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SYSTEMIC CANDIDOSIS DIAGNOSTIC TEST WITH CANDIDA SCORE AND MONOCYTE COUNT IN PREMATURE INFANTS WITH LATE-ONSET SEPSIS: RESEARCH IN LOW RESOURCES COUNTRY

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Abstract. *Introduction*. Candida is the cause of most systemic fungal infections that plays a role in the pathophysiology of sepsis in newborns, especially in premature infants with late-onset sepsis. The Candida score can be used to assess the occurrence of systemic candidosis where a Candida score > 2.5 can accurately identify patients who are at high risk for candidiasis infection. Monocytes also play an important role in preventing candida invasion. *Materials and methods*. This study used a cross sectional research design. Data was collected from premature infants with late-onset sepsis being suspected of systemic candidosis in neonatology inpatient ward. It was submitted from the period of November-December 2021. It takes a minimum of 31 samples to meet the criteria to process and analyze the data. The data obtained were processed and analyzed using the Receiver Operating Characteristic (ROC) method to obtain the Area Under Curve (AUC) value. Based on the AUC curve, the search for the most optimal intersection is carried out to obtain the sensitivity and specificity values. *Results*. Of the 31 research subjects, the number of subjects with positive PCR results was 27 (76.93%) while negative were 4 respondents (12.9%). The mean value of PCR density in the positive group of subjects was 76.93 and the range was 40.23–122.78. Meanwhile, in the group of subjects who were negative, the PCR density value was 0. The results of the Candida score diagnostic test showed that the sensitivity obtained was 81%. Higher sensitivity and specificity > 70% were found in the combined examination of Candida scores and monocyte counts according to cut-off compared with separate examinations. *Conclusions*. The combined examination of Candida score and monocyte count can be used as a diagnostic test for systemic candidosis in premature infants with late-onset sepsis, and could be used to consider the initiation of empirical antifungal therapy, either prophylactically or therapeutically, especially in limited laboratory facilities in Indonesia.

Key words: *Candida score, diagnostic test, late onset sepsis, monocyte count, premature infant, systemic candidosis.*

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СИСТЕМНЫЙ КАНДИДОЗНЫЙ ДИАГНОСТИЧЕСКИЙ ТЕСТ С ИСПОЛЬЗОВАНИЕМ ШКАЛЫ CANDIDA SCORE И ПОДСЧЕТОМ МОНОЦИТОВ У НЕДОНОШЕННЫХ НОВОРОЖДЕННЫХ С ПОЗДНИМ СЕПСИСОМ: ИССЛЕДОВАНИЕ В СТРАНЕ С ОГРАНИЧЕННЫМИ РЕСУРСАМИ

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Резюме. *Введение. Candida* является причиной большинства системных грибковых инфекций, играющих роль в патофизиологии сепсиса у новорожденных, особенно у недоношенных детей с поздним началом сепсиса. Шкала Candida score может использоваться для оценки возникновения системного кандидоза, где балл > 2.5 может точно идентифицировать пациентов с высоким риском заражения кандидозом. Моноциты также играют важную роль в предотвращении инвазии *Candida. Материалы и методы.* В настоящем исследовании использовался дизайн перекрестного исследования. Данные были собраны у недоношенных детей с поздним сепсисом при подозрении на системный кандидоз в стационаре для новорожденных, в период с ноября по декабрь 2021 г. Для соответствия критериям обработки и анализа данных требуется минимум 31 образец. Полученные данные были обработаны и проанализированы с использованием метода рабочих характеристик приемника (ROC) для получения значения площади под кривой (AUC). На основе кривой AUC осуществлялся поиск наиболее оптимального пересечения для получения значений чувствительности и специфичности. *Результаты.* Из 31 обследованных лиц количество с положительными результатами ПЦР составило 27 (76.93%), а с отрицательными — 4 респондента (12.9%). Среднее значение плотности ПЦР в положительной группе испытуемых составило 76.93, а диапазон — 40.23–122.78. При этом в группе испытуемых с отрицательным результатом значение плотности ПЦР равнялось 0. Результаты диагностического теста Candida score показали, что чувствительность составила 81%. Более высокая чувствительность и специфичность > 70% были обнаружены при комбинированном исследовании с использованием шкалы Candida score и подсчетом количества моноцитов в соответствии с отсечкой по сравнению с раздельными исследованиями. *Выводы.* Комбинированное исследование с использованием шкалы Candida score и подсчетом количества моноцитов может быть использовано в качестве диагностического теста на системный кандидоз у недоношенных детей с поздним сепсисом для рассмотрения вопроса о начале эмпирической противогрибковой терапии, как профилактической, так и терапевтической, особенно в условиях ограниченных возможностей лабораторной диагностики в Индонезии.

