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Original Article

Invasive candidiasis among high prevalence neurological patients

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Abstract

Introduction: Invasive candidiasis is a severe form of infection. The incidence of invasive fungal infections has increased, due to the increasing number of patients with impaired immunity who are being treated through prolonged stay in hospital facilities. Neurological patient treatment methods such as antimicrobials, corticosteroid, central venous catheter (CVC), total parenteral nutrition, and mechanical ventilation use are associated with common risk factors for invasive candidiasis. Our study demonstrated invasive candidiasis prevalence among neurological patients.

Methodology: A cross-sectional study was done with consecutive sampling of neurological patients who were hospitalized from January 2017 to February 2020 at the Mahar Mardjono National Brain Center Hospital East Jakarta Indonesia. Patients with sepsis, septic shock, or fever (> 38.5 °C), and who had not received antifungals before culture were enrolled in the study. Clinical specimens were obtained from blood, liquor cerebrospinal or other sterile sites, CVC, respiratory tract specimens, and urine or other non-sterile sites. Socio-demographic data, potential risk factors based on previous studies, clinical, and other tests data were obtained from medical records. Classification of invasive candidiasis was according to the Paphitou classification criteria.

Results: One hundred and two subjects met the study criteria. The prevalence of invasive candidiasis in neurological patients was 13.7%. All of the isolates were *C. parapsilosis*.

Conclusions: The prevalence of invasive candidiasis was high in the samples studied. The infection was associated with septic shock, tracheostomy, and duration of use of central venous catheter, ventilator, and steroids.

Key words: culture, *Candida* spp., invasive candidiasis, invasive fungal infections, neurological patients.

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Introduction

Candidiasis is an infection caused by *Candida* species. It can be a superficial or an invasive infection. A superficial infection is a mild form of infection, and includes oropharyngeal and esophageal candidiasis. Invasive candidiasis is a severe and invasive form of infection, with manifestations that can be seen in the blood (candidemia) or in other organs (disseminated candidiasis) [1].

The diagnosis of invasive candidiasis includes clinical signs and symptoms, culture, radiology, and histopathology [2]. A consensus group of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) published standard definitions for invasive fungal infections for clinical and epidemiological research in 2002, and an updated version in 2008. The group reaffirmed that the definitions should be used only to assist in research and that the integrity of the original definitions with the classifications of "proven", "probable", and "possible" invasive fungal infections would be preserved [3].

However, their utility has not extended beyond patients with cancer, or recipients of stem cell or solid organ transplants. With newer diagnostic techniques available, The European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium

(EORTC/MSGERC) looked closely at imaging, laboratory diagnosis, and special populations at risk of invasive fungal infections. Therefore, the consensus definitions were updated in 2020. There is no change in the classifications of "proven", "probable", and "possible" invasive fungal infections, although the definition of "probable" has been expanded and the scope of the category "possible" has been diminished. The category of "proven" invasive fungal diseases can apply to any patient, regardless of whether the patient is immunocompromised. The "probable" and "possible" categories are proposed for immunocompromised patients only, except for endemic fungal infections [4].

Despite the predictable limitations of any specific set of rules, definitions for clinically relevant and common forms of "proven", "probable", and "possible" forms of invasive candidiasis were developed by Paphitou *et al.* [5]. These definitions were modeled after those proposed by the EORTC/MSG and also followed the principles outlined in the recent Infectious Diseases Society of American (IDSA) guidelines for the management of intravascular catheter-related infections and the concepts proposed by the American College of Chest Physicians Society for Critical Care Medicine (ACCP/SCCM) consensus conference for definition of sepsis [3,5].

The incidence of invasive fungal infections has increased fivefold in the past decade, due to the increasing number of patients with impaired immunity who are being treated through prolonged stay in hospital facilities. Cases of candidiasis began to increase in the 1940s when antimicrobials were used following the increasing incidence of human immunodeficiency virus (HIV) infection, the use of therapeutic modalities for advanced life support, and certain surgical procedures such as organ transplants and implantation of prosthetic devices [2].

The Extended Prevalence of Infection in Intensive Care (EPIC II), 2007 [6] highlighted *Candida* spp as the third most common pathogen, responsible for 17% of all the infections. Invasive candidiasis occurs five to ten times more often (2-6.7 in 1000 admitted patients) in Intensive Care Units (ICUs) than in medical or surgical wards. In the United States, *Candida* sp is the fourth most common cause of blood nosocomial infections. While earlier *Candida albicans* was the dominant pathogen and caused two-thirds of the infections, currently an increasing number of non-albicans species can be noticed and are responsible for almost 50% of the infections. These include *C. glabrata, C. krusei, C. tropicalis*, and *C. parapsilosis* [6].

