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Suitability Analysis of Gram Staining with Blood Culture in Bacteremia

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ABSTRACT

Bacteremia is a serious bloodstream infection, requiring blood culture as the gold standard for definitive diagnosis and rational therapy, but it is time-consuming. The Gram stain should be considered as an alternative test, as it is easier and faster to determine empirical antibiotic therapy in order to minimize the use of broad-spectrum antibiotics. This study aimed to analyze the suitability of Gram staining results with blood cultures in bacteremia. Cross-sectional study design from August to September 2022 at Dr. Wahidin Sudirohusodo Hospital and Labuan Baji Hospital, Makassar. The study sample used specimens from automatic positive blood culture bottles of new bacteremia patients. Gram staining and sub-culture were performed to determine the type of Gram-positive or negative bacteria. Appropriate statistical analysis was used to compare the two methods. A total of 78 research samples were obtained. The results of subculture vs. Gram staining showed Gram-positive bacteria in 45 vs. 42 samples and Gram-negative bacteria in 33 vs. 36 samples, respectively. A comparative test using the Wilcoxon test showed no significant difference for the 2 tests, p-value = 0.257 (p > 0.05). The results of the Gram diagnostic test on culture showed high sensitivity and specificity, respectively: 88.9%, and 93.9% for Gram-positive bacteria, 93.9% and 88.9% for Gram-negative bacteria. Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 95.32 % and 95.32 % for Gram-positive, 86.11%, and 95.23% for Gram-negative bacteria, respectively. There is a suitability between Gram stain and automatic blood culture results with high diagnostic value. Gram staining is expected to be a guide for selecting empirical therapy based on Gram's classification until the AST test results come out.

Keywords: Gram stain, automatic culture, blood, bacteremia

INTRODUCTION

Microbial culture is still the gold standard for definitive diagnosis of a patient that has suspected infection, especially bacteremia, and is an important parameter in choosing rational antibiotic therapy to prevent antibiotic resistance. Rational antibiotic use will decrease morbidity and mortality of patients with bacteremia with effective and rapid antibiotic use, but it takes a minimum of 3 days to receive culture and antimicrobial sensitivity test results. A study by Weinstein et al. showed that there was a relationship between irrational antibiotic therapy and unsatisfactory outcome, showing that positive blood cultures from 707 patients with bacteremia had a 17.5% mortality. Another study by Leibovici *et al*. from 955 bacteremia patients showed a 44% higher death rate due to unidentified organisms, and a still high 25% death rate from known organisms.[1,2]

Minister of Health Regulations no 8, year 2015 regarding the Hospital Antimicrobial Resistance Control Program states that wise antibiotic use is the use of antibiotics in accordance with the cause of infection, suitable with the optimal dose regimen, optimal length of consumption, minimal side effects, and minimal effect towards the emergence resistant microbes. They are the main reasons that antibiotic administration has to be given while searching for the cause of infection.[3]

Rapid diagnosis of the type of pathogen plays an important part in the management of bacteremia. Bacteremia is identified in the laboratory using blood culture. Bacteremia is defined as the presence of bacteria in the bloodstream that is proven by a positive blood culture, as a sign that there is microbe growth in the culture bottle during the time of incubation, that is coherent with the signs and clinical symptoms of infection in a patient (e.g. fever and shivering). The type of pathogen should be identified before therapy is started, but since it takes a long time empirical therapy can be given with guidelines using simpler examination such as Gram staining.[4]

Gram staining can determine two types of microorganisms in bacteremia; either Gram-positive or Gram-negative, this can shorten the time of obtaining results or Turn Around Time (TAT) for 2 days before culture and Antibiotic Sensitivity Testing

(AST) results to choose a drug of choice and it will narrow the choices of rational antibiotic therapy with a faster and using a narrow spectrum antibiotic that could be more effective as an effort to support rational antibiotic therapy and support the antimicrobial resistance program.[5,6]

Specimen for Gram staining is taken from blood culture bottles that are positive (available microorganism growth). Then samples are taken for further culture examination using automatic tools and gram staining. to assess whether there are differences in results between the two methods in order to shorten the examination time.[7]

Antibiotic choice is an important key in the specific therapy of infections. The global problem that facing is the high rates of irrational or inappropriate antibiotic usage. There are various reasons that support the misuse of antibiotics that result in antibiotic resistance. Rational antibiotic use will result in antibiotic therapy effectivity giving optimal results.[8]