Ключевые слова: *шкала Candida score, диагностический тест, поздний сепсис, количество моноцитов, недоношенный ребенок, системный кандидоз.*

Introduction

Neonatal sepsis is one of the complications of preterm birth and is an ongoing global public health challenge with 30% morbidity and 13–56% mortality with 42% of them dying in the first week of birth worldwide. Neonatal sepsis was divided into early-onset sepsis (EOS) and late-onset sepsis (LOS) based on the age at which EOS was due to intrauterine infection, and LOS was associated with postnatal nosocomial infection, with the highest incidence reported between day 10 and day 22 of life [36]. Candida is the cause of most systemic fungal infections that play a role in the pathophysiology of sepsis in newborns.

Despite the current transformational improvements in better diagnostics and management strategies, Increased length of time in hospital and higher management costs are among the concerns that add to the burden on hospitals. The candida diagnosis in newborns with sepsis can be established by the Candida score and a simple examination with the monocyte count which has been developed as a means to identify critical care patients who are at high risk of benefiting from empiric antifungal therapy in limited health facilities [13, 35].

The Candida score can be used to assess which systemic candidosis occurredcandidate score > 2.5 can accurately identify patients at high risk for candidiasis infection with a sensitivity of 81% and a specificity of 74%. Monocytes also play an important role in preventing candida invasion based on studies in mice. Monocytes depend primarily on non-opsonic phagocytosis via Dectin-1and Dectin-2. This phenomenon has the potential to be of major clinical relevance in preterm infants, where b-1,3-glucan is a major constituent of the cells of several microorganisms including candida, which is a commensal fungus commonly found in hospital environments [33, 34].

The combined examination between Candida scores and monocyte counts in limited laboratory facilities is expected to be the gold standard for the diagnosis of systemic candidosis and can be used for early antifungal administration since the patient is diagnosed with sepsis. However, there is no study that combines Candida scores and monocyte counts as a diagnostic of systemic candidosis in premature infants with late-onset sepsis.

Materials and methods

This study used a cross-sectional approach and sampling method with consecutive sampling from data on premature infants with late-onset sepsis clinically suspected of systemic candidosis in November-

Table 1. Results combined diagnostic test between Candida score and monocyte count on PCR

Diagnostic test	PCR Candida		Total
	Positive	Negative	
Candida score > 2.5 + monocytes > 5% (Positive)	20	1	21
Candida score < 2.5/monocyte < 5% (Negative)	7	3	10
Total	27	4	31

December 2021 in the neonatology inpatient ward, Dr. R.S. Saiful Anwar, Malang. The sample size was calculated by using the receiver operating characteristic (ROC) diagnostic test formula. It takes a minimum sample of 31 patients, populations that meet the inclusion criteria are taken as respondents. The inclusion criteria in this study were infants born inside or outside the RSSA with ballard score plots > 28 weeks to < 37 weeks with sepsis criteria > 72 hours; patients receiving antibiotic therapy for bacterial sepsis with LOS; and the patient did not receive previous antifungal drug therapy.

