ORIGINAL ARTICLE

Title
Association between the Biofilm of Double-Lumen Catheter and Blood Culture in Hemodialysis Patients with Suspected Central Line-Associated Bloodstream Infection

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Abstract

Background: Blood culture examination is still the gold standard for diagnosis of central line-associated bloodstream infection (CLABSI). CLABSI is also often associated with biofilm formation which can cause recurrent bacteremia and antibiotic resistance thereby increasing mortality and morbidity.

Objective: This study aims to analyze the relationship between the biofilms of the double-lumen catheter and blood cultures in hemodialysis patients with suspected CLABSI.

Method: This was an analytic observational study with a cross-sectional design to determine the relationship of biofilms and blood culture on patients aged >18 years with routine HD and suspected CLABSI at Dr. Soetomo General Hospital in August 2019 - November 2019. The examination of biofilm on DLC was done using the test tube method was measured with a nephelometer. The biofilm result is positive if ≥ 0.36 MF and negative if < 0.36 MF. Blood was cultured with a 3D BacT/ALERT tool in the Clinical Microbiology Installation Laboratory of Dr. Soetomo General Hospital.

Results: Of the 33 subjects, 45.5% were men and 54.5% were women, with an average age of 49.06 ± 1.5 years. The most common cause of CKD is hypertension (54.5%). The median length of HD was 3 months, ranging from 1-8

months. DLCs were mostly inserted at the right subclavian vein (87.9%) and installed with a mean duration of 77.94 ± 5.22 days. The majority (66.7%) had a normal nutritional status. The mean albumin level was 3.28 ± 0.07 g/dL. There was biofilm growth in 16 subjects (48.5%). The evaluation of blood culture revealed 15 positive results (45.5%). The analysis between biofilm density and CLABSI found significant differences between the two groups ($p=0.024$). From the chi-square test, the association of biofilms with blood culture in HD patients with suspected CLABSI obtained a p-value of 0.001.

Conclusion: Patients with suspected CLABSI and positive bacteria growth in blood cultures had biofilm in DLC. The density of biofilms in DLC is higher in positive blood culture CLABSI cases.

Keywords: chronic kidney disease, CKD, Double lumen catheter, Biofilm, Blood culture, Infection, CLABSI

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INTRODUCTION

Chronic Kidney Disease (CKD) is a major public health problem with an increasing number of events and when it reaches the terminal stage it requires hemodialysis (HD) as a substitute for kidney function to survive.^{1,2} Non-tunneled double lumen catheter (DLC) is one of the most frequently used vascular access for HD patients in Indonesia. Aside from thrombosis, infection is one of the most severe complications of catheter use for dialysis. HD catheter infection is thought increase mortality >50% and also causes significant morbidity in the dialysis population.^{3,4} Data on HD catheters usage showed at least 80,000 reported cases of central line-associated bloodstream infection (CLABSI) occur in intensive care units every year.⁵ In Dr. Soetomo General Hospital Surabaya reported that 32.3% of HD patients with a non-tunneled double lumen catheter had CLABSI.⁶

There are four known mechanisms of CLABSI. The first is microbial migration through catheter insertion lesion. These microbes can originate from the patient's skin, or through medical personnel. Second is the colonization of the catheter lumen. The third is direct contamination by bacteria that circulate in the bloodstream. Meaning the patient already has an infection in the bloodstream, and then the microbes adhere to the catheter when passing through it. Fourth is catheter lumen contamination related to infusion, both at the time of installing infusion (intrinsic) and at the time of usage (medical intervention).⁷ Diagnosis of bloodstream infections mainly relies on blood culture to detect bacteremia because a positive culture is not always followed by clinical symptoms of infection.⁸ However, the examination of culture requires a long time and is expensive, also at the end of incubation, false identification of positive cultures and false-negative results often occur.⁹ In addition to blood culture tests, biofilm examination or bacterial colonization of DLC can help in the diagnosis and management of patients.

