carcinoma.

Sensitivity, specificity, positive predictive value, and negative predictive value

The sensitivity value of immunocytochemistry using 47 kDa polyclonal antibodies was 100.00% (95% CI = 54.07%–100.00%). The specificity value of immunocytochemistry using 47 kDa polyclonal antibodies was 36.36% (95% CI = 20.40%–54.88%). The positive predictive value of the immunocytochemistry examination technique using 47 kDa polyclonal antibodies was 22.22% (95%

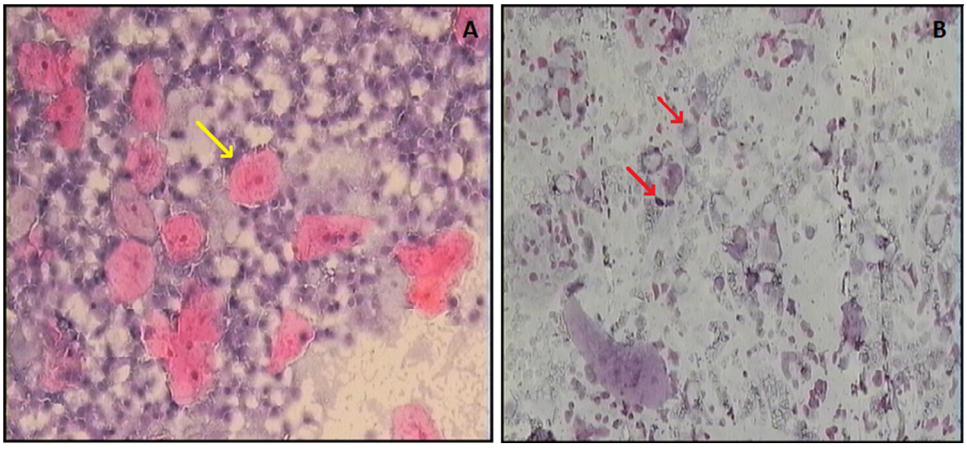

Figure 1 Results of urine cytology test in patients with hematuria complaint. (A) Test value is negative and (B) test value is positive. In figure A, cellularity looks sufficient and consists of scattering epithelial cells in polygonal shape with a nucleus in the middle; the ratio of nucleus: cytoplasm is 1:4, a monotonous micronucleus (the features of normal cell — the yellow arrow); In figure B, cellularity looks quite a lot with cell groups derived from hyperplastic epithelial cells of changed nucleus ratio (1:3 – 1:2), hyperchromatic large nucleus, irregular and polygonal nuclear membrane (the features of malignancy — the Red Arrow). (Observation by Olympus BX51 microscope, magnification 40×).

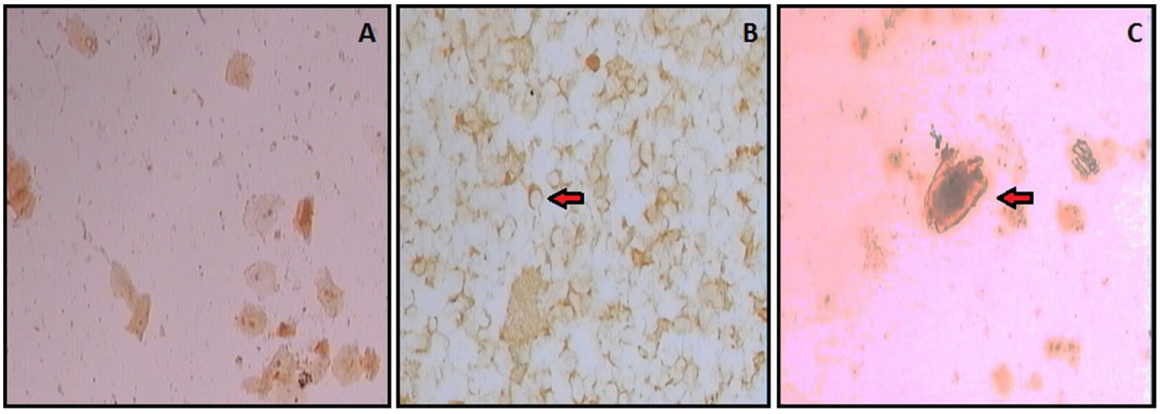

Figure 2 The results of immunocytochemistry tests with 47 kDa polyclonal antibodies in urine of patients with hematuria complaints. (A) test is negative while (B and C) tests are positive, characterized by the features of immunohistochemical reaction (antigen-antibody complexes) on the cell membrane (red arrows). (Observation by Olympus BX51 microscope, magnification 40× (Figure A & B) and 100× (Figure C)).

