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Abstract:

Blood flow infections in HIV/AIDS patients are a major cause of illness and death. Diagnosis of bloodstream infections is mainly established through blood cultures. This study aimed to determine the profile of bacteremia and fungemia in HIV / AIDS patients with sepsis treated at the Intermediate and Infectious Care Unit of Dr. Soetomo General Hospital, Surabaya. Aerobic and fungal blood cultures were examined for HIV/AIDS patients with sepsis using an aerobic bactec bottle and bacterial identification using a BD Phoenix semiautomatic machine, with the identification of fungus by using the semiautomatic machine Vitek 2 compact (BioMerieux). Out of the 51 patients with sepsis which underwent blood cultures as positive blood culture was obtained in eight patients (16% proportion). Bacteria obtained included Staphylococcus aureus, Methicillin-resistant Staphylococcus aureus, Escherichia coli, Klebsiella oxytoca, Acinetobacter baumannii, Non-Typhoid Salmonella, and Micrococcus luteus. The fungus was Cryptococcus spp.

#### 1 INTRODUCTION

AIDS (Acquired immunodeficiency syndrome) patients have a characteristic decrease of CD4 + T lymphocyte cell count, B lymphocyte deficiency, macrophages and other polymorphonuclear cells. Some of these complex immune system abnormalities increase the tendency of HIV patients to have co-infection with opportunistic pathogens (parasites, fungi, and viruses) as well as bacterial agents (Rosas, 2003; Tumbarello, 1995; Nasronudin, 2014).

HIV infection can be affected by a variety of factors: 1. facility of infection exposure; 2. incidence of infection due to an increased ratio of infective microorganisms; 3. changes and shifts in disease patterns; or (4) exacerbations of pre-existing infectious diseases. The potential for opportunistic infections is facilitated by the immunodeficiency underlying HIV infection (Nasronudin, 2014).

Bloodstream infections (BSI) in HIV patients are associated with increased mortality. BSI is a major cause of morbidity in patients with HIV infection. Several studies conducted in several countries with criteria for the recruitment of HIV patients with fever, showed a wide range of BSI prevalence

ranging from 10% to 63% (Peters, 2004; Archibald, 1999; Arthur, 2001; Gilks, 1990).

Hospitalized AIDS patients might also experience a condition of bloodstream infections due to complications from invasive procedures such as the use of urine catheters (Tumbarello, 1995; Petrosillo, 2002).

Information on pathogens associated with bloodstream infections in HIV/AIDS patients admitted to Dr. Soetomo General Hospital does not exist. For this reason, the authors were interested in researching the profile of opportunistic infections of blood flow in HIV/AIDS inpatients (Surabaya, Indonesia).

#### 2 METHODS

This study was descriptive observational to determine the profile of opportunistic infections of blood flow in HIV/AIDS patients. The design of this research was a cross-sectional study. This research was conducted in the Intermediate and Infectious Care Unit and Installation of Clinical Microbiology of the Integrated Diagnostic Center Building at Dr.

Soetomo General Hospital, Surabaya. The data were taken from March to June 2017. The study sample was HIV/AIDS patients with a positive result from 3 methods and had clinical symptoms of SIRS (Systemic Inflammatory Response Syndrome) meeting the inclusion criteria (male and female patients over 21 years of age, and with clinically suspected opportunistic infections of blood flow (sepsis)). There were 51 HIV/AIDS patients who met the criteria of suspected bloodstream infections and underwent two-sided blood cultures. The instruments used in this research were BACTEC engine, incubator, BD Phoenix semiautomatic machine, Vitek 2 Compact (Bio Merieux) semiautomatic machine, biosafety cabinet 2 (BSC 2), light microscope and digital camera for documentation. This study was approved ethically by the Research Ethics Committee of Dr. Soetomo General Hospital, Surabaya.