There is also concern that the increase in invasive fungal infections including invasive candidiasis cases will also occur in neurological patients. Neurological patient cares are associated with common risk factors for invasive candidiasis including use of antimicrobials and corticosteroids, central venous catheter (CVC), total parenteral nutrition (TPN), and ventilator [7]. To our knowledge, this is the first study from our country to obtain invasive candidiasis prevalence data among neurological patients.

Methodology

This cross-sectional study with consecutive sampling was conducted at the Mahar Mardjono National Brain Center Hospital, East Jakarta, Indonesia. Established in 2013, it is the only neurological teaching and tertiary referral hospital in the region. The hospital provides various types of neurological health services.

A sample size of 96 patients was estimated assuming the allowable margin of error (d) of its estimate does not exceed 10% with a 95% confidence level (zα 1.96), and no previously published data showing the rate (p = 0.5). The subjects were adult patients with neurological diseases who were hospitalized from January 2017 to February 2020. The patients who were included has symptoms of sepsis, septic shock, or had clinical symptoms suggestive of infection characterized by fever > 38.5 °C. Culture was performed for these patients. If the patients were treated with antifungals before culture, they were excluded.

Socio-demographic data, potential risk factors based on previous studies of invasive candidiasis, clinical, and other tests data were obtained from the medical records. The risk factors included: 1) Occurrence of interventions (central venous catheters (CVC), total parenteral nutrition (TPN), ventilator, hemodialysis, and surgery), 2) Use of drug therapies (corticosteroids, immunosuppressives, and antibiotics), and 3) Underlying diseases (diabetes mellitus, pancreatitis, and pneumonia) [1,5,7,8,9].

Specimen collection and processing

Neurological patients with sepsis, septic shock, or fever > 38.5 °C who had not received antifungals before culture were enrolled in the study. Clinical specimens were obtained from blood, liquor cerebrospinal or other sterile sites, CVC, respiratory tract specimens, and urine or other non-sterile sites.

Blood specimens were obtained from the left and right arms. Eight to ten milliliters of blood were inoculated into each of two BacT/ALERT® (bioMérieux, Lyon, France). The bottles were

incubated in BacT/ALERT® 3D 60 instrument (bioMérieux, Lyon, France). Bottles flagged by the instrument as positive were removed. Bottles that were not flagged by the instrument were incubated for a total of 5 days or until reflagged by the instrument. An aliquot of the blood-broth mixture was removed from the bottle with a sterile needle and syringe. A portion was used for a Gram stain, and the remainder was sub-cultured on sheep blood agar.

Table 1. Subject characteristic related to invasive candidiasis.

Variable	Total (n = 102)
Age, years	
Mean ± SD	48.0 ± 14.9
Gender, n (%)	
Male	69 (67.7)
Female	33 (32.3)
Weight, n (%)	
Underweight	14 (13.4)
Normal	51 (50.0)
Overweight	37 (36.3)
Referral, n (%)	
Home	87 (85.3)
Hospital	15 (14.7)
Nursing home	0 (0)
Care unit, n (%)	
Non-intensive	56 (54.9)
Intensive	46 (45.1)
Length of stay, days	
Mean ± SD	16.8 ± 13.1
Length of stay, n (%)	
1–7 days	26 (25.4)
8–14 days	26 (25.5)
> 14 days	50 (49.0)
Bed rest, n (%)	42 (41.2)
Primary diagnosis, n (%)	
Haemorrhagic stroke	27 (26.5)
Cerebral infarction	21 (20.6)
Brain tumour	14 (13.7)
Nervous system malignancy	8 (7.8)
Nervous system tuberculosis	6 (5.8)
Nervous system abscess	5 (4.9)
Secondary diagnosis, n (%)	
Fever ≥ 38.5 °C	92 (90.2)
Sepsis	33 (32.4)
Septic shock	3 (2.9)
Pancreatitis	-
Pulmonary infection	67 (65.7)
Diabetes mellitus	33 (32.4)
HIV	1 (0.9)
Leukocyte ≥ 12 000/uL, n (%)	80 (78.4)
Neutropenia < 500/uL, n (%)	1 (0.9)
***Candida* spp. colonization, n (%)**	
Skin	0 (0)
Sputum	1 (0.9)
Intravascular catheter	0 (0)
Exudate	0 (0)
Urine	0 (0)
Feces	0 (0)
Medical device, n (%)	
CVC	40 (39.2)
Ventilator	49 (48.0)
Dialysis	0 (0)
Therapy, n (%)	
Antibiotics	97 (95.1)
Steroid	61 (59.8)
Chemotherapy/immunosuppressive	0 (0)
TPN, n (%)	5 (4.9)
Surgery, n (%)	
Abdominal	0 (0)
Head	35 (34.3)
Tracheostomy	28 (27.6)

SD: standard deviation; HIV: Human Immunodeficiency Virus; CVC: central venous catheter; TPN: total parenteral nutrition.

