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Diagnostic Value of Myeloperoxidase Index in Bacterial Infections

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ABSTRACT

Infectious diseases remain a serious problem in Indonesia. Myeloperoxidase (MPO) is a substance released by neutrophils, which activates the synthesis of hypochlorous acid (HOCL) from hydrogen peroxide (H2O2) and chloride ion (Cl-). Hypochlorous acid plays a vital role in the body's defense against infection. Myeloperoxidase Index (MPXI) is a parameter in the hematology analyzer Advia 2120i based on the principle of flow cytometry. This study aimed to determine the diagnostic value of MPXI in patients with bacterial infections. The study was a cross-sectional observational analysis. The samples consisted of a group of patients with bacterial infection and a group of healthy subjects. The specimens used in this study were whole blood + anticoagulant (EDTA) in a purple tube with a volume of 3 mL to determine the MPXI value in both groups using ADVIA 2120i hematology analyzer. The study subjects consisted of a group of patients with bacterial infections (69 patients) and a group of healthy subjects (33 people). Analysis of the MPXI ROC curve with a cut-off ≥ -5.8 and < -5.8, showed AUC of 0.323 (CI=95%, p=0.004), sensitivity of 34.8%, specificity of 39.4%, Positive Predictive Value (PPV) of 54.5%, and Negative Predictive Value (NPV) of 22.4%. Due to its low diagnostic value, the MPXI value was not recommended to be used as a diagnostic instrument for bacterial infections. Also, further research was highly needed.

Keywords: Myeloperoxdase index, myeloperoxidase, sensitivity, specificity, positive predictive value, the negative predictive value

INTRODUCTION

Infectious disease is caused by various pathogenic microbes, one of which is bacteria. Infection is generally caused by bacterial or viral, fungal, and parasitic infections. According to the WHO, in 2014, infectious diseases kill 3.5 million people per year. Infectious disease is one of the significant health problems in developing countries, including Indonesia. From this data, around 83% of deaths are caused by contagious diseases. This problem is of particular concern to all health workers. Infectious diseases also cause death around the world, including lower respiratory tract infections (3.9 million), HIV/AIDS (2.8 million), diarrheal diseases (1.8 million), malaria (1.3 million), and nosocomial infection.[1-3]

Rapid diagnosis and treatment using appropriate antibiotics are essential to reduce morbidity and mortality due to infection. Fever is the most common infection symptom; however, microbiological tests are still needed for a definitive diagnosis of bacteremia. Microbiological tests such as blood culture require a long time of 24-48 hours. Therefore, a rapid laboratory test will be beneficial to overcome bacterial infections.[3]

The diagnosis of bacterial infection is determined by observation of one or more signs of infection. Medical examinations such as physical examination and appropriate laboratory tests are needed. Bacterial infections are characterized by hyperthermia/febrile (temperature > 38°C), hypothermia (temperature < 36°C), leukocytosis (leukocyte count>12,000/mm^3), leukopenia (leukocyte count <4,000/mm^3), procalcitonin value ≥ 0.5 ng/mL and or positive or negative cultures.[4]

Over the past few years, several biomarkers have been used as corresponding to bacteria; however, these markers cannot distinguish bacteria or inflammatory processes that are not due to infection. Therefore, specific markers to identify typical causes of infectious diseases are required.[5]

Myeloperoxidase (MPO) is a substance released by neutrophils, which activates the synthesis of hypochlorous acid (HOCL) from hydrogen peroxide (H2O2) and chloride ions (Cl-). Hypochlorous acid plays a vital role in the body's defense against bacterial infections. Neutrophils extracted from individuals with MPO deficiency exhibit lower microbial activity than individuals with normal MPO activity.[6]

Myeloperoxidase Index (MPXI) is one of the parameters in Advia 2120i, an automatic hematology analyzer that works on the principle of flow cytometry by peroxidase staining, use of reagents that can assist the peroxidase activity of each cell, and use of 4-chloro-1-naphthol, a substrate for MPO and granulocytes that will be needed for selected granulocyte cells. Granulocytes that have been stained will pass through the flow cell, the scattering of light (y-axis) and absorbance (x-axis) are captured using a tungsten-halogen light source. The difference between the mean values of the x-axis and the normal population is referred to as MPXI by counting from the instruments.[6,7]

Myeloperoxidase index is a marker of infection; however, there has been no research that compares the value of MPXI in patients with and without bacterial infections. It was expected from this study that MPXI would be able to help early diagnosis of the disease by examination of the complete blood parameters.