Blood sampling was carried out once, namely at the beginning of the study in the RSA neonatology inpatient ward. The volume of blood taken from each study sample was 5 ml.Blood monocytes were obtained by differential centrifugation of heparinized blood on a Ficoll-Hypaque gradient. The monocyte lymphocyte layer was harvested and washed 2× with buffered saline containing 0.5 IU heparin per ml, after which the cell suspension was approximately 1.0×10^7 to 1.5×10^7 monocytes per ml, 3.5×10^7 in Hanks balanced salt containing containing 0.1% (wt/vol) gelatin was prepared.Furthermore, serum samples were made by centrifugation at 3000 rpm for 5 minutes, and stored at 4°C for further measurement of serum PCR and candida culture. Measurement of Candida score based on rounded Candida score = 1x (total parenteral nutrition) + 1x (post surgery) + 1x (multifocal species candida colonization) + 2x (severe sepsis). PCR measurement Using 1 pair of primers which amplifies the target genome sequence. PCR uses real time. Measurements were carried out once, namely at the beginning of the study. This research is a diagnostic test study, which is to test the accuracy of a diagnostic tool by comparing the results of the examination using the tool with other examination standards. Furthermore, The data obtained were processed and analyzed using the receiver operating characteristic (ROC) method to obtain the area under curve (AUC) value of the tool being tested. The AUC curve is then searched for the best intersection point to be processed in order to obtain the sensitivity and specificity of a diagnostic tool.

Results

In this study, two methods were used to diagnosed systemic candidosis, namely using the Candida score and monocyte profile then compared with the results of the examination. Polymerase Chain Reaction (PCR) for identification of *Candida* species. The PCR

Table 2. Characteristics of research subjects

Characteristics	Score
Gender, n (%)	
Male	17 (54.8)
Female	14 (45.2)
Born, n (%)	
In	10 (32,3)
Outside	21 (67.7)
Gestational age, n (%)	
< 28 weeks	2 (6.5)
28 – < 32 weeks	9 (29)
32 – < 37 weeks	20 (64.5)
Mode of Birth, n (%)	
Sectio Caesarea	27 (87.1)
Vaginal spontaneous	4 (12.9)
Treatment Room, n (%)	
NICU	21 (67.7)
Perinatology	10 (32,3)
Breathing apparatus, n (%)	
CPAP/CNO	9 (29)
Endotracheal tube ventilator	11 (35.5)
Low flow nasal canule	1 (3,2)
Non-invasive ventilator	10 (32.23)
Birth weight, n (%)	
< 1000 grams	1 (3)
1000–2000 grams	23 (74)
2100–2500 grams	7 (23)
Days of treatment at the previous hospital, n (%)	
< 5 days	11 (55)
> 5 days	9 (45)
Surgery, n (%)	
Not	19 (61.3)
Yes	12 (38.7)
Administration of antibiotics, n (%)	
< 15 days	17 (54.8)
> 15 days	14 (45.2)
IV Line Installation, n (%)	
Central (CVC, umbilical catheter)	30 (96.8)
Peripheral	1 (3,2)
Length of stay in hospital, n (%)	
< 15 days	17 (54.8)
> 15 days	14 (45.2)
Infant nutrition, n (%)	
Breast milk	14 (45.2)
Breast milk + formula milk	17 (54.8)
Formula milk	–

