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Evaluation of Urine Procalcitonin, Interleukin-6, Heparin Binding Protein, Leukocyturia, and Bacteriuria for Early Detecting Urinary Tract Infection

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KEYWORDS

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Abstracts Urinary Tract Infection (UTI) is a common infectious disease. Urine culture as a gold standard is less practical and time- consuming. Examination of urine dipstick and microscopic is less accurate. Procalcitonin (PCT), IL-6 and Heparin Binding Protein (HBP) have known increase after infection, which potentially used for early detection of UTI. The aim of this study was to determine the diagnostic performance of urine IL-6, HBP, PCT, leukocyturia and bacteriuria level as biomarkers of UTI. The study was conducted on 51 UTI patients with positive urine culture, 16 UTI patients with negative urine culture, and 16 healthy control. Urine IL-6, HBP, and PCT level were determined using ELISA. Leukocyturia and bacteriuria were measured using automated flowcytometry and fluorescent dye analyzers. The gold standard for UTI diagnosis was urine culture. The sensitivity and specificity of urine PCT, IL-6, HBP, leukocyturia and bacteriuria were 60.78% and 65.62%; 72.55% and 56.25%; 96.07% and 50.00%; 80.39% and 78.12%; 80.39% and 59.09% respectively. The AUC of urine PCT, IL-6, HBP, leukocyturia and bacteriuria were 0.600; 0.605; 0.666; 0.877 and 0.830 respectively. For combinations of two biomarkers, the best sensitivity and specificity was demonstrated by urine PCT and leukocyturia.

Introduction

Urinary Tract Infection (UTI) is a common infectious disease and characterized by significant bacteriuria (>10^5 CFU/mL urine) via clean catch mid-stream technique (Longo *et al*., 2012). This disease often occurs without symptoms, it is often recurrent and can cause severe complications if not treated properly (Smelov *et al.*, 2016). Adult women around 50-80% have been reported to have at least one UTI episode during their lifetime. About 20–30% of women who have had one episode of UTI will have recurrent episodes (Longo *et al.*, 2012; Mazzulli, 2012).

Early diagnosis of UTI based on clinical symptoms is often difficult because most of the cases are asymptomatic and require laboratory testing. Urine culture is a gold standard for UTI diagnosis, but it is less practical and the results are often negative (De Rosa *et al*., 2010; Brilha *et al.,* 2010). Urinalysis by dipstick method gives fast results but is still influenced by interference factors. Examination of microscopic urine sediments requires skilled labor and give subjective results, so that other rapid, accurate and non-invasive methods of diagnosis are required to confirm the diagnosis of UTI (Strasinger & Di Lorenzo, 2014).

Urine sediment analysis using automatic urine analyzer is based on flow cytometry methods and fluorescent dye currently widely developed. This method provides promising results to identify UTI (Strasinger & Di Lorenzo, 2014). The advantages of Urine analyzer are more practical, give faster results, and economical cost. However, the diagnostic performance of flow cytometry methods in

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various studies are still inconsistent (Shang *et al*., 2013).

Other UTI examination markers currently under investigation are Interleukin (IL-6), Heparin Binding Protein (HBP), and Procalcitonin (PCT) urine (Nuri *et al.*, 2017; Kjolvmark *et al*., 2014; Leroy & Gervaix, 2011). Interleukin-6 (IL-6) urine is synthesized by activated urothelium cells due to response to infection and inflammation. IL-6 is generally not found in the urine of healthy people and significantly increases in urine of UTI patients (Sundvall *et al*., 2014). Heparin Binding Protein (HBP) is a protein of 37 kDa stored in secretory and azurophilic granules of human neutrophils. Heparin-binding proteins have been evaluated in clinical trials as biomarkers for bacterial infections. Increased HBP levels were associated with UTI occurrence (Kjolvmark *et al.,* 2014). Procalcitonin is a calcitonin prehormone that is normally produced by thyroid C cells. Procalcitonin is known as a bacterial infection marker and can be used for UTI diagnosis (Leroy & Gervaix, 2011).

According to the previous explanation, there is an opportunity to investigate the diagnostic capability of urine IL-6, urine HBP, and urine PCT level with leukocyturia and bacteriuria count using flowcytometric method as an alternative method for identification of UTI before urine culture results are obtained. The aim of this study is to assess the sensitivity and specificity of urine IL-6, urine HBP, urine PCT, leukocyturia and bacteriuria count for detecting UTI in adult population compared to urine culture.