The Gram staining method was found in 1884 by a microbiologist from Denmark named Han Christian Gram while observing the differences between *Pneumococcus* and *Klebsiella pneumoniae*, causing the staining technique to be named Gram. Other than the conventional way, there are also various Gram staining methods, such as the water washing method, thin blood smear, and drop and rest method. The last two methods are the most recommended methods. Thin blood smear and drop and rest method distributes the samples evenly and separates the charcoal and resin so the smear only has microorganisms and simplifies the interpretation, but technically there are differences in how these methods work.[9]

Several studies show that by reporting Gram results as soon as possible is very useful in selecting rapid and rational antibiotic therapy for the patient. A study by Harindina *et al.* about the suitability of Gram staining with blood culture as a predictor of critical value of bacteremia cases shows that Gram staining can be used as the basis of empiric therapy due to the high suitability value with positive blood culture results.[10,11] Hara *et al.* found that the effect of reporting Gram staining results from positive blood culture bottles towards the choice of antibiotic therapy facilitates the empiric antibiotic therapy while waiting for the culture identification and resistance test, showing perfect suitability with the upcoming culture identification.[12]

Based on the reasons above, this study was conducted to evaluate the suitability of Gram staining results with automatic blood culture testing, whether Gram staining results can be used as an alternative test to shorten TAT of culture and AST results of the cause of bacteremia causing a more rapid decision of antibiotic therapy that is rational and evidence-based as an effort in antimicrobe resistance control and contributing in decreasing the mortality rate of patients with bacteremia.

METHODS

This study used an observational analytic cross-sectional design. The study was held at the Laboratory of the Clinical Pathology Installation of Dr Wahidin Sudirohusodo and Labuan Baji Hospital, Makassar. It was held on during August – September 2022. The study population was all the BacT Alert/BACTEC© bottles that detected microorganism growth examined at Laboratory of the Clinical Pathology Installation of Dr Wahidin Sudirohusodo and Labuan Baji Hospital. Samples detected with microorganism growth were then cultured and underwent Gram staining, and afterward both results were compared to see the suitability of the 32 tests. This study was done in one population to describe the validity of Gram staining towards blood culture tests in bacteremia. Both methods were examined during the same period of time to guarantee the same disease conditions.

Data analysis used SPSS. The statistic method used was the Wilcoxon test to see if there were significant differences between Gram staining and blood culture in patients with bacteremia. A comprehensive assessment of the diagnostic accuracy of Gram-negative and Gram-positive bacteria using the Gram stain method was carried out in this study.

This research was carried out after obtaining ethical clearance by considering respect for the subject, beneficence, non-maleficence, and justice from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Hasanuddin University Hospital/RSPTN UH/Dr. Wahidin Sudirohusodo Hospital, Makassar with article number: 430/UN4.6.4.5.31/PP36/2022.

RESULTS AND DISCUSSIONS

This study involved 78 positive blood culture samples from patients with bacteremia. The research results showed that of the 78 samples, there were 46 (59%) male and 32 (41%) female with an age range of 1–79 years. The samples were then grouped according to the results of Gram-positive and Gram-negative bacterial cultures. In the blood culture test, 45 (57.7%) samples of Gram-positive

bacteria were obtained, and 33 (42.3%) samples of Gram-negative bacteria. Meanwhile, in the Gram staining test, 42 (53.8%) samples of Gram-positive bacteria were obtained, and 36 (46.2%) samples of Gram-negative bacteria (Table 1).

Table 1. Characteristics of the study samples

Criteria	n	(%)	Mean±SD
Gender			
Male	46	59	
Female	32	41	
Age	78	100	30.72±27
Blood culture			
Gram (+) bacteria	45	57.7	
Gram (–) bacteria	33	42.3	
Gram staining			
Gram (+) bacteria	42	53.8	
Gram (-) bacteria	36	46,2	

Source: Primary data

Table 2 shows a comparison of the results of Gram staining for Gram-positive and Gram-negative bacteria using the Wilcoxon difference test. The test results showed that there was no statistically significant difference between Gram staining and blood culture results in identifying bacteria in blood samples from bacteremia patients, where the p-value was> 0.05 (p=0.257). Thus, it can be said that Gram staining gives the same results or in other words are in accordance with the blood culture results.

Statistical test results of diagnostic tests using culture results as the gold standard in Table 3 towards Gram staining. The diagnostic ability of Gram staining for Gram-positive bacteria has a sensitivity of 88.9%, specificity of 93.9%, Positive Predictive Value (PPV) of 95.32%, and Negative Predictive Value (NPV) of 95.32%.

Table 4 shows that the diagnostic value of Gram staining for Gram-negative bacteria has a sensitivity of 93.9%, specificity of 88.9%, PPV of 86.11%, and NPV of 95.23%.