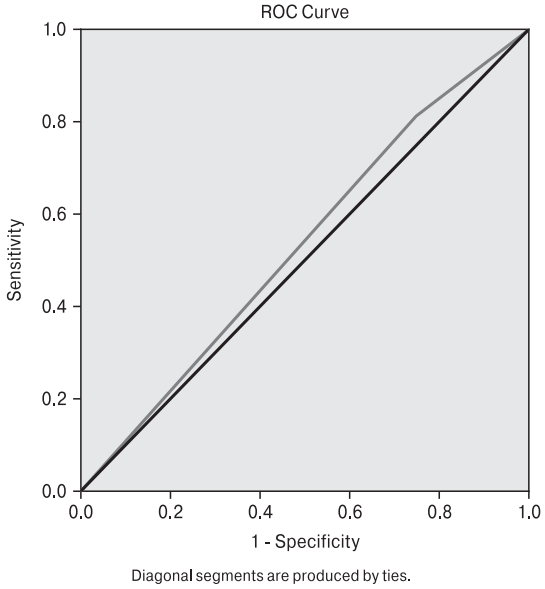

Figure 1. ROC graph of Candida scores compared with PCR

Note. The interpretation of this figure indicates that the candidate score can be used as a diagnostic test in the poor/weak category. Based on the cut-off test for the cut-off value of the Candida score of 2.5.

result which combined with the monocyte count is shown in Table 1. To find out the distribution of data on Candida scores and monocyte profiles, normality testing was carried out using the Shapiro–Wilk test (number of samples < 50). The data is declared normal if sig > 0.05. The data were also tested for homogeneity to find out whether the data in this study had homogeneous/heterogeneous variance. The variance of the data is homogeneous if sig > 0.05. The characteristics of research subject are being shown in Table 2. The results of the normality test showed that the Candida scores were not normally distributed (sig < 0.05) while the monocyte profiles were normally distributed (sig > 0.05). Both variables have homogeneous variance (sig > 0.05). Of the 31 research subjects, the number of subjects with positive PCR results was 27 (76.93%) while negative were 4 respondents (12.9%). The mean value of PCR density in the positive group of subjects was 76.93 and the range was 40.23–122.78. Meanwhile, in the group of subjects who were negative, the PCR density value was 0. The results of the Candida score diagnostic test showed that the sensitivity obtained was 81%. These results illustrate the ability of the Candida score in diagnosing systemic candidosis of 81%. The resulting positive predictive value is also quite high (88%). However, the RKP value is still very small, namely 1.08 where the diagnostic result is said to be good if the RKP > 10. While the RKN value is still high where the diagnostic result will be good if the RKN < 0.1. So that the Candida score diagnostic test is still not fully accurate. The results of the AUC Candida score resulted in an area value of > 50%, namely 53.2% (p = 0.837) which indicated that the diagnostic value of the Candida score was still very weak (Fig. 1). The results of the monocyte profile diagnostic test showed that the sensitivity obtained was 93%. These results illustrate the ability of monocyte profiles in diagnosing systemic candidosis which is 93% higher than the Candida score, and it is shown in Fig. 2. The positive predictive value is equal to the sensitivity of 93%. However, judging from the RKP value < 10 which indicates the diagnostic results are still not good even though the RKN is around 0.1. The results of the AUC monocyte profile resulted in an area value of > 50%, namely 71.2% (p = 0.175) which indicated that the diagnostic value of the monocyte profile was still quite good. RKN 50%, which is 71.2% (p = 0.175) which indicates that the diagnostic value of the monocyte profile is still quite good. RKN 50%, which is 71.2% (p = 0.175) which indicates that the diagnostic value of the monocyte profile is still quite good. RKN = (1–sensitivity)/specificity = (1–0.75)/0.74 = 0.35. Candidate score diagnostic test results, monocyte profile against candida PCR. Table 2 shows a sensitivity of 74% which tends to be lower than the results of separate diagnoses. However, the specificity value obtained is higher, namely 75%. The Positive Presumptive Value of the combined examination of Candida scores and monocyte profiles was 95% increased and higher than the results of separate diagnoses. The positive probability ratio of 2.96 also increased even though it was still < 10. The combined AUC results of Candida scores and monocyte profiles resulted in an area value of > 50%, namely 74.5% (p = 0.118) which indicated a fairly good diagnostic value and a higher value than the separate examination.