Materials and methods

Study subjects

A cross sectional study was conducted between September 2015 and February 2017, in internal medicine polyclinic, Internal ward and emergency room in Dr. Saiful Anwar General Hospital, Malang, Indonesia. Eighty three subjects were divided into 3 groups, 51 UTI patients with positive urine culture, 16 UTI patients with negative urine culture, and 16 healthy control. The patient aged 18-75 years old who are clinically suspected of having UTI, signing informed consent, not being diagnosed with malignant disease, post physical trauma, and autoimmune diseases (Systemic Lupus Erythematosus) by an Internal Medicine physician, not taking antibiotics and ascorbic acid within <24 hours prior to collection of specimens and leukocyturia ≥5/HPF were classified into UTI group. Colony count of Bacteria $>10^5$ CFU/mL from urine culture results from clean catch midstream collection classified as UTI with positive urine culture and if $<10^5$ CFU/mL were classified into negative urine culture. Urine samples that were obtained more than 1 hour were excluded. Healthy control is a healthy volunteer based on the results of a medical examination by an Internal Medicine physician, with negative leukocyturia in urinalysis and negative urine culture results. Ethical approval was approved by the Ethics Committee, Faculty of Medicine, University of Brawijaya/Dr. Saiful Anwar General Hospital, Malang, Indonesia.

Urine culture

Midstream urine sample is taken aseptically into a sterile container and homogenized urine specimens were inoculated on Nutrient agar medium and MacConkey agar medium. This culture media was then incubated for 24 hours at 37°C of temperature. The number of bacterial colonies grown were counted with colony counter and the results were multiplied by 10^2. Specimens without bacterial growth or specimens with bacterial growth $\leq 10^5$ CFU/mL are considered negative, while $>10^5$ CFU/mL were considered positive (Longo et al., 2012). Identification of Gram-negative and Gram-positive bacteria used VITEX 2 automated microbiology system analyzer.

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Urinalysis

Rutine urinalysis used urine dipstick and urine automated analyzer Sysmex UX-2000. Urine automated analyzer Sysmex UX-2000 worked was based on flow cytometry method using a semi-conductor laser diode with 635 nm wavelength. It has two separated channels that use for sediment and bacteria analysis. Uncentrifugated urine sample with volume arround 0.8 mL-1.2 mL were aspirated through the probe sample. The sample diluted in 2 different reaction channels, one channel for urine sediment and one for bacteria. Urinary sediment as erythrocyte, leucocytes, epithelial cells, crystals, and cylinders in the channel will be diluted at temperature 35°C, afterwards the nucleus, cytoplasm, and cell membrane will be stained with polymethine fluorescent dye. In a special bacterial channel (BACT), urine will be mixed with special diluents at 42 °C which will increase the permeability of the cell membrane and facilitate staining of bacterial nucleic acid by polymethine fluorescent dye. The particles then passed in a flow cell, one by one, and shot by a laser beam. Based on the intensity of light emitted, erythrocytes, leukocytes, epithelial cells, crystals, cylinders and bacteria will be classified and counted respectively. The results are presented in the form of numbers, histograms, and scattergrams.

Biomarker Assay

Midstream urine sample is taken aseptically into a sterile container, then immediately centrifuged, and stored the container in the refrigerator at -80°C of temperature until examination. All biomarkers (IL-6, HBP, and PCT) were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method using ELISA IL-6 (E0090Hu, BT Laboratory) kit, U-HBP kit (E-EL-H0540, Elabscience), and the ELISA PCT kit (BT Laboratory). All assays were performed strictly according to the manufacturer's instruction. Results were reported in ng/ml.

Statistics.

Statistical analysis used SPSS version 20.0. Normality test for distribution of characteristics data used Kolmogrov-Smirnov and Shapiro-Wilk test. Statistical test of difference of level IL-6 urine, HBP urine, PCT urine, leukocyturia and bacteriuria in group of positive urine culture UTI, negative urine culture UTI, and healthy control used Kruskal-Wallis test with statistically significant difference if p value less than 0,05. The cut-off values of urinary IL-6, urinary HBP, urinary PCT, leukocyturia and bacteriuria for identification of UTI were determined by receiver operating characteristic (ROC) curve analysis. A diagnostic test using tables 2x2 is performed to determine sensitivity, specificity, positive predictive value, and negative predictive value of level urine IL-6, urine HBP, urine PCT, leukocyturia and bacteriuria.