This research used a cross-sectional design involving 78 samples. The characteristics of the samples in Table 1 show that 46 (59%) were male and 32 (41%) were female ranging from 1–79 years old. 42 (53.8%) samples from Gram staining were band Gram-positive bacteria and 45 (57.7%) of the samples from blood culture were Gram-positive bacteria, while 36 (46.2%) samples from Gram staining were Gram-negative bacteria and 33 (42.3%) samples from the blood bottle culture were Gram-negative bacteria.

The results from Gram staining compared to blood culture show a few differences in a couple

Table 2. Analysis of the suitability of the Gram stain method with automated blood culture

Gram Staining	Blood Culture		Total	p
	Gram (+) Bacteria	Gram (-) Bacteria		
Gram-positive bacteria	40	2	42	
Gram-negative bacteria	5	31	36	0.257*
Total	45	33	78	

Source: Primary data *Wilcoxon test (p > 0.05)

Table 3. Evaluation results of the Gram staining diagnostic test on Gram-positive bacteria based on bacterial culture results

Gram Staining	Blood Culture		Total of Cultures	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative					
Positive	40	2	42				
Negative	5	31	36	88.9	93.9	95.32	86.11
Total	45	33	78				

Source: primary data

Table 4. Evaluation of the Gram stain diagnostic test results on Gram-negative bacteria based on the results of bacterial culture

Gram Staining	Blood Culture		Total	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative					
Positive	31	5	36				
Negative	2	40	42	93.9	88.9	86.11	95.23
Total	33	45	78				

Source: Primary data

samples, but the differences were not statistically significant when tested using the Wilcoxon suitability test, showing there were no significant differences between Gram staining with blood culture with p of 0.257 (p> 0.05), indicating that Gram staining is in accordance with blood culture. The test shows that 40 samples were detected to be Gram-positive in Gram staining and in blood culture, 5 samples were detected to be Gram-negative in Gram staining but had positive blood culture results, 2 samples were detected to be negative in blood culture while being positive in Gram staining, 31 samples were detected to be Gram-negative in both culture and Gram staining. The results of this study is in accordance with a study done by Uehara *et al*. showing a perfect suitability between Gram staining with culture identification, the impact of these results are facilitating empiric antimicrobe therapy before the results of culture identification and antibiotic sensitivity tests are available.

The results of this study also show that Gram staining results for Gram-positive bacteria has an 88.9% sensitivity, 93.9% specificity, 95.32% PPV and 95.32% NPV. Gram-negative had 93.9% sensitivity, 88.9% specificity, 86.11% PPV and 95.23% NPV.

Blood cultures are the gold standard in laboratory examinations used to identify types of microorganisms in bacteremia. Detection of bacteremia should be reported as soon as possible for purposes of selecting antibiotic therapy. This study compared Gram staining with the results of automatic culture on culture bottles that showed microorganism growth. Samples were taken from patients suspected of having an infection (bacteremia).[12] The types of bacteria found in this study based on culture results were Gram-positive bacteria consisting of *Acinetobacter baumannii, Staphylococcus vitulinus, Staphylococcus epidermidis, Aerococcus viridans,* and *Klebsiella pneumoniae.* Gram-negative bacteria were *Burkhodeleria cepacia, Aeromonas veronii, Aeromonas caviae, Salmonella spp*. and *Serratia marcescens.*

Reporting Gram staining results can have a positive effect on faster and more precise administration of antibiotics than the results of AST, which is time-consuming. Gram staining is a simple, quick procedure and can be reported earlier than the final results of AST. Research conducted by Barenfanger *et al*. showed that delayed reporting of blood culture results resulted in increased mortality. The current study showed that the proportion of suspected bacteria does not differ much between the results of automatic culture and a fairly short Gram stain.

It is hoped that this research can be a reference for clinicians in the management and selection of antibiotic therapy in cases of bacteremia and is expected to be a basis for further research related to Gram staining with different sample populations and specimen types.

CONCLUSIONS AND SUGGESTIONS

There is a suitability between the Gram staining and automatic blood culture results of bacteremia patients. Diagnostic test results for Gram staining showed a high sensitivity and specificity as follow: 88.9% and 93.9% for Gram-positive bacteria and 93.9% and 88.9% for Gram-negative bacteria.

Gram staining on positive blood cultures is hoped to be considered to guide the selection of empiric therapy based on the classification of Gram antimicrobials in the hospital until the AST results come out. Further research on Gram staining with different types of specimens is needed.

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