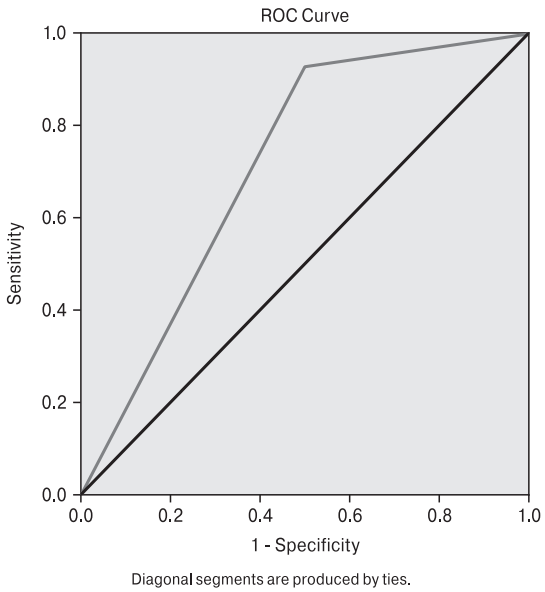

Figure 2. Graph of ROC of monocyte count compared to PCR

Note. The interpretation of this figure shows that monocyte count can be used as a diagnostic test with good category. Based on the cut-off test for the monocyte count cut-off value > 5%.

Discussion

Based on the characteristics of the research subjects, samples of premature infants (boys and girls) were diagnosed with late onset sepsis. More male subjects than female subjects (54.8% and 45.2%). In accordance with research conducted by Avila et al in 2019, premature infants with late onset sepsis accompanied by candida infection were more male subjects than female subjects, namely 60% while the study conducted by Aziz et al in 2020 contained male and female subjects. women with a ratio of 1.9:1 [1, 2, 30, 31, 32].

Research conducted by El-atawi et al 2017 states Low birth weight (> 2500 g) and earlier gestational age (29–32 weeks) were found to be commonly associated with systemic candida in neonates.In this study, 31 study subjects had a gestational age range of < 28–36 weeks, with the number of subjects with gestational age < 28 weeks as much as 6.5%, gestational age 28–32 weeks as many as 29%, and gestational age between 33–36 week as much as 64.5%. Among the 31 subjects, patients found 3% with birth weight (bbl) < 1000 grams, 74% with 1000–2000 grams, and 23% with 2100–2500 grams. Infants with very low birth weight (LBW < 1000 grams) are at the highest risk of developing systemic candidosis [4, 34].

Systemic candidosis is an important cause of LOS in premature neonates in the Neonatal Intensive Care Unit (NICU). In this study, 67.7% of the samples were treated in the NICU Hospital and 32.3% of the samples were treated in the Neonatology Hospital. Among them 32.3% were born in the RSSA, and 67.7% were born outside the RSSA. With the length of treatment < 15 days as many as 54.8% and > 15 days as much as 45.2%. Where prospective multicenter cohort study of 2847 infants in the NICU found a significant association between catheter and antibiotic use (particularly in relation to invasive procedures and treatments) and the incidence of systemic candidosis.

In this study, 54.8% of samples with antibiotic use > 15 days and 45.2% of samples with < 15 days of antibiotic use were obtained. It was previously known that the type and amount of antibiotics, such as the third generation cephalosporins, vancomycin or carbapenems, can predispose to candida infection, especially in the host with a compromised immune system. Repeated or long-term use of antibiotics will damage the normal flora balance, causing the proliferation of *Candida albicans* [25, 26, 27, 28, 29].

Central intravenous insertion (CVC) can be a predilection risk factor for fungal colonization. In 31 research subjects, there were 96.8% of patients using CVC and 3.2% of patients using peripheral venous access. Neonates with LBW often require central vascular catheterization (CVC) for parenteral nutrition and administration of antibiotics during long periods of hospitalization. The main types of CVC and means of access to the neonate are umbilical catheters, central catheters inserted through a peripheral vein (PICC), central venous catheters through direct puncture of the femoral, jugular or subclavian vein, and central venous catheters inserted surgically by means of venous dissection. The most common life-threatening complication of CVC is catheter-associated bloodstream infection (CR-BSI), and CR-BSI is also associated with high costs [24, 25].