#### 3 RESULTS

The total number of samples were 51 patients (Table 1). Samples of the study that had not received ARV was 32 (62.75%) patients while 19 (37.25%) patients had previously received ARV. Only 31 patients had CD4 + lymphocyte count data. Out of these 31 patients, 90% had a CD4 + T cell lymphocyte count < 100 cells /  $\mu$ l, 3.33% had a CD4 + T cell lymphocyte count > 100 cells /  $\mu$ l, 3.33% had a CD4 + T cell lymphocyte count > 200 cells /  $\mu$ l, and 3.33% had arithmetic lymphocytes T CD4 + > 300 cells /  $\mu$ l. There were unknown virus load values for all patients due to no routine virus load examination.

Fifty-one patients with sepsis clinical symptoms underwent double-sided blood cultures with asepsis and closed-system action, and there were 11 patients with a positive blood culture. Three of the 11 patients with positive blood cultures were found to have bacteria that were general contaminants (coagulase-negative Staphylococcus (CONS)), and being only positive on one side showed less significance as a cause of sepsis. Other bacteria found in eight patients with other positive blood cultures were Staphylococcus aureus, Escherichia coli, Klebsiella oxytoca, Acinetobacter baumannii, Non-Typhoid Salmonella, and Micrococcus luteus with the mean time to positivity (TTP) of 15.3 hours. Cryptococcus fungus was found in two patients. A significant proportion of positive blood cultures was 16% (8 patients out of a total of 51 patients with sepsis symptoms).

The eight patients with a positive blood culture were men with an average age of 40.5 years. Three out of eight had received antiretroviral therapy, but not routinely. Five of the eight patients had diagnoses associated with lower respiratory tract infections, while three of the eight patients had gastrointestinal-related diagnoses. One of seven patients was found to be polymicrobial in both blood cultures. Two of seven patients with a positive blood culture died.

Table 1: Patients characteristics.

	Amount (Patient)	%
Age range (year)	(Fatient)	
21-30	18	35.3
21-40	18	35.3
41-50	8	15.7
51-60	5	9.8
61-70	1	4
>71	1	4
Degree (°C)		
<36	13	25.5
36-38	20	39.2
>38	18	35.3
Heart beat	//	
(x/Minute)		
<90	8	15.7
>90	43	84.3
Breath (x/minute)	7	
<20	10	19.6
>20	41	80.4
Leukocytes (103/µ	ıl)	
<4.00	13	25.5
4.00-12.00	34	66.7
>12.00	4	7.8
Neutrophils (%)		
<70.5	8	15.7
>70.5	43	84.3

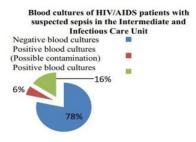


Figure 1: Blood cultures of HIV/AIDS patients with clinical sepsis.

T-test independent of positive and negative blood culture groups was observed for age, length of the day of care, heart rate, count of breath, temperature, count of leucocytes and neutrophils. There were no significant differences in all of these aspects in the positive and negative blood culture groups.

Table 2: Bacterial sensitivity pattern obtained from positive blood cultures of HIV/AIDS patients.

Organism	Cn	Amp	Sam	Caz	Ctx	Cro	Sxt	Lev	Cip	Mem	VA
Staphylococcus Aureus			R	_ 3463	- //-		S	S		R	S
Methicillin Resistant											
Staphylococcus		15									
Aureus	S	R	S				S R	S S			
Micrococcus	R						R	S			S
Luteus	8										
Non Typhoid	R	S	S	S	S	S	S			S	
Salmonella											
Klebsiella	R	R	S	R	R	R	S	S	S		
Oxytoca											
Acinetobacter	R	R	R	R	R	R	R	R	R	R	
Baumannii		13									
Eschericia Coli	S	R	S	S	S		R	S	S	S	
Eschericia Coli	R	R	R	R	R	R	R	R	R	S	
ESBL											

Noted: CN: gentamycin; AMP: ampicillin; SAM: ampicillin sulbactam; CAZ: ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; SXT: cotrimoxazol; LEV: levofloxacin; CIP: ciprofloxacin; MEM: meropenem; VA: vancomycin.