Liquor cerebrospinal was centrifuged to concentrate any microorganisms in the sediment. A small quantity of the sediment was emulsified into a drop of India ink on a microscope slide, overlaid with a coverslip, and was examined directly under a microscope for confirmation of suspicious encapsulated microorganisms. The remainder was cultured on sheep blood agar.

Each CVC had to be removed aseptically after sterilization of the skin puncture site. The distal 1-inch (2.54 cm) segment of the catheter tip was cut off while keeping it sterile and by the technique used by Maki *et al.* [10]. The catheter tip was placed on the surface of a sheep blood agar, and rolled back and forth four times with sterile forceps.

Respiratory tract specimens could be sputum or bronchial washings. A portion of sputum specimen was used for a Gram stain. The remainder was cultured on sheep blood agar. Sputum specimens were used for culture according to Murray and Washington's grading system [2].

The urine specimens were processed using calibrated loops for plating to allow for colony-forming unit per milliliter (CFU/mL) findings as well as the isolation of colonies for identification and susceptibility testing. The urine specimens were cultured onto sheep blood agar.

Direct Gram's stain of clinical material was used to determine whether a specimen is representative of the site of infection and to identify bacterial forms. The incubation time on agar was 18 to 24 hours at 35 °C. Colony morphology on the agar was observed after incubation. The colonies were selected from the plate, and identified using Gram stain.

A homogenous organism suspension was prepared by adding a sufficient number of morphologically similar colonies to sterile saline with density equivalent to a McFarland No. 1.80 to 2.20 using a calibrated VITEK® 2 DensiCHEK™ Plus. The suspension was processed using the VITEK® 2 Yeast identification card (YST) in conjunction with an automated VITEK system (bioMérieux, Lyon, France).

Definitions

The diagnosis of invasive candidiasis was made based on the classification criteria of Paphitou *et al* [5]. The definitions also followed the principles outlined in

the European Organization for Research on Treatment of Cancer Mycoses Study Group (EORTC/MSG) for use in trials of invasive mycoses of cancer patients; the recent Infectious Diseases Society of American (IDSA) guidelines for the management of intravascular catheter-related infections; and the concepts proposed by the American College of Chest Physicians Society for Critical Care Medicine (ACCP/SCCM) consensus conference for definitions of sepsis [6].

Data Analysis

The characteristics of the research subjects are presented descriptively. Analyses were performed using IBM® SPSS® Statistics version 20. Normally distributed data were reported as means and standard deviation (SD), while abnormally distributed data were reported as medians and ranges. Chi square analysis was used to assess the difference between demographic variables and invasive candidiasis and non-invasive candidiasis. Bivariate analysis was performed to identify risk factors for invasive candidiasis.

Ethics statement

The study has received ethical approval from the Research Ethics Committee of the National Brain Center Hospital Number UM.01.05/12/029/2020.

Results

During the study period, there were 110 adult hospitalized patients with neurological diseases, and sepsis, septic shock, or fever > 38.5 °C. Eight patients were excluded from the analysis because they had received antifungal therapy. The number of subjects who met the study criteria was 102.

The mean age was 48 ± 14.9 years. There were 46 (45.1%) intensive and 56 (54.9%) non-intensive care patients. The mean length of hospital stay was 16.8 ± 13.1 days. Subject characteristics are provided in Table 1.

Invasive candidiasis developed in 14 (13.7%) subjects, who included 9 (64.3%) males and 5 (35.7%) females. Of the 102 subjects, there were 5 subjects with positive *Candida* sp blood culture and 9 subjects with positive *Candida* sp cerebrospinal fluid culture. Thus, the prevalence of invasive candidiasis in our study was 14/102 (13.7%). All of the isolates were *C. parapsilosis*. The percentage of subjects who met the classification of invasive candidiasis according to the criteria of Paphitou *et al.* [5] based on EORTC/MSG, are listed in Table 2.

Bivariate analysis determined that some of the risk factors were significantly different between the invasive and non-invasive candidiasis groups. Using the significance level of $p < 0.25$ in bivariate analysis, the risk factors for invasive candidiasis in our study were septic shock, use of CVC, duration of use of CVC < 24 hours, duration of use of ventilators < 48 hours, duration of steroid use 1-7 days, duration of steroid use > 14-21 days, duration of steroid use > 28 days, head surgery, and tracheostomy. The risk factors of invasive candidiasis are listed in Table 3.

