C- REACTIVE PROTEIN LEVELS OF SEPSIS

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C-REACTIVE PROTEIN LEVELS OF SEPSIS PATIENTS: A COMPARISON OF THREE IMMUNOASSAY METHODS

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ABSTRACT

C-Reactive Protein (CRP) is a marker to aid in diagnosing sepsis. Currently, there are not many studies about the comparison of CRP levels in sepsis patients with a variety of instruments and methods. This study aimed to analyze differences of CRP results using Particle Enhanced Turbidimetric Immunoassay (PETIA), Sandwich Immunodetection and Reflectometry Immunoassay, and to compare the characteristics of the instruments based on their specifications and practicality. The study used a total of 65 serum of sepsis patients who fulfilled the qSOFA criteria and were treated in the Emergency Department, Intensive Observation Room, Intensive Care Unit (ICU) and Internal Medicine Wards of the Dr. Soetomo Hospital Surabaya from May to September 2018. All samples were measured the CRP levels measured using the three methods. There were significant differences of CRP levels between the PETIA and Reflectometry immunoassay methods (p=0.003), suggesting a supportive role of both methods. There was no significant difference of CRP levels between PETIA and Sandwich Immunodetection (p=0.172) as well as Reflectometry immunoassay and Sandwich Immunodetection (p=0.251). The selection of instruments and methods for CRP examination must be adjusted to laboratory needs and facilities.

Key words: Sepsis, CRP, PETIA, sandwich immunodetection, reflectometry immunoassay

INTRODUCTION

Sepsis is a life-threatening medical condition due to dysregulation of the immune response to infection resulting in dysfunction of host organs with a very high mortality.[1,2] Sepsis is the cause of death of patients in the ICU and all ages worldwide, with a mortality rate of 25%. Death of sepsis patients is higher in developing countries than in developed countries.[3,4]

The definition of sepsis has changed several times. The last update was in the Third International Consensus in 2016 by The Society of Critical Medicine (SCCM) and The European Society of Intensive Care Medicine (ESICM). The meeting also recommended the use of the Sequential criteria (sepsis-related) Organ Failure Assessment (SOFA) and "quick" (q) SOFA to replace previously used criteria for systemic inflammatory response syndrome (SIRS).[5,6]

C-Reactive Protein (CRP) is an acute-phase protein from the pentraxin family produced by the liver when stimulated by several cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1 and IL-6. C-Reactive Protein (CRP) increases in 6-12 hours after infection or inflammation and reaches the highest levels in 24-48 hours. Increased CRP levels in symptomatic patients can also help diagnose sepsis, thus CRP is one of the most widely used markers during the treatment of sepsis patients.[7-9] C-reactive protein measurements can be performed using a variety of commercially available instruments, including automatic, semi-automatic, manual or with a Point of Care Test (POCT). The instrument used in the Clinical Pathology laboratory Dr. Soetomo Hospital is an integrated automatic Dimension RxL Max with a PETIA method.[10] The AFIAS-6 is a compact, automatic fluorescent immunoassay using the Sandwich Immunodetection method with an all-in-one cartridge in a practical form.[11] The Standard F 200 is a POCT device with Reflectometry Immunoassay technology.[12] The advantages of the POCT examination method are directly performed on the patient's side, use of less blood volume, faster results, can help provide earlier therapy, and more cost-effective compared to standard laboratory equipment.[13] Currently, there are not many studies about the comparison of CRP level in sepsis patients with a variety of instruments and methods. This study aimed to compare the characteristics of the

instruments to measure CRP levels based on specifications and practicality. The instruments we used applied PETIA, Reflectometry Immunoassay, and Sandwich Immunodetection methods.

METHODS

This study was a cross-sectional study conducted from May to September 2018 using the remaining serum samples from patients of the Clinical Pathology research resident with suspected sepsis in the Emergency Department, Intensive Observation Room, Intensive Care Unit (ICU) and Internal Medicine Ward of the Dr. Soetomo Hospital who fulfilled the inclusion and exclusion criteria. The inclusion criteria were adult subjects (≥ 18 years old) with a qSOFA score ≥ 2, while the serum of patients with hemolysis, jaundice and lipemic (HIL), history of liver abnormalities, kidney abnormalities, malignancy, and HIV infection were excluded. The process and examination of samples were carried out in the Clinical Pathology Laboratory of the Dr. Soetomo Hospital.