Biofilm is a mucopolysaccharide matrix produced by the colonization of micro-organisms on the surface of the catheter. Biofilms can protect pathogenic microorganisms from innate immunity, antimicrobial agents, and disinfectants, making these pathogens difficult to eradicate.^{10,11} Biofilms

will undergo dispersion (release) which causes microbial biofilms to enter the bloodstream and leads to bacteremia.^{12,13} It is reported that 65% of the incidence of infection in developed countries is related to the formation of microbial biofilms which further aggravates the condition of bacteremia into recurrent bacteremia.¹⁴ Ramanathan et al. reported biofilm density is related to CLABSI conditions. Biofilm examination can be an option because it gives faster results than blood culture, and besides that biofilms are not affected by the surrounding environment (host and environment).¹⁰

Research data on the relationship between biofilms and CLABSI are still minimal in Indonesia. The author intends to prove that there is a correlation between the non-tunneled double lumen catheter biofilm of HD patients with CLABSI incidents based on blood culture. It is expected that with this research, the relationship between biofilms and blood cultures of those suspected with CLABSI can be more evident so that better management can be applied in CKD patients with HD that experience CLABSI.

METHODS

This study has a cross-sectional design and a population of CKD patients with a double-lumen HD catheter in the outpatient, inpatient and HD installation of Dr. Soetomo General Hospital Surabaya during August – November 2019. Patients aged >18 years and have undergone routine HD with suspected CLABSI. The exclusion criterion for this study was if other sources of infection were found such as respiratory and urinary tract infections.

Suspected CLABSI patients are patients with DLC as dialysis vascular access and experience one of the following symptoms: fever (>38°C), chills, or unexplained hypotension, and no signs of infection elsewhere. Then the diagnosis of CLABSI is based on the 2017 CDC criteria, by fulfilling one of the Laboratory Confirmed Bloodstream Infection criteria: a) Patients who are identified having pathogenic organisms from one or more blood specimens through a culture method based on microbiological methods & organisms that are identified in the blood and are not related to infections elsewhere; b) All patients who experi-

ence one of the following symptoms: fever ($>38^{\circ}\text{C}$), chills, or hypotension & organisms identified in the blood are not related to infection elsewhere & the same commensal organism is found on two or more examinations at the same time differ through the method of culture based on microbiological methods.¹⁵

Biofilm examination: The isolates obtained from DLC were put into BHI liquid, incubated 24 hours at 37°C and then cultured in MH media. Biofilm measurement was carried out using a nephelometer machine with positive results if ≥ 0.36 MF, negative results if < 0.36 MF.^{16,17}

The data collected will be processed and presented in textual, tabular and graphic forms. All research data were tested for normality with the Shapiro-Wilk test. A Chi-square test is used to analyze the relationship between two nominal scale variables. A significant result is achieved if the p-value < 0.05 with a 95% confidence interval. All data will be analyzed using the SPSS program version 25.0.

RESULTS

Of the 33 total subjects, the majority (54.5%) were female. The mean age of the study subjects was 49.06 ± 1.5 years with a range between 34-73 years. The etiology of CKD in this study was mostly caused by hypertension (54.5%). The median duration of HD was 3 months with a range of 1-8 months. The most frequent location of double lumen catheter placement in this study was the right subclavian vein found in 29 subjects (87.8%). The complete profiles of subjects could be observed in table 1.

Based on blood culture examination, there were 15 subjects (45.5%) with positive results and confirmed the diagnosis of CLABSI. Regarding the pathogenic bacteria in blood cultures, 10 specimens showed growth of gram-positive bacteria with *Staphylococcus aureus* as the most pathogens (20%) revealed. Furthermore, from the antibiotic sensitivity test, 100% of bacteria are sensitive to several antibiotics including Teicoplanin, Vancomycin, Linezolid and Trimethoprim. On the other hand, an antibiotic with a 100% resistant rate was Cephazolin. Data on blood culture results and antibiotic sensitivity tests are summarized in table 2 and table 3.

In this study, from 33 subjects, biofilm growth was obtained in 16 subjects (48.5%). Biofilm growth was then evaluated based on several parameters that can affect it including DLC insertion location, duration of DLC attachment, and antibiotic use during double lumen catheter placement. There were no significant differences resulted in all parameters (Table 4).