Discussion

Characteristics of research subjects

A total of 102 individuals were studied, the majority of whom were male (67.7%).

Table 2. Percentage of invasive candidiasis (according to the classification criteria of Paphitou *et al*. [5])

Classification	Criteria	Subject n (%)
I. Proven	.Candidemia with clinical symptoms suggestive of infection	5 (4.9)
	.*Candida* spp. from sterile sites other than (a) urine or (b) peritoneal fluid in setting of GI perforation	9 (8.8)
II. Probable	.Candidemia without clinical symptoms suggestive of infection	-
	.*Candida* spp. from ≥ 2 non-sterile sites, plus fever ≥ 38.5 °C, plus WBC ≥ 12 000/uL, plus no bacterial pathogens at any other site	-
	.*Candida* spp. from urine > 100 000 CFU/mL, plus fever ≥ 38.5 °C, plus WBC ≥ 12 000/uL, plus no bacterial pathogens in the urine or any other site	-
	.*Candida* spp. from central venous catheter (CVC) tip at ≥ 15 CFU, plus fever ≥ 38.5 °C or WBC ≥ 12000/uL, plus no bacterial pathogens at this or any other site	-
	.Empiric treatment with systemic antifungals, initiated due to persistent fever ≥ 38.5 °C or WBC ≥ 12000/uL despite broad spectrum antibiotics and:	
	A. *Candida* spp. from ≥ 1 non-sterile site or from a CVC tip at < 15 CFU, plus no bacterial pathogens at the non-sterile site or any other site	1 (0.9)
	B. *Candida* spp. from ≥ 2 of any combination of non-sterile sites or CVC tips at < 15 CFU	-
III. Possible	.*Candida* spp. from a CVC tip at > 15 CFU, but not satisfying all criteria of II.iv	-
	.Empiric treatment with systemic antifungals initiated due to persistent fever ≥ 38.5 °C or WBC ≥ 12000/uL despite broadspectrum antibiotics but not meeting the criteria of II.v.a or II.v.b	87 (85.4)

CFU: colony forming unit; CVC: central venous catheters; GI: gastrointestinal.

Table 3. Risk factors of invasive candidiasis.