METHODS

The study was an observational analysis with a cross-sectional design. The study samples consisted of 2 groups, 69 patients with bacterial infection and 33 healthy subjects as controls.

Inclusion criteria for patients with bacterial infection were derived from the diagnosis by the doctor responsible and observed signs of infection with one or more infections, hyperthermia/febrile (temperature > 38°C), hypothermia (temperature < 36°C), leukocytosis (> 12,000 mm^3), leukopenia (< 4,000 mm^3), procalcitonin value ≥ 0.5 ng/mL and or positive/negative culture. The patients agreed to the study and signed informed consent. Exclusion criteria were immunocompromised patients, patients with a history of liver abnormalities, kidney disorders, malignancies, HIV infection, and patients receiving immunosuppressant therapy.

Inclusion criteria were subjects in healthy groups of adults (age ≥ 18 years), and healthy condition based on physical examination with no complaints and with no symptoms of the disease, normal results of physical and laboratory tests (systolic blood pressure of 118-130 mmHg, diastolic of 80-90 mmHg, leukocyte count of 3,200-10,000 mm^3, breathing frequency of 18-20/minute), no signs of infection were obtained, and signed informed consent. Exclusion criteria were subjects who looked ill with reported complaints or observed symptoms of the disease and or signs of infection.

The sample group of bacterial infection patients was adult patients ≥ 18 years old who had just arrived at the Emergency Ward and or were treated at the Resuscitation Room, Intensive Observation Room, Internal and Pulmonary Inpatients at the Dr. Soetomo Hospital, Surabaya.

All groups of bacterial infection patients who fulfilled the requirements were culture-based (results were obtained from secondary data). All blood specimens of the bacterial infection group and the healthy group were determined by the MPXI using an ADVIA 2120i hematology analyzer.

The study was conducted in the Emergency Ward Laboratory, Dr. Soetomo Hospital. The research samples used were whole blood + anticoagulant (EDTA) in a purple tube with a volume of 3 mL.

A total of 69 samples were used in the group of patients with a bacterial infection. This number has fulfilled the sample size determined in this study, while the sample group of Healthy subjects used 33 samples.

This study was approved by the Health Research Ethics Committee of Dr. Soetomo Hospital, Surabaya, with the ethical number 07/Panke.KKE/I/2018.

RESULT AND DISCUSSION

The samples were collected from December 2017 to February 2018 in the Emergency Room, Intensive Observation Room, Resuscitation Room, and Internal and Pulmonary Inpatient Room of Dr. Soetomo Hospital Surabaya. Data were presented in tables, figures/graphs, and written information. The cut-off of MPXI was determined using the ROC curve. Based on the cut-off value results, a 2x2 table was made to determine the diagnostic values. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were then obtained.

Characteristics of research subjects in the group of patients with the most bacterial infections were males (50.7%). The healthy group was dominated by females (33 people). The mean age of the groups of patients with bacterial infection and healthy subjects was almost the same (Table 1).

The median MPXI value in patients with bacterial infection was -7.6 (-25-4.6), whereas the MPXI value in the healthy group was -5.1 (-11-0.1). Receiver operating characteristic curve analysis of MPXI with cut-off ≥ -5.8 and <-5.8 showed AUC 0.323 (CI=95%, p=0.004).

The results of MPXI values in all samples in the group of patients with bacterial infection and the healthy subjects with positive and or negative cultures can be seen in Table 2.