Of the 16 subjects with biofilm growth, 12 (80%) of them had positive blood cultures while 4 subjects (22.2%) did not. Whereas in the other 17 subjects with negative biofilms, the majority (77.8%) of subjects did not have bacterial growth in their blood cultures. Before analyzing the relationship of biofilms with CLABSI events, researchers found that there were significant differences in biofilm density ($p=0.024$; 95% CI) between patients with positive and negative culture in CLABSI groups (Figure 1), in which the biofilm density the negative CLABSI group were lower than the positive CLABSI group (table 5). Further analysis with the chi-square test (table 6) showed a significant relationship between biofilms and the incidence of CLABSI based on blood cultures ($p=0.001$; 95% CI).

DISCUSSION

From the basic characteristics, there are more female subjects than men. Research by Saleh et al. also showed the dominance of female subjects (61%).¹⁸ The mean age of the subjects was 49 years, with the youngest age at 34 years and the oldest at 73 years. This is consistent with the 2013 Basic Health Research data and also data from the Indonesian Renal Registry (IRR).^{19,20} Other studies also obtained an average age of 48.9 years.²¹ As for the etiology of CKD, this study reported hypertension as the most common cause (54.5%). Data from IRR 2017 support these results where 51% of CKD patients with HD are caused by hypertension.

Regarding the catheter, 87.8% of the double lumen catheters were inserted at the subclavian vein. In contrast, other studies have more insertion in the femoral²² and jugular^{8,23}. The rationale of selecting subclavian insertion site is due to easier mobilization and lower risk of infection compared to femoral and jugular insertion sites.^{8,22-24}

Table 1. General Characteristics of the patients

Category	Total (%)
	n=33
Gender, n (%)	
Male	15 (45.5)
Female	18 (54.5)
Age, years	
Mean $\pm$ SD	49.06 $\pm$ 1.5
Range (Min-Max)	34 – 73
ESRD etiology, n (%)	
DM	13 (39.4)
HT	18 (54.5)
Autoimmune	2 (6.1)
Duration of HD, month	
Median	3
Range (Min-Max)	1-8
Double Lumen Catheter Location, n (%)	
Right subclavian vein	29 (87.8)
Right jugular vein	2 (6.1)
Femoral vein	2 (6.1)
Double Lumen Catheter Duration of Installment, days	
Mean $\pm$ SD	77.94 $\pm$ 5.22
Range (Min-Max)	31 – 151
Nutritional Status, n (%)	
Underweight	3 (9.1)
Normal	22 (66.7)
Overweight	7 (21.2)
Obese	1 (3.0)
Albumin, g/dL	
Mean $\pm$ SD	3.28 $\pm$ 0.07
Range (Min - Max)	2.5 – 3.9

Table 2. Blood Culture Result

Types of microorganism	n
Gram-positive bacteria	
<i>Staphylococcus aureus</i>	3 (20%)
<i>Staphylococcus epidermidis</i>	2 (13.3%)
<i>Methicillin-resistant staphylococcus aureus</i>	2 (13.3%)
<i>Staphylococcus haemolyticus</i>	2 (13.3%)
<i>Enterococcus faecalis</i>	1 (6.7%)
subtotal	10 (66.7%)
Gram-negative bacteria	
<i>Enterobacter cloacae</i>	2 (13.3%)
<i>Acinetobacter baumannii</i>	1 (6.7 %)
<i>Escherichia coli</i>	2 (13.3 %)
subtotal	5 (33.3%)
Total	15 (100 %)

Table 4. Characteristic of Biofilm Growth

Category	Total (%)	Frequency		p value
		Biofilm positive	Biofilm negative	
	n=33	n= 16 (48,5%)	n= 17 (51,5%)	
Double Lumen Catheter Location				
Subclavia Dextra	29 (87,8)	12 (75)	17 (100)	0,68
Jugular Dextra	2 (6,1)	2 (12,5)	0 (0)	
Femoral	2 (6,1)	2 (12,5)	0 (0)	
Double Lumen Catheter Duration of Installment, days				
Mean $\pm$ SD	77,94 $\pm$ 5,22	87,69 $\pm$	71,12 $\pm$	0,11
Range (Min - Max)	(31-151)	8,79	5,00	
Prior use of Antibiotics				
Yes	10 (30,3)	7 (43,75)	3 (17,65)	0,10
No	23 (69,7)	9 (56,25)	14 (82,35)	