Variable	Total n, (%)		Invasive candidiasis n, (%)		Non invasive candidiasis n, (%)		*p* value	OR (95% CI)
Age, years	**48.0 ± 14.9**		**46.69 ± 10.0**		**50.5 (18–75)**		**0.506**	
Gender								
Male	69	(67.6)	9	(64.3)	60	(68.2)		
Female	33	(32.4)	5	(35.7)	28	(31.8)	0.772	1.2 (0.3–3.9)
Weight, n (%)								
Underweight	14	(13.7)	2	(14.2)	12	(13.6)	0.800	0.8 (0.1–4.5)
Normal	51	(50.0)	6	(42.9)	45	(51.2)		
Overweight	37	(36.3)	6	(42.9)	31	(35.2)	0.550	1.4 (0.4–4.9)
Referral, n (%)								
Home	89	(85.6)	12	(85.7)	77	(85.6)	0.962	1.04 (0.2–5.2)
Hospital	15	(14.4)	2	(14.3)	13	(14.4)		
Nursing home	0	(0)	0	(0)	0	(0)	-	-
Care unit, n (%)								
Non-intensive	56	(54.9)	6	(42.9)	50	(56.8)		
Intensive	46	(45.1)	8	(57.1)	38	(43.2)	0.334	1.8 (0.6–5.5)
Length of stay, days								
< 3	14	(13.7)	0	(0)	14	(15.9)		
4–6	9	(8.8)	0	(0)	9	(10.2)	0.999	-
> 7–13	27	(26.5)	4	(28.6)	23	(26.2)	0.999	-
> 14	52	(51.0)	10	(71.4)	42	(47.7)	0.627	0.7 (0.2–2.6)
Bed rest	42	(41.2)	6	(42.9)	36	(40.9)	0.891	1.1 (0.4–3.4)
Length of bed rest, days			**45 (47)**		**20 (83)**			
No bed rest	60	(58.8)	8	(57.1)	52	(59.1)		
1–6	5	(4.9)	0	(0)	5	(5.7)	0.532	0.7 (0.2–2.3)
> 7–13	10	(9.8)	1	(7.2)	9	(10.2)	0.999	-
> 14	27	(26.5)	5	(35.7)	22	(25.0)	0.539	0.5 (0.1–4.8)
Diagnosis								
Fever	92	(90.2)	12	(85.7)	80	(90.9)	0.547	0.6 (0.1–3.2)
Sepsis	33	(32.4)	6	(42.9)	27	(30.7)	0.369	1.7 (0.5–5.4)
Septic shock	3	(2.9)	2	(14.3)	1	(1.1)	0.034*	14.5 (1.2–172.3)
Pancreatitis	0	(0)	0	(0)	0	(0)	-	-
Pulmonary infection	67	(65.7)	11	(78.6)	56	(63.6)	0.282	2.1 (0.5–8.1)
Malignancy	8	(7.8)	1	(7.2)	7	(7.9)	0.916	0.9 (0.1–7.8)
Diabetes Mellitus	33	(32.4)	5	(35.7)	28	(31.8)	0.772	1.2 (0.4–3.9)
HIV	1	(1.0)	0	(0)	1	(1.1)	-	-
Leukocyte, uL	**18 404 ± 10 704**		**16 071 ± 11 132**		**18 775 ± 11 132**		0.420	-
Leukocyte ≥ 12000 uL	80	(78.4)	9	(64.3)	71	(80.7)	0.174	0.4 (0.1–1.5)
Neutrofil, uL	**15 278 ± 9 981**		**13 029 ± 6317**		**15 636 ± 10 429**		0.907	-
Neutropenia < 500/uL	1	(1.0)	1	(7.14)	0	(0)	1.000	-
***Candida* spp. colonization**								
Skin	0	(0)	0	(0)	0	(0)	-	-
Sputum	1	(1.0)	0	(0)	1	(1.1)	1.000	-
Intravascular catheter	0	(0)	0	(0)	0	(0)	-	-
Exudate	0	(0)	0	(0)	0	(0)	-	-
Urine	0	(0)	0	(0)	0	(0)	-	-
Feces	0	(0)	0	(0)	0	(0)	-	-
Medical device								
CVC	40	(39.2)	10	(71.4)	30	(34.1)	0.013*	4.8 (1.4–18.7)
Ventilator	49	(48.0)	10	(71.4)	39	(44.3)	0.069	3.1 (0.9–10.8)
Dialysis	0	(0)	0	(0)	0	(0)	-	-
Length of CVC								
No CVC	47	(46.1)	1	(7.2)	46	(52.3)		-
< 24 hours	9	(8.8)	2	(14.2)	7	(7.9)	0.014	0.6 (0.007–0.6)
> 24–48 hours	6	(5.9)	1	(7.2)	5	(5.8)	0.815	0.8 (0.1–5.2)
> 48- < 72 hours	1	(1.0)	0	(0)	1	(1.1)	0.633	0.6 (0.05–6.0)
3–4 days	19	(18.6)	5	(35.7)	14	(15.9)	1.000	-
4–5 days	1	(1.0)	0	(0)	1	(1.1)	1.000	1.0 (0.2–4.2)
5–6 days	0	(0)	0	(0)	0	-	-	-
> 6 days	19	(18.6)	5	(35.7)	14	(15.9)	1.000	-

(continues on next page)

This is consistent with observations of Paphitou *et al*. (68.0%) [5], Leon *et al.* (67.3%) [8], Leroy *et al.* (75.5%) [9], Umberger *et al*. (94.8%) [11], Ahmed *et al.* (63, 0%) [12], Dash *et al.* (62.9%) [13], Mermutluoglu *et al*. (63.1%) [14], Moreira *et al.* (58.8%) [15], and Singh *et al.* (52.7%) [16]. The mean age of the subjects was 48.0 ± 14.9 years. No significant differences between age and gender indicated no influence of age and gender on the occurrence of invasive candidiasis.

Most of the diagnoses were stroke, either hemorrhagic or infarction, in line with the data of the 10 inpatients at this hospital. Secondary diagnosis is a diagnosis that accompanies the primary diagnosis at the time of patient care, including comorbidities or complications associated with invasive candidiasis. Secondary diagnoses that were most frequently associated with invasive candidiasis in our study were pulmonary infection, diabetes mellitus, malignancy, septic shock, and human immunodeficiency virus (HIV).

In addition, 13.4% of the subjects were underweight. A risk factor for the incidence of invasive candidiasis is malnutrition, which can lead to immunosuppression. The data on the use of TPN in subjects was 4.9%, indicating that the nutritional status of the subjects was normal. Use of TPN leads to the development of mucosal atrophy and loss of mucosal epithelial barrier function, which might affect the relationship between microorganisms in the gut and the possibility of gaining access to blood vessels [17,18]. TPN also has a profound effect in the gastrointestinal microbiome [18].