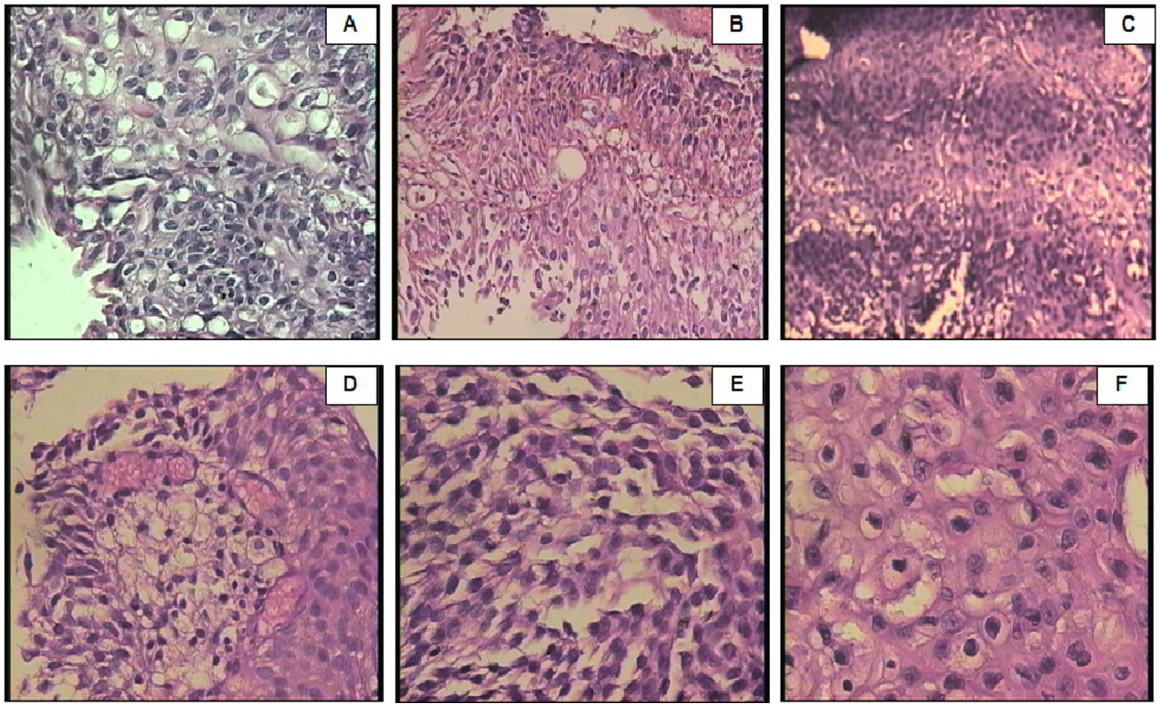

Figure 3 The results of histological examination on the six samples that were positive in the cytology test. All of these tissues are tissues with cells that have become tumors, tumors originate from transitional epithelium with hyperchromatic polygonal cell forms, there is an increase in the nucleus to cell ratio, dominant and hyperchromatic nuclei, all diagnosed as transitional bladder cell carcinomas well-differentiated bladder (Observation with Olympus BX51 microscope, 40× magnification).

CI = 8.62%–42.26%). The negative predictive value of the immunocytochemistry using 47 kDa polyclonal antibodies was 100.00% (95% CI = 73.54%–100.00%) (Table 2).

Table 2 Results of cytology and immunocytochemistry tests of 47 kDa polyclonal antibodies.

Immunocytochemistry	Cytology		Total
	Positive	Negative	
Positive	6	21	27
Negative	0	12	12
Total	6	33	39

Discussion

Biomarkers for cancer should have unlimited capability, not only for detecting early stage tumors with high accuracy, but also for monitoring recurrence, tumor progression, and tumor prediction for therapeutic approaches [18]. Human body fluids play a very significant role in proteomics research related to bladder cancer. As part of body fluids, urine samples can be easily obtained and is one of the samples most frequently analyzed clinically [19].