Results and discussions

The demographic and clinical characteristics of subjects were shown in table 1. Patients in UTI group with positive urine culture were 51 subject, UTI group with negative urine culture were 16 subject, and healthy control were 16 subjects. The UTI group is dominated by women. There were statistically significant differences in urinary IL-6, PCT, leukocyturia, bacteriuria, and HBP in the three group with p value <0.05.

Urinary Tract Infection (UTI) is an infectious disease commonly occurring following respiratory tract infections. UTI is more common in women because the anatomy location of urethra had a short distance from the anus. It is considered to be the main reason why UTI is predominantly suffered by women compared to men (Longo *et al.,* 2012; Dos Santos *et al.,* 2007). This study also showed the number of women infected with UTI was more than men.

In this study, there were significant differences on HBP, IL-6, PCT, leukocyturia, and

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bacteriuria levels between UTI patients with positive culture, UTI patients with negative culture and healthy control group with p value <0.05. The results were consistent with several studies showing that there was a significant increase of IL-6, HBP, PCT, leukocyturia, and bacteriuria levels in patients with UTI compared with healthy control with p-value <0.05 (Kjolvmark *et al.,* 2014; Duong *et al.,* 2016; Manoni *et al.,* 2009).

Tabel 1. Subjects Characteristics

Characteristics	UTI positive culture (n=51)	UTI negative culture (n=16)	Healthy control (n=16)
Age (Year)	50±17	41±15	32±3
Sex (n, %)			
- Male	15 (29.41%)	2 (12.5%)	1(6%)
- Female	36 (70.59%)	14 (87.5%)	15(94%)
Bacteriuria (/µL) *	14,838,630±2,045,493	1,410,310±157,800	44,500±31,230
Leukocyturia (/µL) *	1566.5±3674.9	238.4±532.5	4.5±3.3
IL-6 (ng/mL) *	351.9 ± 305.3	614.4 ± 1129.6	89.5 ± 39.6
HBP (ng/mL) *	1.05±1.06	1.24 ± 0.73	0.09±0.016
PCT (ng/mL) *	950.16±554.73	1023.84±268.61	617.14±66.19

Note: Values are presented as mean ± standard deviations. UTI, urinary tract infection; ng/ml, nanogram/milliliter.
*p < 0.05

The result of urine positive culture mostly dominated by gram negative bacterial infection as much 86.27 %, while infection of gram-positive bacteria as much 15.69%. The most common bacteria in this study were *Escerichia coli* (31.37%) followed by *Staphylococcus epidermidis* (13.73%) and *Pseudomonas stutzeri* (13.73%). The results of urine culture can be seen in table 2.

The most common bacteria species in urine culture based on this study were Escerichia coli (31.37%). The results of this study are in line with the research by Dos Santos *et al.* (2007) which showed the most common bacteria that cause UTI is E. coli (67%), followed by S. saprophyticus (9.3%). Other literature also states that the most common UTI uropathogen is E. coli (75-90%) followed by Staphylococcus saprophyticus (5-15%), Klebsiella sp, Proteus sp, Enterococcus sp, Citrobacter sp, and other organisms around 5-10% (Sundvall *et al.,* 2014).

Table 2. Result of Urine Culture

Bacteria	Distribution in UTI group (n, %)
Gram Positive	**8 (15.69%)**
- *Staphylococcus aureus*	1 (1.96%)
- *Staphylococcus epidermidis*	7 (13.73%)
Gram Negative	**43 (84.31%)**
- *Escherichia coli*	16 (31.37%)
- *Enterobacter sakazakii*	1 (1.96%)
- *Pseudomonas aeruginosa*	3 (5.88%)
- *Pseudomonas fluorescens-25*	6 (11.76%)
- *Pseudomonas pseudomallei*	6 (11.76%)
- *Pseudomonas stutzeri*	7 (15.70%)
- *Proteus mirabilis*	1 (1.96%)

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Bacteria	Distribution in UTI group (n, %)
- *Serratia liquefaciens*	2 (1.96%)
- *Enterobacter aerogenes*	1 (1.96%)
Total	(51, 100%)

The ROC curve of urine Procalcitonin, IL-6, Heparin Binding Protein, leukocyturia and bacteriuria counts can be seen in Figure 1.