The majority of the subjects were patients who came directly, and there were no referral patients from the nursing home. In contrast the study by Guillamet *et al*. [7], found that 20.2% of the subjects were referred by the nursing homes and were elderly with a mean age of 61.4 ± 16 years [7]. In western countries, nursing homes are the facilities of choice for the elderly because they allow patients to remain independent without having to depend on children, while in Asia, including Indonesia, most elderly live at their homes.

Candida colonization of central venous catheter (CVC) might provide a route for entering into the bloodstream. Candida catheter-related bloodstream infections grow quickly, possibly because patients with catheter-related infection have a higher inoculum [18]. The use of CVC and ventilators in our study was lower than most of the previous studies, because at the time of culture examination, most of the subjects were non-intensive inpatients (54.9%) [6,8]. This shows that the

Table 3 (*continued*). Risk factors of invasive candidiasis.

Length of ventilator, hours								
No ventilator	53	(52.0)	4	(28.6)	49	(55.7)		
< 48	12	(11.8)	1	(7.1)	11	(12.5)	0.013*	0.2 (0.1–0.7)
48–96	8	(7.8)	1	(7.1)	7	(7.9)	0.159	0.2 (0.02–1.9)
96- < 144	3	(2.9)	0	(0)	3	(3.4)	0.324	0.3 (0.03–3.1)
> 144	26	(25.5)	8	(57.1)	18	(20.5)	0.999	-
Therapy								
Antibiotic	97	(95.1)	13	(92.9)	84	(95.5)	0.679	0.6 (0.1–5.9)
Steroid	73	(71.6)	14	(100)	59	(67.5)	0.344	0.6 (0.2–1.9)
Chemotherapy	0	(0)	0	(0)	0	(0)	-	-
Length of antibiotics, days			**19.69 ± 14.52**		**13.43 ± 12.32**			
No drugs	4	(3.9)	0	(0)	4	(4.5)		
1–5	28	(27.5)	1	(7.2)	27	(30.7)	0.999	-
6–13	29	(28.4)	5	(35.7)	24	(27.3)	0.085	0.2 (0.02–1.3)
> 14	41	(40.2)	8	(57.1)	33	(37.5)	0.810	0.9 (0.3–2.9)
Length of steroid, days			**19.853**		**14.24 ± 14.26**			
No steroid	38	(37.3)	2	(14.4)	36	(40.9)		
1–7	25	(24.5)	4	(28.6)	21	(23.9)	0.175	3.4 (0.6–20.3)
> 7–14	13	(12.7)	1	(7.1)	12	(13.6)	0.766	1.5 (0.1–17.6)
> 14–21	8	(7.8)	3	(21.4)	5	(5.7)	0.021*	10.8 (1.4-81.3)
> 21–28	6	(5.9)	1	(7.1)	5	(5.7)	0.330	3.6 (0.3–47.3)
> 28	12	(11.8)	3	(21.4)	9	(10.2)	0.069	6.0 (0.9–41.4)
TPN	5	(4.9)	0	(0)	5	(5.7)	0.999	-
Surgery								
Abdominal	0	(0)	0	(0)	0	(0)	-	-
Head	35	(34.3)	8	(57.1)	27	(30.7)	0.061	3.0 (0.9–9.6)
VP *shunt*	15	(14.7)	3	(21.4)	12	(13.6)	0.449	1.8 (0.4–7.1)
Tracheostomy	28	(27.5)	9	(8.8)	19	(18.6)	0.002*	6.5 (1.9–21.8)

* *p* value ≥ 0.05; OR: odds ratio; CI: confidence interval; HIV: Human Immunodeficiency Virus; CVC: central venous catheter; TPN: total parenteral nutrition; VP shunt: Ventriculoperitoneal shunt.

level of *Candida* sp infection in the intensive care unit is not different from that in the non-intensive care unit.

The length of stay before culture in our study was longer than that of Paphitou *et al.* (13.0 days) [5]. The length of stay before culture was 16.8 days in majority of the cases. This indicates that neurological patients have a longer length of stay, and that there was the possibility of bed rest. Bed rest was found in 41.2% of the subjects, thus increasing the likelihood of pulmonary infection. Most of the studies showed that pulmonary infection occurs due to aspiration in addition to dysfunction of the swallowing mechanism due to neurological damage [19]. This also caused the incidence of invasive candidiasis in tracheostomy subjects in our study to be higher than that of Singh *et al*. (4.1%) [16]. The risk of invasive candidiasis in tracheostomy occurs because in tracheostomy there can be a disturbance in the integrity of the mucosa, which becomes the pathway for *Candida* sp to access the bloodstream from the lumen [18].