Table 1. Characteristics of groups of patients with bacterial infection and groups of healthy subjects

Characteristics	Patients with Bacterial Infection	Healthy Subjects
Male	35 (50.7%)	16 (48.5%)
Female	34 (49.3%)	17 (51.5%)
Mean age ± SD	46.53±13.84 y.o	44.18±14.11 y.o
Age range	18-77 y.o	23-48 y.o

Table 2. The results of MPXI values in the group of patients with bacterial infection and the healthy group using the 2x2 table

MPXI Value	Patients with Bacterial Infection	Healthy Subjects	Total
≥ -5.8	24 (24/69=34.8%)	20 (20/33=60.6%)	44 (44/102=43.1%)
< -5.8	45 (45/69=65.2%)	13 (13/33=39.4%)	58 (58/103=56.9%)
Total	69 (100%)	33 (100%)	102 (100%)

The diagnostic sensitivity of MPXI to detect patients with bacterial infections was 34.8% (24 of 69 samples) with a diagnostic specificity of 39.4% (13 of 33 samples).

Table 3. The diagnostic values of MPXI

Diagnostic Value	%
Sensitivity	34.8
Specificity	39.4
Positive predictive value	54.5
Negative predictive value	22.4

The diagnostic value of MPXI had low sensitivity and specificity. There was a great variation of MPXI value in the Advia 2120i hematology analyzer in a group of patients with bacterial infection, depending on patients' condition and the number of leukocytes. The myeloperoxidase index value is negative if the number of peroxidases in patient samples was smaller. It usually occurs in the process of infection that has decreased with normal or low leukocyte counts. Natural immune response to extracellular bacteria is mainly through the mechanism of phagocytosis by neutrophils, monocytes, and macrophages, which are phagocytic cells with the primary function of finding and destroying microorganisms, increasing the number of leukocytes accompanied by the presence of granulocytes in bacterial infections. An increased number of leukocytes, predominantly neutrophils, and macrophages, is needed to kill bacteria through the process of phagocytosis. Neutrophils also contain granules that have antimicrobial activity (defensins, lysozyme, lactoferrin, metalloprotease), resulting in an increase in the number of leukocytes accompanied by immature granulocytes in bacterial infections.[8-10]

It should be noted that peroxidase reaches high levels at 4-8 hours of infection. After more than 4-8 hours, the granules will come out of cells as a defense mechanism against bacteria. Conversely, while the infection process is still ongoing, toxic granules will be produced in high leukocyte counts. This is thought to be the cause of the wildly varying results in this study, leading to a low diagnostic value. This study showed that the mean MPXI levels examined by the ADVIA 2120i method between patients with bacterial infection and healthy controls showed significant differences ($p < 0.05$); however, the diagnostic value of MPXI was low. These results were following research conducted by Yonezawa *et al*., which obtained MPXI values of -2.40 (-19.60-9.60) in the group of patients with a bacterial infection. This value was significantly lower compared to the healthy group of 0.20 (-8.70-8.40). Yonezawa *et al*. also stated that the MPXI value in bacterial infections fluctuated during the study. The fluctuations depended on the cause of the disease and the medical intervention. In severe bacterial infections, active neutrophils release large amounts of MPO for bactericidal activity, and increased neutrophil degranulation can reduce MPXI values. Myeloperoxidase index is thought to be controlled by a balance between synthesis and release of MPO. Also, cytokine storm during bacterial infections can interfere with the natural immune system, causing neutrophil dysfunction and reducing the value of MPXI.[8]

This study has several limitations, including

patients' primary clinical condition with the bacterial infection, the varying length of illness before the patient was referred to the Dr. Soetomo Hospital, and the previous antibiotic administration before patients were admitted to the hospital. This allowed a considerable bias. The varying sample test time in this study was estimated to be > 8 hours and presumed to have affected the MPXI results.

CONCLUSION AND SUGGESTION

The MPXI value was not recommended as a diagnostic instrument for bacterial infection due to its low diagnostic value. Further research was needed, with sampling before giving antibiotics, sampling time in the first 4-8 hours after diagnosis of bacterial infection, and there was no comparison for healthy subjects as controls.

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