#### 4 DISCUSSION

HIV/AIDS is associated with an increased risk of bacterial and fungal infections due to damaged cellular, humoral and mucosal immunity with both qualitative and quantitative neutrophil damage (Declercq, 2015). Blood flow infections in HIV/AIDS patients are 7 more common and associated with increased morbidity and mortality than in patients without HIV/AIDS (Huson, 2014; Declercq, 2015). Blood flow infections in HIV/AIDS patients mainly occur in patients who have reached the AIDS stage (Declercq, 2015).

A study conducted in the Intermediate Nursing Unit of Infectious Diseases of Dr. Soetomo found a proportion of positive blood cultures in sepsis patients of 16%. Research conducted in Lagos Nigeria found a prevalence of bloodstream infections in HIV/AIDS patients of 12.9% (Adesida, 2017). A higher prevalence was found by a study conducted by Oluyege in Nigeria with a prevalence of 22.9% HIV/AIDS patient blood flow infections. In a study conducted in Malawi, the prevalence of HIV/AIDS patient blood flow infections was 30% (Bonadio, 1998).

In this study, three patients with positive CONS blood culture on one side showed less significance as the infecting agent. Blood cultures taken in this study used a closed system to minimize the acquisition of contaminant germs. The presence of three blood cultures with a CONS result and positive on one side only (indicating comorbidity) may be due to a lack of disinfection when taking blood with a closed system at that time.

In this study, the HIV/AIDS patients with positive blood cultures were all male. This is similar to other studies that show men are more commonly affected by bloodstream infections than women (Bryce, 2014; Afessa, 2001; Haddy, 2012; Kiertiburanakul, 2012; Declercq, 2015; Buchacz, 2016). However, it is different from the studies performed by Oluyege in Nigeria, where the prevalence of female HIV / AIDS patients was higher than in men (Oluyege, 2015).

In this study, five out of eight patients with positive blood cultures had symptoms of lower respiratory tract infections (lung), resembling other studies (Afessa, 2001; Bryce, 2014; Declercq, 2015). In addition to the lungs, sources of bloodstream infections in HIV/AIDS patients included skin, subcutaneous tissues and intravenous catheters (Afessa, 2001).

We found one case of a patient with polymicrobial blood culture results. The study conducted by Haddy et al. showed about 14.5% sepsis due to polymicrobial and mortality of

HIV/AIDS patients with polymicrobial-induced sepsis was 25%, whereas due to monomicrobial it was 8.5%. The mortality of non-HIV/AIDS septic patients with polymicrobial was 45%, whereas the mortality of sepsis non-HIV/AIDS patients with monomicrobial was 18% (Kiani, 1979). Mortality being not significantly different in polymicrobial or monomicrobial in HIV/AIDS patients is possibly due to the severe painful condition of HIV/AIDS patients, resulting in less significant amounts of bacteria in the blood as a cause of death. Septicemia whether monomicrobial or polymicrobial is a marker of severity of HIV/AIDS patients (Haddy, 2012).

The types of organisms obtained in this study were Escherichia coli (19%), Non-typhoidal Salmonella (12%), Acinetobacter baumannii (12%), Klebsiella oxytoca (6%), Staphylococcus aureus (13%), Coagulase-negative Staphylococcus (13%), Micrococcus luteus (6%), and Cryptococcus spp (19%). The pattern of the organism causing blood flow infections of HIV/AIDS patients differs depending on the geographical location (Huson, 2014).

In general, the main pathogens around the world are NTS, Streptococcus pneumoniae, Eschericia coli, and Staphylococcus aureus; however, there are different frequencies between regions (Taramasso, 2016). Mycobacteria and fungal (most commonly Cryptococcus) are also important pathogens that cause bloodstream infections. Various studies in Asia suggest a small gain in Streptococcus pneumoniae (0.8%), and high NTS (47.1%) infection in bloodstream infections of HIV/AIDS patients (Archibald, 1999; Hung, 1998; Kiertiburanakul, 2012; Mootsikapun, 2007; Phe, 2013).