From the 31 research subjects, the number of subjects with positive PCR results was 27 (76.93%) while negative were 4 respondents (12.9%). The mean value of PCR density in the positive group of subjects was 76.93 and the range was 40.23–122.78. Meanwhile, in the group of subjects who were negative, the PCR density value was 0. In a study conducted by Ratridewi et al., PCR had a sensitivity rate of 69.2% and a specificity of 71% in diagnosing systemic candidosis in children with malignancy with severe neutropenia. In this study, real time PCR was used where 1 set of primers was used for DNA identification and then the amplified sequence was connected to a fluorescent probe that emits light when it binds to the amplification product [23, 26, 35].

Non-culture based methods, such as DNA detection by PCR, have been developed to aid in the rapid diagnosis of infection, enabling the initiation of empiric antifungal therapy at 6 hours after the onset of sepsis. A scoring system has been developed to guide empirical antifungal therapy for patients colonized with *Candida* spp. using a Candida score [17, 18, 19, 20, 21, 22].

From the 31 study subjects, 80% of patients were positive for candida PCR, and 20% of patients were negative for candida PCR with Candida scores > 2.5. Meanwhile, patients with a Candida score < 2.5 found about 22% of patients with positive PCR *Candida* and 78% of patients with negative PCR *Candida*. In this study, a candidate score > 2.5 had a sensitivity of 81% and a specificity of 25% with a positive predictive value of 88% and a negative predictive value of 16.67%. In a study conducted by Lambiotte in 2017 stated the relationship between initiation of antifungal agents and Candidate scores are quite satisfactory in this study. The frequency of empirical antifungal therapy in Lambiotte's study were 2.3%, 27.6%, 41,2%, and 75% in patients with Candida score of 2, 3, 4, and 5 respectively. Furthermore, in a study conducted by Ratridewi et al. in 2020 in patients with malignancy and severe neutropenia, the Candida score had a sensitivity of 84.6% and a specificity of 71.1% in diagnosing systemic candidosis [17, 26].

Scoring systems such as the Candida score have been developed as a means to identify high-risk critical care patients who may benefit from empiric antifungal therapy. A scoring system was conducted to evaluate potential antifungal uses which could have economic, and medical implications. A Candida score of more than 3 is an indication for early antifungal administration [13].

From the 31 study subjects, 27 patients had monocyte values > 5% and 4 patients with monocytes < 5%. Where of the 27 patients with monocytes > 5%, 92% of the patients were positive for candida PCR and 8% with negative for candida PCR. While 4 patients with monocytes < 5%, 50% of them with positive PCR candida and 50% with negative PCR candida. With a sensitivity value of 93% and a specificity of 50% in this test. These results illustrate the ability of the monocyte profile to screen for systemic candidosis which is 93% higher than the Candida score.

Monocytes have lower numbers in peripheral blood than neutrophils, but very important in defense against bacterial and fungal infections, as phagocytosis to kill pathogens in the body, regulate inflammatory responses through the release of chemoattractants, inflammatory cytokines, and activate adaptive immune responses. Monocytes are produced early in pregnancy (before 20 weeks) and represent 7–38% of all circulating mononuclear cells in the vasculature of term infants. The cut off of the normal monocyte count is < 5% [15, 16].

Premature infants have immature immune systems, with low innate and acquired immunity. The immune system in premature infants compared to term infants only has low levels of monocytes and neutrophils, is unable to kill pathogens and low cytokine production is limited to T cell activation and decreased ability to fight bacteria and detect viruses in cells. Intrauterine inflammation is a major cause of preterm birth resulting in premature immune system activation and cytokine production. This can lead to immune tolerance leading to decreased immune function of the newborn. Intrauterine inflammation is associated with an increased incidence of early-onset sepsis and has long-term adverse immune consequences [14].