Table 5. Distribution of biofilm density with and without CLABSI

	Total	CLABSI		p value
	Mean	Positive	Negative	
	n=33	n=15	n=18	
Biofilm density	0.31 $\pm$ 0.02	0.38 $\pm$ 0.04	0.26 $\pm$ 0.03	0.024

Table 3. Antibiotic Sensitivity Test Results

Antibiotic	Sensitive	Intermediate	Resistant	Total
Aminoglycoside				
Amikacin	5 (83,3%)	-	1 (16,7%)	6
Tobramycin	-	-	-	-
Gentamycin	6 (40%)	1 (6,7%)	8 (53,3%)	15
Beta-lactam Penicillin				
Astreonam	1 (16,7%)	-	5 (83,3%)	6
Amoxicillin clavulanic acid	2 (14,3%)	1 (7,1%)	11 (78,6%)	14
Ampicillin	1 (7,7%)	-	12 (92,3%)	13
Ampicillin-Sulbactam	1 (16,7%)	-	5 (83,3%)	6
Penicillin G	1 (12,5%)	-	7 (87,5%)	8
Oxacillin	2 (25%)	-	6 (75%)	8
Piperacillin	2 (40%)	1 (20%)	2 (40%)	5
Piperacillin tazobactam	4 (66,7%)	2 (33,3%)	-	6
Beta-lactam Cephalosporin				
Generasi I				
Cephazolin	-	-	5 (100%)	5
Generasi III				
Ceftazidime	2 (33,3%)	-	4 (66,7%)	6
Cefotaxime	2 (28,6%)	-	5 (71,4%)	7
Ceftriaxone	2 (28,6%)	-	5 (71,4%)	7
Cefoperazone-Sulbactam	5 (83,3%)	-	1 (16,7%)	6
Sulfa-Trimethoprim				
Cotrimoxazol	6 (40%)	-	9 (60,%)	15
Trimethoprim	1 (100%)	-	-	1
Tetracyclin	5 (35,7%)	1 (7,1%)	8 (57,1%)	14
Chloramphenicol	10 (76,9%)	-	3 (23,1%)	13
Macrolides				
Erythromycin	5 (55,6%)	2 (22,2%)	2 (22,2%)	9
Clindamycine	5 (62,5%)	-	3 (37,5%)	8
Quinolone				
Quinopristin-dalfopristin	5 (83,3%)	-	1 (16,7%)	6
Ciprofloxacin	4 (28,6%)	-	10 (71,4%)	14
Moxifloxacin	7 (70%)	1 (10%)	2 (20%)	10
Levofloxacin	4 (36,4%)	1 (9,1%)	6 (54,5%)	11
Lain-lain				
Fosfomycin	7 (70%)	-	3 (30%)	10
Vancomycin	2 (100%)	-	-	2
Linezolid	2 (100%)	-	-	2
Imipenem	4 (80%)	1 (20%)	-	5
Meropenem	5 (83,3%)	-	1 (16,7%)	6
Ertapenem	-	-	-	-
Teicoplanin	9 (100%)	-	-	9