The study involved 39 patients with hematuria complaints, i.e. 35 (89.74%) male and 4 (10.25%) female patients. This indicated that patients with hematuria complaint affected more men than women

due to various risk factors. The average age of male patients was 56.91 ± 15.71 years. While the average age of female patients was 48.75 ± 10.87 years. This suggested that elderly patients (high risk group) experienced many hematuria complaints. In the population of China, the average age of bladder cancer cases (14.260 cases) was higher, i.e. 63.5 years [20]. Our study consistent with two previous studies in Indonesia, that the average age of bladder cancer patients was 54 years and 57.8 years [4,5].

According to the European Association of Urology, it is believed that urine cytology is intended for high-risk patients. However, the meta-analysis report finds that the sensitivity value of cytology is low. The sensitivity value in a low-grade tumor is between 4%–31% [21–24]. In this study, the positive urine cytology analysis showed 6 cases of 39 cases (15.38%). Meanwhile the use of immunocytochemistry technique with 47 kDa polyclonal antibodies showed positive values in 27 of 39 cases (69.23%). This indicated that 47 kDa polyclonal antibodies could enhance the diagnostic value of urine cytology. We suspected that 47 kDa polyclonal antibodies probably would react with the antigen expressed by pre-cancerous cells. Of the six positive cytology results, it is followed by transurethral resection of the bladder in which the anatomical pathology results showed transitional bladder cell carcinoma.

The relationship between urine cytology results and the reaction results of 47 kDa protein polyclonal antibody against bladder epithelial cells in patients with hematuria concluded that there was a significant relationship ($p < 0.05$; $r = 0.552$) between the examination results of urine cytology and the examination results of the reaction of 47 kDa protein polyclonal antibody against bladder epithelial cells in patients with hematuria. This indicated that the higher value of the reaction of a 47 kDa protein polyclonal antibody, the higher the value of urine cytology.

The sensitivity of immunocytochemistry using 47 kDa polyclonal antibodies was 100.00%. The specificity of immunocytochemistry using 47 kDa polyclonal antibodies was 36.36%. The positive predictive and negative predictive values of immunocytochemistry using 47 kDa polyclonal antibodies were 22.22% and 100.00%, respectively. Various studies revealed that the sensitivity, specificity, positive predictive value, and negative predictive values of cytologic analysis were 50%; 100.00%; 100.00%; and 63.45% [21,22]. Thus, the sensitivity and negative predictive values of immunocytochemistry technique of 47 kDa polyclonal antibodies were higher than cytology. Even though cytological were determined by the expertise of a pathologist and the interpretation depended on the expert [25]. Meanwhile immunocytochemistry technique of 47 kDa polyclonal antibodies was easily performed by trained personnel and their interpretation did not depend on the presence of a pathologist to identify malignant cells, so it could be done in primary care in Indonesia.

Conclusion

Urine detection with 47 kDa polyclonal antibodies has higher sensitivity value and negative predictive value (100.00%) than urine cytology. This technique becomes an alternative early detection of bladder cancer for high-risk populations which can be easily and specifically carried out in primary care in Indonesia.

Conflict of interest

All authors agree to publish in this journal and equally contribute to this study and there is no conflict interest.

Ethical committee approval

This research has been approved by research ethics committee Faculty of Medicine University of Brawijaya, Malang, Indonesia.

Authors' contributions

Conceived and designed the experiments: Heru Prasetya, Basuki Bambang Purnomo, Karyono Mintaroem, Sumarno Reto Prawiro.

Performed the experiments: Heru Prasetya, Basuki Bambang Purnomo, Karyono Mintaroem, Sumarno Reto Prawiro.

Analyzed and interpreted the data: Heru Prasetya, Basuki Bambang Purnomo, Karyono Mintaroem, Sumarno Reto Prawiro.

Contributed reagents, materials, analysis tools or data: Heru Prasetya.

Wrote the paper: Heru Prasetya, Basuki Bambang Purnomo, Karyono Mintaroem, Sumarno Reto Prawiro.

Consent from the patient

Yes

Source of funding

None funding.

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