Colonization data was obtained based on examination of specimens from the skin (axilla), tracheal aspiration (or brush, bronchoalveolar lavage), vascular catheter, wound or drainage exudate, urine, feces (or rectal swab), or other infected areas. Ahmed *et al.* [12] performed examinations on admission, day three, and thereafter weekly. In contrast to most other studies, colonization was only found in sputum in our case [11,12]. This is because the examination for colonization in our study was not an active surveillance examination.

Neutropenia < 500/uL was found in only 0.98% of the subjects. Neutropenia is a major problem in most immunocompromised patients. Neutropenia at > 7 days is a high risk for bacterial and viral infections. Invasive candidiasis is more common during neutropenia or > 2 weeks after neutropenia [17]. Neutropenia can occur in malignant patients receiving chemotherapy. In our study, although subjects with nervous system malignancies were present, there was no treatment with chemotherapy.

Antibiotics were used in the majority (95.1%) of study subjects, higher than those of Hermsen *et al.* (84.7%) [1], Leon *et al.* 14 (88.6%) [8], and Ostrosky-Zeichner *et al.* (75.0%) [20]. Antibiotic therapy was higher than other studies because fever in neurological patients can be caused by infectious and non-infectious factors, including neurogenic factors. Therefore, the management of fever began with the treatment of infection and other symptoms that could be treated. The possibility of neurogenic fever was considered when no microbial and fungal source was found [21]. Antibiotics affect the microbiota and can cause dysfunction of epithelial tissue, especially gastrointestinal tissue. Several studies have shown the effect of antibiotics on increased colonization. Antibiotics also have an effect over gut microbiota, and some studies have had impact from antibiotics with anti-anaerobic effect, and those with higher gastrointestinal concentration. They contribute to the observed increased colonization over time that is observed in patients. With continued antibiotic effect, there is a net decrease in the number of species in the gastrointestinal tract, an increase in the number of patients colonized, and the proportion of them being heavily colonized [18].

Steroids were used in 59.8% of study subjects, similar to Umberger *et al.* (50.7%) [11], and Gaspar *et al*. (57.0%) [22]. The use of steroids causes immunosuppression which was a risk factor of invasive candidiasis [17].

A major surgery that was found to be a risk factor for invasive candidiasis in several studies is the abdominal surgery. In abdominal surgery, gastrointestinal manipulation may occur, and this may lead to invasive candidiasis. Abdominal surgery is not performed at the National Brain Center Hospital Jakarta because it is a tertiary hospital for neurological diseases. The major surgeries that may be performed at this hospital are head surgery and ventriculoperitoneal (VP) shunt. Infection is a complication of a VP shunt. In our study, there was 2.9% incidence of invasive candidiasis in VP shunt, higher than that observed by Bradakar *et al.* [23].

Prevalence of invasive candidiasis

Based on the calculation of percentage of subjects who had *Candida* spp growing culture divided by the total number of subjects tested, the prevalence of invasive candidiasis in our study was 14/102 (13.7%), higher than Singh *et al.* (5.8%) [16]. Our study included all of neurological patients with sepsis, septic shock, or fever > 38.5 °C who had not received antifungals before culture, whereas Singh *et al*. [16] study only took a population of patients with HIV.

Based on our study, although the prevalence of invasive candidiasis at the Mahar Mardjono National Brain Center Hospital was only 13.7%, the ratio of the "proven" with "probable" and "possible" invasive candidiasis was high (14/83, 16.8%). Thus, further management is needed for this matter. Invasive candidiasis has non-specific signs and symptoms and a positive culture result may be obtained in late infection. Histopathological diagnosis is difficult because it requires an invasive procedure to collect specimens of

organs or fluids, whereas radiological examination is not specific for a particular pathogen [24].

There were 14 (13.7%) "proven", 1 (0.9%) "probable", and 87 (85.4%) "possible" invasive candidiasis subjects. All of the isolates were *C. parapsilosis*. *C. parapsilosis* is a nosocomial infection that grows on biofilms in CVC, implanted devices, and TPN [3]. The increase in the frequency of *C. parapsilosis* infections has been attributed to a variety of risk factors, including the organism's selective growth capabilities in TPN and its affinity for intravascular devices and prosthetic materials. Immunocompromised individuals such as HIV patients and surgical patients, are at high risk for infection with *C. parapsilosis*. Patients requiring prolonged use of a central venous catheter or indwelling device, are at increased risk for infection with *C. parapsilosis* [25].

The number of cases of "proven" invasive candidiasis in our study were higher than Paphitou *et al.* [5] and Ahmed *et al*. [12], who included only intensive care patients in their study. This suggests that the incidence of invasive candidiasis in non-intensive care unit is as likely as in the intensive care unit.