Still related to the catheter, the mean duration of the double lumen catheter installation in this study was 77.94 days. This differs from 2 other studies that have a longer mean duration of 138 days¹⁰ and 214 days²⁵. However, both studies used tunneled DLC. Based on blood culture results, 15 subjects were confirmed CLABSI positive with 10 of them having growth of gram-positive bacteria. Several other studies have shown similar results with the percentage of gram-positive bacteria growth ranging from 61.5 – 75%.^{10,23,26} *Staphylococcus aureus* becomes gram-positive bacteria which accounts for 40 – 80% in the majority of catheter infection cases.²⁷⁻²⁹ This is in line with this research. In the sensitivity tests, all pathogens are still sensitive to some antibiotics. One research by Gafor et al. with a period of 6 months in 28 study subjects, also obtained antibiotics with 100% sensitivity, including vancomycin, meropenem, imipenem, amikacin, and trimethoprim. The vancomycin regimen is recommended as an empirical systemic antimicrobial therapy. A study in India states that teicoplanin is effective with a 100% sensitivity test on CLABSI caused by *S. Aureus*. As for gram-negative, meropenem, imipenem, and amikacin can be an option.^{4,26,28}

Figure 1. Distribution of Biofilm Density between CLABSI Groups

Figure 1. Distribution of biofilm density between subjects with positive and negative blood culture results.

Table 6. Analysis of Biofilm Growth and Blood Culture

	Culture		Total (%)	p value
	(+)	(-)		
Biofilm positive	12 (80%)	4 (22,2%)	16 (48,5%)	0,001
Biofilm negative	3 (20%)	14 (77,8%)	17 (51,5%)	
Total	15(100%)	18(100%)	33 (100%)	

In this study, the majority (48.5%) of subjects had results of biofilm growth. In theory, almost all (99.9%) microorganisms can form biofilms on various surfaces. The ability to form biofilms has been reported in a large number of bacterial species such as *P. Aeruginosa*, *S. Epidermidis*, *E. Coli* spp, *S. Aureus*, *E. Cloacae*, *K. Pneumoniae*.³⁰ Researchers also analyzed several factors that could influence colonization and biofilm growth and it was discovered that the duration of DLC insertion, location, duration of CDL installation, and history of antibiotic use did not produce significant differences. Research by De Freitas et al. ($p = 0.11$)⁸ and Kang et al. ($p = 0.304$)³¹ also got insignificant results on the duration of DLC installation. However, a study in Brazil reported that the location of the insertion gave a significant effect ($p=0.03$).⁸ Different types and brands of DLC could affect the results of the study, whereas previous studies used tunneled DLC which has a longer duration of installation than that of non-tunneled DLC.

The results of the analysis present that there is a significant

difference in density ($p=0.024$; 95% CI) between subjects with positive and negative blood culture results. Furthermore, the chi-square test showed a significant relationship between biofilms and the blood culture of suspected CLABSI patients ($p=0.001$; 95% CI). These results are similar to studies in America ($p=0.03$) and Canada ($p=0.02$). Both studies also found significant differences in density and explained that biofilm thickness could increase by up to two-fold in subjects with CLABSI.^{10,25} This proves that biofilms formed from bacterial colonization at the catheter hub can lead to bacteremia.⁵ Biofilms are said to be responsible for 65% of the incidence of catheter-related infections.¹⁴ After the formation of biofilms, bacteria are thought to leave the biofilm itself regularly. By doing this, bacteria can undergo multiplication and rapid spread.³⁰ Biofilms regularly release bacteria into the bloodstream and can cause recurrent bacteremia. Antibiotic resistance in biofilms is also a threat of concern.²⁵

Several limitations of this study are the cross-sectional design with a narrow observation time of the study, so it cannot firmly explain the causal effect between biofilms and blood cultures of CLABSI patients. This study was also conducted at a single center so that it can describe the overall population of HD patients.

CONCLUSION

Patients with suspected CLABSI and positive bacteria growth in blood cultures had biofilm in DLC. This is supported by the results of this study that biofilm is significantly associated with CLABSI based on blood culture. Patients who were confirmed to have CLABSI had significantly higher biofilm density values than the negative CLABSI group. Biofilm could be an alternative examination in evaluating patients with suspected CLABSI. Faster results and its resistance to the surrounding environment are added values for biofilm examinations.

ETHICAL CLEARANCE

This research had been approved by the Ethics Committee before the study conducted.

CONFLICT OF INTEREST STATEMENT

The authors declare that there was no conflict of interest in this research.

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AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, preparation, drafting, review, and approval of this manuscript

DISCLOSURE

The author reports no conflicts of interest in this research.

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