Two recent studies have demonstrated that both classical and nonclassical monocytes from preterm infants are relatively unresponsive to stimulation of the Toll-like receptor (TLR), which monocytes also function for phagocytes. Monocyte CD64 expression was similar between preterm and term infants, and increased at early gestational age of 24–31 weeks. Preterm birth is driven by various inflammatory factors, particularly infection, which can alter monocyte phenotype and function at birth, as well as in the postnatal period and beyond. Three main subsets of monocytes have been described in humans, based on the cell surface expression of CD14, coreceptors for cell wall components of gram-negative lipopolysaccharide (LPS), and CD16 (IgG Fc FcgR-III receptor). In adult peripheral blood, monocytes ($CD14^{++}CD162$) constitute the majority (0.80%) of monocytes [11, 12].

The remaining monocyte pool consists of classical ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{+}$) monocytes. Prior to classification into two distinct subsets, $CD16^{+}$ monocytes were collectively referred to as proinflammatory monocytes due to their capacity to produce higher levels of inflammatory cytokines compared to classical monocytes ($CD14^{++}CD16$). $CD16^{+}$ monocytes also develop in neonates, children, and adults in sepsis, suggesting an active role for these cells during invasive infection. However, although non-classical and classical $CD16^{+}$ monocytes are closely related at the molecular level (determined by the microarray gene expression profile), the former has been shown to produce higher levels of proinflammatory cytokines (e.g., TNFα and IL-1β0 [3, 8, 9, 10, 37].

Monocytes too plays an important role in preventing candida invasion based on studies in mice. Monocytes depend primarily on non-opsonic phagocytosis via Dectin-1and Dectin-2. Recent studies have suggested that substimulator doses of a candida pattern recognition receptor (PRR) agonist have been shown to induce epigenetic changes in human monocytes, but also in other innate immune cells leading to an increased response to re-stimulation after a period of rest. This phenomenon is called "immune innate training" by Natea and is strongly induced by stimulation of the C-type lectin receptor (CLR) dectin-1 by b-1,3-glucan. This phenomenon has the potential to be of major clinical relevance in preterm infants, where b-1,3-glucan is a major constituent of the cells of several microorganisms including candida, which is a commensal fungus commonly found in the hospital setting [5, 6, 7].

From the 31 research subjects, 21 patients with Candida scores > 2.5 and monocytes > 5% and 10 patients with Candida scores < 2.5 and monocytes < 5%. Where of the 21 patients with Candida scores > 2.5 and monocytes > 5% there were 95% of patients with positive PCR Candida and 5% patients with negative PCR Candida. Meanwhile, from 10 subjects with a Candida score < 2.5 and monocytes < 5%, 70% of the patients were positive for Candida PCR and 30% of patients were negative for Candida PCR. The results of the diagnostic test of Candida scores and monocyte profiles against Candida PCR resulted in a sensitivity of 74% which tended to be lower than the results of separate diagnoses. However, the specificity value obtained is higher, namely 75%.

There has been no study that has tested the Candida score and monocyte profile simultaneously for early diagnosis of systemic candidosis. However, from previous studies, these two tests were carried out separately, it was found that there was a high sensitivity with the same cut-off value, and can be used as a tool for early detection of systemic candidosis in premature infants with late-onset sepsis. In this study, these two tests when carried out in combination had a sensitivity rate of 74% lower than the diagnostic results separately. This is possible because the sample size is not representative of the population of premature infants with sepsis and the research time is too short, as well as the baby's immune response to candida is different for each individual. However, the speci-

ficity value obtained is higher, namely 75% compared to separate diagnostic tests. Where these two tests can be considered as an early diagnostic test for systemic candida in premature infants with late onset sepsis.

This research has several shortcomings. The sample in this study could not represent the total sample size of premature infants treated in Neonatology Department of Saiful Anwar Hospital Malang, and the samples used in this study were only premature infants with late onset sepsis. Another drawback is that this study was not used for evaluation before and after empirical antifungal administration. Only done for inspection at a time only.

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