Staphylococcus aureus is also an important pathogen found quite frequently in Asia (14.1%), Europe and the United States (19.6%), but only occasionally found in Africa (3.8%) (Huson, 2014). Intravenous catheter infections, skin tissue infections, and endocarditis are significantly associated with Staphylococcus aureus. In a multicenter study performed by the Center for Diseases Control (CDC), Staphylococcus aureus accounted for 35% of the nosocomial causes of bloodstream infections (Stroud, 1997). The frequency of staphylococcal bacteremia in HIV/AIDS patients was 16.5 times greater than for patients without HIV/AIDS (Adesida, 2017). This study recorded 13% of staphylococcal bacteremia.

Methicillin-resistant Staphylococcus aureus (MRSA) increases the probability of occurrence of endocarditis and death when compared to other

Staphylococcus aureus strains (Furuno et al., 2011). Increased incidence of MRSA is due to increased use of intravenous drug use (IVDU). IVDU, hemodialysis and CD4 + T cell lymphocyte count < 200 cells/µl are risk factors for MRSA (Burkey, 2008)

The low frequency of Streptococcus pneumoniae as a cause of bloodstream infections in Asia HIV/AIDS patients might be because these patients have previously received antibiotics before going to a health facility, or purely the geographic distribution differences of pathogens (Deen, 2012).

The immune response due to NTS infection in patients with or without HIV is very different. Normally, Th17 cells secrete interleukin-17 which works effectively to inhibit NTS invasion of the intestine. HIV infection results in reduced Th17 cells and decreased interleukin concentration 17 thus disrupting the function of bowel mucosal defense against NTS. If NTS enters the patient's bloodstream without HIV, IgG antibodies bind to the outer membrane protein NTS and allow complement attachment (C1q), followed by the formation of MAC (membrane attack complex) on the bacterial surface and causes bacteria to lyse and die. In contrast to HIV patients, NTS bacteria are bound by antibodies that result in the persistence of NTS in the blood. Antibodies are not attached to NTS outer proteins, but on NTS lipopolysaccharides, so MACs and bacteria do not die (Huson, 2014; Palardini,

HIV/AIDS patients have very high numbers of harmful bacteria which lead to deterioration of their immune systems. HIV/AIDS patients have high levels of salmonella, Escherichia, Shigella and staphylococcus bacteria in their digestive tract. These bacteria possibly lead to food poisoning in HIV/AIDS patients more than in people without HIV/AIDS but is not serious. Symptoms of food poisoning include demand, diarrhea, abdominal pain, and dehydration. The researchers believe that the high number of harmful bacteria in the digestive tract of HIV/AIDS patients attacks the patient's immune system so that the immune system does not function (HIV resource for health professionals, 2013). GI-related infections were found in 42.85% of patients in the study.

This study found cryptococcus fungus on the patient's blood culture. However, there was no fungus other than that. Cryptococcus spp and Candida spp are the most commonly found fungal species (Garbino, 2001). In a retrospective study conducted from 2004-2008 in Thailand, Cryptococcus neoformans was the most commonly

isolated pathogen (20.8% of all cases). A total of 140 cases had 35 cases of bloodstream infections due to fungi (25%). Predicting factors of blood flow infections due to fungus are old age, local area infection, renal insufficiency, high HIV-RNA viral load and low CD4 + T lymphocyte count (Kiertiburanakul et al., 2012). The absence of Candidemia in this study was similar to that of the prevalence of candidemia in the study by Garbino et al., where the mushrooms found were Penicillium marneffei, Histoplasma capsulatum, and Cryptococcus neoformans (Garbino, 2001).