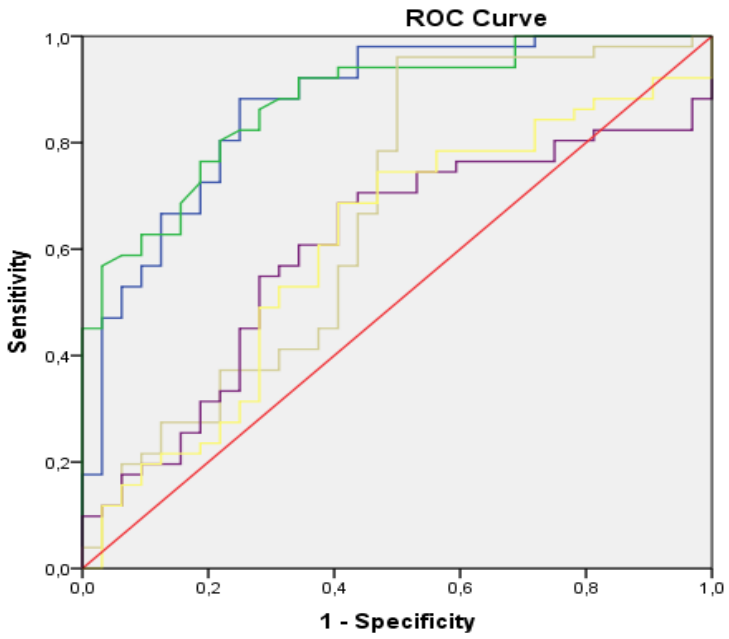

Figure 1. The ROC curve of urine Procalcitonin, IL-6 , Heparin Binding Protein, leukocyturia and bacteriuria counts. Red line: reference line; yellow line: urinary interleukin-6; blue line: leukocyturia; green line: bacteriuria; purple line: urinary procalcitonin; grey line: urinary heparin binding protein.

The ROC curve of urine PCT, IL-6, HBP, leukocyturia and bacteriuria counts were 0.600; 0.605; 0.666; 0.877 and 0.830 respectively. Diagnostic value of the leukocyturia counts is better than urine IL-6, HBP, PCT levels and bacteriuria counts (table 3).

The cut-off values of IL-6, leukocyturia, PCT and bacteriuria in this study were higher than that in previous studies. The cut off values for leukocytes in this study were 76.6 leukocytes/ µL, (sensitivity 80.39% and specificity 78.12%), bacteriuria 755/µL (sensitivity 80.39% and specificity 59.09%), PCT 0.86 ng/ml (sensitivity 60.47% and specificity 43.75%) and IL-6 >134.95 ng/mL (sensitivity 72.55% and specificity 56.25%). Nuri *et al.* (2017) used cut-off values for IL-6 5.584 pg/ml which gave sensitivity 53.3% and specificity 53.3%. Another study obtained cut off value bacteriuria 14.2 cells/µL that demonstrated sensitivity 95.76% and specificity 44.88% (Jatupon *et al.,* 2012).

The high cut-off value in this study compared to previous studies can be caused by several possibilities. The first is difference in the study population. Study by Peters *et al.* (2013) showed that an Asian population had higher levels of IL-6 than Europe population (Peters *et al.,* 2013). In developing countries, especially Southeast Asia, the level of infectious disease is high to affect the response of individual

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infections (Duong *et al.,* 2016). Second, the types of germs that cause infections may be impact on result of this study. Bacterial type also affected the levels of IL-6, and PCT where in gram-negative bacterial infections, PCT and IL-6 levels are higher than the infection of gram-positive bacteria (Leroy & Gervaix, 2011; Manoni *et al.,* 2009).

Table 3. Diagnostic value of urine Procalcitonin, IL-6 , Heparin Binding Protein, leukocyturia, bacteriuria counts and combination each variable biomarker.