The majority of clinical manifestations of invasive candidiasis in our study were disseminated candidiasis, similar to that of Paphitou *et al.* [6]. In contrast to the study of Ahmed *et al.* [12], "proven" invasive candidiasis subjects consisted of 6.6% blood manifestations, 0.5% intraabdominal manifestations, and 1.5% concurrent blood and intraabdominal manifestations. This suggests that blood manifestations are not the most common clinical manifestation of invasive candidiasis in neurological patients. No subject showed concurrent blood and other organs manifestations, because fungal infections of the nervous system can occur without preceded blood manifestation [26].

In our study, positive cultures for *Candida* sp of "probable" invasive candidiasis were only obtained from sputum in contrast to most other studies [11,12,27]. This is probably because our colonization examination is not an active surveillance examination. Active surveillances were conducted twice a week with specimens of oropharyngeal secretions, tracheal secretions, gastric fluid, perineum, feces, urine, surgical wounds, abdominal drains, and catheter insertion areas [28]. Colonization was obtained if *Candida* sp positive culture was obtained from > 3 specimens taken from > 1 area at 2 consecutive screenings [29]. Ahmed *et al*. [12] performed blood culture on admission, the third day, and thereafter weekly.

The "possible" invasive candidiasis in our study was high because we used the invasive candidiasis criteria developed by Paphitou *et al.* [6], which can be seen in Table 2. The EORTC/MSG criteria were initially invasive candidiasis criteria for malignant patients, which consisted of host factors and clinical criteria. The invasive candidiasis criteria of Paphitou *et al*. follows the criteria for EORTC/MSG invasive candidiasis, and also considers the management of candidiasis and intravascular catheters infection according to the Infectious Diseases Society of America (IDSA) guidelines, as well as the sepsis consensus of the American College of Chest Physicians Society for Critical Care Medicine (ACCP/SCCM). Besides, fever in neurological patients can be caused by infectious and non-infectious factors, including neurogenic factors. Diffuse axon and frontal lobe dysfunction can cause central fever. Management of fever begins with treatment of infection or other treatable causes. The possibility of neurogenic fever is considered if no microbial source is found, including ruling out fungal infection [29]. Therefore, in our study, it was found that there were more subjects with fever or persistent leukocytosis even though they were given broad-spectrum antibiotics.

Risk factors of invasive candidiasis

The risk factors for invasive candidiasis identified in our study were septic shock, use of CVC, duration of use of CVC < 24 hours before, duration of use of a ventilator < 48 hours before, duration of use of steroids > 14-21 days before, and tracheostomy. The incidence of invasive candidiasis in septic shock increased 14.5 times compared to without septic shock. In patients with septic shock, the likelihood of hospital stay is prolonged and requires vascular equipment or other catheters [23]. Leon *et al.* [8] included sepsis in the risk assessment of the *Candida* score.

The use of CVC increased the risk of invasive candidiasis 4.8 times compared to without using CVC. Hermsen *et al*. [1] and Ostrosky-Zaichner *et al.* [20] included the use of CVC in days 1 to 3. In our study the duration of use of CVC for < 24 hours reduced the risk of invasive candidiasis by 60%. Use of a ventilator for < 48 hours reduced the risk of invasive candidiasis by 20% compared to those without a ventilator. Ostrosky-Zaichner *et al.* [20] included at least 48 hours of ventilator use in the risk assessment.

Duration of steroid use for > 14-21 days increased the risk of invasive candidiasis 10.8 times compared to that without using steroids. In Ostrosky-Zeichner *et al.* [20] study, steroid use from 7 days before to day 3 was

a risk factor, whereas Hermsen *et al.* [1] included steroid use 7 days before ICU admission to day 3.

Leon *et al.* [8] and Ostrosky-Zeichner *et al.* [20] included surgery, whereas Hermsen *et al.* [1] included abdominal surgery 7 days before to day 3. In our study, the major surgery performed was head surgery, including VP shunt, but had not been proven to be a risk factor of invasive candidiasis. A significant surgical risk factor for the incidence of invasive candidiasis was tracheostomy which increased the risk 6.5 times compared to without tracheostomy.

Conclusions

The prevalence of invasive candidiasis was considered to be high in the neurological patients in Indonesia. Despite characteristics such as old age, underweight, and longer length of stay, the infection might be influenced by septic shock, duration of use of CVC, duration of use of the ventilator, duration of use of steroids, and tracheostomy. Further research is needed on the early diagnosis of invasive candidiasis in neurological patients given the risk factors for invasive candidiasis. Such information can be useful to neurological patient management.

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