In this study, we did not see the presence of Mycobacterium germs in the blood of HIV/AIDS patients due to the unavailability of service using a MycoF-lytic bottle in the installation of the microbiology laboratory. Then, 74% of HIV/AIDS patients in this study also had tuberculosis diagnoses. HIV/AIDS patients with tuberculosis do generally present with symptoms resembling sepsis (Huson, 2014). Mycobacteria infection should always be suspected in HIV/AIDS patients in countries with a high prevalence. Mycobacteria in the bloodstream were found in 17% to 54% of the various studies conducted in endemic areas (Taramasso et al., 2016). Indonesia is a country with a high prevalence of tuberculosis (WHO, 2013).

This study shows a high burden of HIV/AIDS and the presence of a gap in HIV diagnostics and its treatment. All patients in this study fell under the AIDS conditions (WHO stage 3 and stage 4) and all patients with CD4 + T lymphocyte data exhibited results below 350 cells / µl, which is the WHOdefined threshold for immediate antiretroviral therapy. In addition, 62% of the patients in this study were newly diagnosed patients and had not received antiretroviral therapy, while 38% of patients who had received antiretroviral drugs had low adherence to taking antiretroviral drugs so that CD4 + T lymphocytes  $< 200 sel / \mu l$  were still found. In patients with ESBL bacterial blood cultures, one patient recovered because the treating clinician could replace empiric antibiotics. One other patient died before the outcome of his culture due to the slow culture performed on the patient. In patients with positive blood culture results, left Klebsiella oxytoca ESBL and right positive blood cultures were Micrococcus luteus, then Klebsiella oxytoca ESBL was significant. In this patient who had previously received ceftriaxone therapy the ESBL results then suggested to the treating clinician immediately to replace it with antibiotics that were still able to treat ESBL. The choice taken was levofloxacin because the patient also had pneumonia infection, where

levofloxacin is a respiratory fluoroquinolone that acts as gram-negative and gram-positive. Patients with MRSA blood culture results were injecting drug users, according to several other studies suggesting that more Staphylococcus aureus is found in injecting drug users. In these patients MRSA was also in the sputum, urine and swab nose. These patients received intravenous vancomycin therapy for one week, nasal decontamination with mupirocin ointment and a chlorhexidine bath. In patients with polymicrobial blood, culture results found Acinetobacter baumannii, Coagulase-negative staphylococcus and Cryptococcus suggesting the possibility of Coagulase negative Staphylococcus only as a contaminant. Likewise the results of left blood cultures were Cryptococcus neoformans and for right blood cultures were Staphylococcus hominis, suggesting the possibility of Staphylococcus hominis as the contaminant. The sensitivity of some of the antibiotic drugs tested in this study included gentamicin (37.5%), ampicillin (14.3%), ampicillin-sulbactam (57.1%), ceftazidime (40%), cefotaxim (40%), ceftriaxone (25%), cotrimoxazole (50%), levofloxacin (71.4%), ciprofloxacin (50%), meropenem (60%), and vancomycin (100%).

The factor that affected the results was unrelated i.e., blood volume and the number of tubes taken, the lack of disinfection during blood collection, and the difficulty of taking blood cultures prior to the administration of antibiotics. In some patients, especially in patients with small blood vessels, it was difficult to take two sides of the blood culture with the volume of blood in each bottle of 8-10 cc. This may reduce the positivity of blood cultures. The disinfection process by the nurse was not correct so there were contaminants in the blood culture results. Then, the difficulty of taking blood culture before antibiotics was consumed by a microbiological laboratory that was not operating for 24 hours during the study.

#### 5 CONCLUSION

The proportion of positive blood cultures in this study was 16%. Bacteria obtained from a positive blood culture of HIV/AIDS patients included Escherichia coli (20%), Non-typhoidal Salmonella (14%), Acinetobacter baumannii (13%), Klebsiella oxytoca (7%), Staphylococcus aureus (13%), Coagulase-negative Staphylococcus (13%), and Micrococcus luteus (7%). The fungus found in a positive blood culture of HIV/AIDS patients at Dr.

Soetomo General Hospital was Cryptococcus spp (13%). MRSA and two ESBL germs were obtained in this study. Blood cultures assist clinicians in providing definitive antibiotic therapy appropriate to reduce morbidity and mortality.

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