All samples were examined for the CRP levels using all three methods. For the Particle Enhanced Turbidimetric Immunoassay (PETIA) method the Dimension RxL Max was used, Flex C-reactive protein extended range (RCRP) reagent with a CRP detection limit for serum samples between 0.5-250 mg/L, samples with results more than 250 mg/L had to be repeated by dilution. C-reactive protein levels of less than 0.5 mg/L (0.05 mg/dL) were reported as <0.5 mg/L. For the Reflectometry Immunoassay method, a standard F 200 Biosensor was used. The SD Biosensor Standard F CRP used reagent with a CRP detection limit for serum samples between 1-130 mg/L. The results below 1 mg/L were reported as ↓1 mg/L and above 130 mg/L were reported as ↑130 mg/L. The Sandwich Immunodetection method used the AFIAS 6, Boditech reagent CRP AFI with a detection limit of CRP devices in serum samples between 0.5 - 200 mg/L. Sample with results exceeding the detection limit of Standard F 200 and AFIAS-6 was not diluted due to no explanation in the reagent insert kit guidelines.

Statistical analysis was performed using SPSS ver. 17.0. The results of the study were tested for the data normality using Kolmogorov-Smirnov. Repeated ANOVA analysis was carried out if the data were normal, but if the results of the data were abnormal, Friedman's analysis was used with a significance level of $p < 0.05$. Ethical clearance of this research was submitted to the Research Committee of Health Dr. Soetomo Hospital with No: 0604/KEPK/IX/2018.

RESULT AND DISCUSSION

The samples in this study were 56 serum of patients with suspected sepsis who met the qSOFA criteria collected from May to September 2018. Patients came from the Emergency Department, Intensive Observation Room, Intensive Care Unit (ICU) and the Internal Medicine Ward of the Dr. Soetomo Hospital.

Characteristic data of the subjects included sex, room origin, age, systolic blood pressure, leukocyte count, and respiratory rate shown in Table 1. Sepsis patients were mostly males 37 (66.1%). The average age of sepsis patients was 52.82 years old with an age range of 20-84 years old. Based on the origin of most rooms came from ED as many as 19 patients (33.9%).

Table 1. Characteristics of study subjects

Number of Research Subjects	56
Male [a]	37 (66.1)
Female [a]	19 (33.9)
Origin of Rooms	
Intensive Care Unit [a]	5 (8.9)
Intensive Observation Rooms [a]	15 (26.8)
Emergency Department [a]	19 (33.9)
Internal Medicine Ward [a]	17 (30.4)
Age (years) [b]	52.8 (± 14.3)
Systolic blood pressure (mmHg) [b]	114.82 (± 27.1)
Leukocyte count (thousand/µL) [b]	16.168 (± 7)
Respiration rate (x/minutes) [b]	26.55 (± 4.1)

[a] Percentage [b] Mean

Male patients were reported more than female patients with a proportion of 37 (66.1%) and 19 (33.9%). This was in line with a research on 13 hospitals in Southeast Asia in 2017 where the percentage of sepsis among adulthood patients was greater in males than females (57% and 43%).[14] The incidence of sepsis was higher in males, it was possibly due to hormonal conditions, where androgens stimulate cellular immune responses, while female hormones are protective which reduce the cellular immune response and cardiovascular damage.[15]

The average age of sepsis patients in this study was 52.82 years with an age range of 20-84 years, with the highest number of patients aged between 51-60 years. The incidence of sepsis was higher in the elderly, with more than 60% of patients diagnosed with sepsis were 65 years old and sepsis was the most common cause of elderly patients treated in the ICU. Old age is associated with a decrease of immunity, for example, decreased of cytokine production

influences the expression and function of TLR, changes of the adaptive immune system, and the production of antibodies with lower affinity, suggesting a higher risk of more severe infections.[16]

C-reactive protein levels measurement of 56 serum of sepsis patients using PETIA method revealed 40 samples (71.42%) with CRP levels within analysis range and 16 samples (28.57%) with CRP levels of more than 250 mg/L. CRP levels measurement using Reflectometry Immunoassay method found that 28 samples (50%) were within the detection range of the device, of 1-130 mg/L. Meanwhile, 25 samples (44.64%) showed results above the device detection range >130 mg/L, and 3 samples (5.35%) were below the device detection range of 1 mg/L. CRP levels measurement using the Sandwich Immunodetection method showed 36 samples (64.28%) within the device detection range of 0.5-200 mg/L, 19 samples (33.9%) with results above the device detection range of 200 mg/L, and 1 sample (1.78%) with result below the detection range of 1 mg/L.

The CRP levels of 56 sepsis patients were not entirely able to be statistically analyzed because some of the results were below or above the range of detection and did not appear in absolute numbers. Statistical analysis could merely be performed on a total of 27 samples. The analysis showed significant differences of CRP levels using the PETIA and Reflectometry immunoassay methods, with an average CRP level of 41.37 mg/L and 35.6 mg/L (p=0.003), respectively. There was no significant difference of CRP levels between PETIA and Sandwich Immunodetection method with a mean of 41.37 mg/L and 38.77 mg/L (p=0.172), respectively. The CRP levels measured with Reflectometry Immunoassay and Sandwich Immunodetection showed no significant differences with p=0.251 (Table 2).