Biomarker	Sensitivity (%)	Specificity (%)	PPV	NPV
IL-6 (ng/mL)	72.55	56.25	72.55	56.25
HBP (ng/mL)	96.07	50.00	75.38	88.89
PCT (ng/mL)	60.78	65.62	73.81	51.22
Leukocyturia (/µL)	80.39	78.12	85.42	71.43
Bacteriuria(/µL)	80.39	59.09	82.00	56.52
IL-6 and Leukocyturia	70.59	84.38	75.90	87.80
IL-6 and Bacteriuria	92.00	43.75	72.29	71.88
IL-6 and or Leukocyturia	90.20	59.38	78.31	77.97
IL-6 and or Leukocyturia and or Bacteriuria	98.04	50.00	79.52	75.76
Leukocyturia and Bacteriuria	70.59	84.38	75.90	87.80
HBP and Leukocyturia	100.00	50.00	80.72	76.12
HBP and Bacteriuria	96.08	46.88	77.11	74.24
HBP and or Leukocyturia and or Bacteriuria	98.04	46.88	78.31	74.63
PCT and Leukocyturia	92.16	65.63	81.93	81.03
PCT and Bacteriuria	96.08	53.13	79.52	76.56
PCT and or Leukocyturia and or Bacteriuria	100.00	50.00	80.72	76.12
IL-6 and or HBP	100.00	50.00	80.72	76.12
HBP and or PCT	100.00	50.00	80.72	76.12
IL-6 and or PCT	72.13	53.13	73.49	74.58
IL-6 and or HBP and or PCT	100.00	50.00	80.72	76.12

Note: Positive predictive value (PPV), and Negative predictive value (NPV).

The best sensitivity of biomarker in this study was obtained by HBP (96.07%), followed by leukocyturia (80.39%), bacteriuria (80.39%), IL-6 (72.55%), and PCT (60.78%). These results indicate that urine HBP, leukocyturia and bacteriuria are superior when used as screening tests compared to urine IL-6 and PCT because they have good sensitivity values. The best specificity was obtained in leukocyturia (78.12%), followed by PCT (65.62%), bacteriuria (59.09%), IL-6 (56.25%) and HBP (50%). These results indicate that leukocyturia is still superior when used as screening and diagnostic testing compared to other biomarkers. The sensitivity values of HBP, IL-6, leukocyturia, and bacteriuria in this study were better than those of previous studies. A study conducted by Kjolvmark *et al.* (2014), obtained a lower sensitivity of urine HBP (89.2%) but higher specificity (89.8%). Study by Nuri et al (2017) obtained sensitivity of IL-6 (57%) and specificity (53.3%). The sensitivity of urinary PCT in this study was lower than study by Leroy and Gervaix (2010) where the sensitivity and specificity of serum procalcitonin ranged between 70-100% and 70-97%.

In this study we tried to combine several biomarkers to obtain the best sensitivity and specificity. The best sensitivity of two biomarkers combination was found in urine HBP and leukocyturia, HBP and/or PCT, and IL-6 and/or HBP (results considered positive if one variable has a

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value above cut off). The combinations increase sensitivity values by up to 100% but decrease in specificity values by up to 50%. The combination between IL-6 and leukocyturia showed decrease in sensitivity up to 70.59% and an increase in specificity to 84.38%. These results indicate a combination of these two biomarkers can be used for diagnosis test. The combination of IL-6 and/or PCT increased sensitivity to 72.13% and decreased the specificity to 53.13%, so the combination of these two biomarkers was less effective when used as screening test and for diagnostic enforcement. For combinations of two biomarkers, the best sensitivity and specificity was displayed by the combination of PCT and leukocyturia (sensitivity 92.16% and specificity 65.63%). Combination of 3 biomarkers showed that the best sensitivity was found in combination between IL-6 and/or HBP and/or PCT, and a combination of PCT and /or Leukocituria and/or Bacteriuria, but the specificity decreases up to 50%.

Conclusions

There were significant differences in levels of IL-6, HBP, and PCT urine as well as the number of leukocyturia and bacteriuria in UTI patients with positive culture, UTI patients with negative culture and healthy control group. The use of leukocyturia count based on flowcytometry as a single biomarker provides optimal diagnostic value for UTI diagnosis compared to IL-6, HBP, PCT, and bacteriuria. The best sensitivity and specificity of two biomarkers combinations was obtained by urine PCT and leukocyturia.

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