The difference of CRP levels referred to PETIA and the Reflectometry Immunoassay method indicated that the two instruments could not replace each other. The difference of CRP levels between the PETIA and Sandwich Immunodetection as well as Reflectometry Immunoassay and Sandwich Immunodetection methods were not significant, suggesting the potential of each instrument to be used in the same laboratory and substitute each other.

Significant differences between the CRP results of the PETIA (Dimension RxL Max) and Reflectometry Immunoassay (Standard F 200) methods were possibly due to the manual process on the Standard F 200 including sampling with syringe, mixing samples and reagents contained in syringe, and the penetration of samples and reagents, suggesting a higher risk of human error which affected the results of the examination, where the inaccurate amount of samples taken and mixing samples with unfavorable reagents will give false and high false low results. The results of this study were not in accordance with the internal research which compared the results of CRP levels between Standard F devices with reference devices in the laboratory, Roche Cobas c111, and other POCT devices, Nycocard. The study found a good correlation between the Standard F and the two devices.[13]

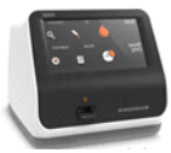
Detailed information and characteristics of the Dimension RxL Max (PETIA), Standard F 200 (Reflectometry Immunoassay) and AFIAS-6 (Sandwich Immunodetection) were shown in Table 3.

Information and characteristics of the three instruments can help the selection of instruments according to the needs and facilities owned by the laboratory.

Table 2. The difference of CRP levels result measured using PETIA, reflectometry immunoassay and sandwich immunodetection of sepsis patients

	Instruments	Mean	Standard Deviation	p
n =27	CRP PETIA	41.37	33.30	0.003
	CRP reflectometry immunoassay	35.62	55.68	
	CRP PETIA	41.37	33.30	0.172
	CRP sandwich immunodetection	38.62	29.94	
	CRP sandwich immunodetection	38.62	38.62	0.251
	CRP reflectometry immunoassay	35.62	55.68	

Table 3. Information and characteristics of the Dimension RxL Max, Standard F 200 and AFIAS-6 for CRP measurement

Characteristic of instruments	Dimension RxL Max	Standard F 200	AFIAS-6
Instruments			
Display	7 inch, touch screen	7 inch, color touch screen	7 inch, color touch screen
Size, Length x width x height (mm)	1590 x 810 x 1120	200 x 240 x 205	420 x 336 x 293
Weight (kg)	400	2,5	15,1
Printer	Built-in	Built-in	Built-in
Throughput/ hours	~800	~20	~36
Price of instrument (Rupiah)	1.700.000.000	37.180.000	252.000.000
Cost of CRP/test (Rupiah)	18.000	70.500	57.750
Reagent storage temp (^{0}C)	2 - 8	2 - 30	2 - 8
CRP analysis	Quantitative	Quantitative	Quantitative
Sample for CRP	serum/heparin plasma	whole blood, serum/plasma	whole blood, serum/plasma
Sample quantities (µL)	10	5	General mode:100 C-tip mode: 10
Length of measurement (minutes)	7	3	3
Detection range of CRP level (mg/L)	0.5 – 250	1-130	0.5 - 200

Based on the specifications of the Dimension RxL Max, it is a standard laboratory instrument with the largest size, weight, and throughput compared to the other two instruments, leading to its large use in many laboratories and with a high number of examination requests. The minimum quantity of samples required is 10 µL for both serum and plasma heparin samples. The RxL Max has the longest duration of examination and the highest throughput compared to the other two instruments. It has the highest detection range of 0.5 - 250 mg/L compared to the other two devices.

The F 200 Standard is one of the smallest POCTs in size, weight, and throughput compared to the other two instruments. Its small size and lightweight makes it quite easy to put in a place that is less spacious and easily moved. The Standard F 200 instrument throughput with the POCT method is the lowest compared to the other two devices, suggesting its compatibility to be used a laboratory with a lower number of examination requests. The advantages of POCT in clinical and economic aspect compared to standard laboratory equipment are its direct use at the examination site to early help diagnose and provide therapy to patients, alleviate workload and examination costs.[17] Standard F 200 detects CRP levels quantitatively, but the procedure includes manual procedures such as mixing samples with reagents, the homogenization process and sample uptake in the instrument, suggesting its incompatibility for laboratories with a high number of examination requests. The types of samples used

are more various, including whole blood (capillary, venous blood), serum and plasma with heparin anticoagulants, EDTA or Na citrate. The minimum quantity of samples it requires is 5 µL, making it compatible for pediatric or neonatal patients.

The AFIAS-6 has a compact size with 15.1 Kg in weight so that it can be placed in a less spacious room and it is easy to move. AFIAS-6 detects CRP levels quantitatively in spite of its need of manual addition of sample. The advantage of AFIAS-6 is the more various types of samples that can be chosen, including whole blood, serum or plasma. The device is in the form of an all-in-one cartridge containing all reagents and test strips so it is more practical. AFIAS-6 throughput is up to 36 measurement/hours with 6 slots that enable single or simultaneous tests with same or different parameters. The AFIAS-6 requires the highest amount of serum samples for CRP measurement compared to the other two devices which is 100 µL. For whole blood samples, a capillary tip can be used for a smaller sample size of 10 µL. The length of the measurement is 3 minutes per examination.

The limitations of this study were no written instructions given on the AFIAS and the CRP Standard F insert kit reagent guidelines to handle samples that exceeded the detection range so that sample dilution was not carried out on both devices. There was no synchronization of the results of CRP measurement on the three instruments using the third party control, leading to inability toassess the best CRP levels measurement method among the three devices. Furthermore, there was no examination of the confounding compounds in the sample.

CONCLUSIONS AND SUGGESTIONS

There were significant differences of CRP levels between the PETIA and Reflectometry Immunoassay method, suggesting the inability of both methods to substitute each other (p=0.003). There were insignificant differences of CRP levels between the PETIA and Sandwich Immunodetection methods (p=0.172) as well as the Reflectometry Immunoassay method and the Sandwich Immunodetection method (p=0.251). The specifications of instruments and methods for CRP measurement must be adjusted to laboratory needs and facilities. Further research on CRP levels was required by including the equalization of treatment for samples with CRP levels that exceed the instrument detection range, synchronization of three devices, and elimination of the possibility of confounding compounds in the sample.

REFERENCES

1. Stearns-Kurosawa DJ, Osuchowski MF, Valentine C, Kurosawa S, Remick DG. The pathogenesis of sepsis. Annu. Rev. Pathol. Mech. Dis. 2011; 6: 19-48.
2. Taeb AM, Hooper MH, Marik PE. Sepsis: Current definition, pathophysiology, diagnosis, and management. Nutrition in Clinical Practice XX(X). 2017; 1-13.
3. Chehade AH, Chebi RB, Majzoub I, Dagher GA. Assessment of sepsis in developing country: Where do we stand?. Health Care Current Reviews 2015; 3(2): 1-4.
4. Limongi D, Agostini CD, Ciotti M. New sepsis biomarker. Asian Pac J Trop Biomed, 2016; 1-4.
5. Simpson SQ. New sepsis criteria a change we should not make. Chestnet org, 2016; 149(5): 1117 - 1118.
6. Daniels R, Nutbeam T, Berry E. BMJ best practice sepsis in adults. The United Kingdom, BMJ Publishing Group Ltd. 2018; 4-25.
7. Rogic D, Juros GF, Petrik J, Vrancic AL. Advances and pitfalls in using laboratory biomarkers for the diagnosis and management of sepsis. eJIFCC 2017; 114-121.
8. Vincent JL, Beumier M. Diagnostic and prognostic marker in sepsis. Expert Rev. Anti Infect. Ther, 2013; 11(3): 265-275.
9. El-Shafie ME, Taema KM, El-Hallag MM, Kandeel AM. Role of presepsin compared to C-reactive protein in sepsis diagnosis and prognostication. The Egyptian Journal of Critical Care Medicine, 2017; 1-12.
10. Dimension Flex reagent cartridge C-Reactive Protein Extended Range (RCRP) insert kit. Siemens Healthcare Diagnostic. 2015.
11. AFIAS CRP. Boditech Med Incorporated. 2016 (cited 2018 May 2). Available from: http://www.boditech.co.kr/eng/tests/crp.asp
12. Standard F CRP. SD Biosensor. 2017 (cited 2018 May 2). Available from: http://www.sdbiosensor.com/xe/product/2637?category=2310
13. Lee SJ, Lee JH, Choi HG. Comparison of the standard F CRP point-of-care test to Roche Cobas c111 and NycoCard Tests. SD Biosensor Inc. 2018.
14. Limmathurotsakul D. Causes and outcomes of sepsis in Southeast Asia: A multinational multicentre cross-sectional study. Lancet Glob Health, 2017; 5: e157-167.
15. Angele MK, Pratschke S, Hubbard WJ, Chaudry IH. Gender differences in sepsis cardiovascular and immunological aspect. Virulence, 2014; 5(1): 12-19.
16. Rowe TA, McKoy JM. Sepsis in older adults. Infect Dis Clin N Am, 2017; 31: 731-742.
17. Prestegard E. The future of point-of-care testing using C-reactive protein-an ideal tool for diagnosis, prognosis and therapy management. European Endocrine Review. 2006; 1-4.

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5