RESEARCH ARTICLE

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Effectivity of Topical Quinolones and Metronidazole on Cancer Ulcers in Patients with Locally Advanced Breast Cancer: A Randomized Controlled Trial

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Abstract

Introduction: Malignant Fungating Wound (MFW) has a significant role in increasing quality of life. The secondary infection could cause as well as Hemorrhage, Odor, Pain, Exudate & Superficial infections (HOPES) and reduced activity. Worseness of MFW could be caused by a combination of aerobic and anaerobic bacterial infections. This study aimed to prove the effectiveness of the topical antibiotic solution toward wound repairment secondary to MFW. **Methods:** This study was a pre and post-test randomized controlled trial in which inclusion and exclusion criteria were predefined. Patients who suffered locally advanced breast cancer were given chemotherapy and then randomized into the treatment group of MFW management treated with Ciprofloxacin and Metronidazole solution, and the non-treatment group treated with 0.9% NaCl. The dependent variable was the number of Colony Forming Unit (CFU) and the degree of fibrosis. Mann Whitney-U and Kendall's tau-b test was carried out to examine the difference and correlation test. Statistical significance was defined as $p < 0.05$. **Result:** The study involved 40 patients who were randomized into the treatment group dan the non-treatment group. Paired T-tests of the CFU in the non-treatment group and the treatment group are significant ($p<0.001$). The Mann Whitney-U test in delta CFU in both groups is significant ($p<0.001$). Mann Whitney-U test in the degree of fibrosis in both groups is significant ($p=0.046$). Kendall's tau-b correlation test shows a significant negative correlation between the CFU and the degree of fibrosis ($p=0.027$, $r=-0.36$). **Conclusion:** The treatment of MFW in locally advanced breast cancer treated with metronidazole and ciprofloxacin solution gives a better rate of improvement in terms of reducing the number of CFU and increasing the degree of fibrosis. This study proves that there is a significant negative correlation between a decrease in CFU and the degree of fibrosis in the treatment group.

Keywords: Breast Cancer- MFW- CFU

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Introduction

The MFW has a significant role in increasing quality of life. The authors see this aspect as a long process in which many intermediate variables must be considered. These variables require a long observation and research roadmap, starting from the effect of giving an agent on improving MFW, the effect of MFW on quality of live improvement, and the effect of quality of life due to improving MFW on survival [1, 2, 3]. The most common location for MFW formation is on the breast and chest (39%). MFW causes several signs and symptoms, including bad odor, exudation, pain/itching, bleeding, superficial infection process like HOPES, as well as reduced activity. Bad odor (malodor) can be caused by a combination of aerobic and anaerobic bacterial infections, necrotic tissue, tissue hypovascularization, and excessive exudate [4, 5].

General management of MFW includes dressing (wound closure with 0.9% NaCl) and the use of a single topical antibiotic, but its effectiveness in reducing malodor and improving quality of life is still considered low [6, 7]. Hypovascularization and tissue necrosis in MFW also create a good environment for the growth of infections, namely gram-positive bacteria (Staphylococcus

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aureus), gram-negative bacteria (*E coli*, Klebsiella sp, Pseudomonas aeruginosa), and anaerobic bacteria (Clostridia perfringens, C septicum, C tertium). Infections occurred can be treated with antibiotics. For anaerobic bacteria, protozoa, and microaerophilic bacteria, infection, metronidazole is the recommended drug to reduce infection, while for aerobic bacterial infections (gram-positive and gram-negative) quinolones may be used (eg. ciprofloxacin) [8, 9, 10].

The use of topical antibiotics in MFW is expected to reduce secondary infections so that MFW can improve during chemotherapy administration, reduce patient subjective complaints related to HOPES, improve quality of life, and reduce secondary infection [6, 11]. One of the parameters used in determining the decrease in secondary infection is calculating the decrease in bacterial colonies. Meanwhile, the parameters used for wound healing and repair are by using an increase in the degree of fibrosis, which starts from the proliferation of fibroblasts [12, 13, 14, 15]. This research aims to prove that topical administration of Ciprofloxacin 200 mg/100 ml and metronidazole 500 mg/100 ml in the treatment of cancer wounds will reduce the number of bacterial colonies, increase the degree of fibrosis, and there is a relationship between a decrease in the number of bacterial colonies and an increase in the degree of fibrosis.

Materials and Methods

Patients

Patients from the dr. Kariadi Hospital in Semarang, Central Java, who had locally advanced breast cancer stage III-IV and MFW and had Fluorouracil, doxorubicin and cyclophosphamide (FAC) regiment chemotherapy up until the second chemotherapy were selected. They ranged in age from 50 to 70 years old. Subjects were then selected by the following inclusion criteria include patients with locally advanced breast cancer stage III-IV who underwent FAC chemotherapy with cancerous wounds, laboratory leukocyte count >4000/mm3, platelets 150,000/mm3, Hemoglobin (Hb) level 10 mg/dL. Exclusion criteria include patients with a history of immune disorders or allergies, patients undergoing corticosteroid therapy, patients undergoing radiation therapy or other therapy, patients with chronic systemic disease (diabetes mellitus, hypertension, anemia, heart disease, liver, and kidney dysfunction), and those who refused to be included in the study.

Trial Design

This study was conducted with doubled-blind pre-test and post-test, parallel-group, randomized controlled trials used to demonstrate clinical benefit between two groups in terms of efficacy. Patients were randomly assigned in a 1:1 ratio to receive MFW wound management using either ciprofloxacin-metronidazole or 0.9% NaCl solution. Randomization was performed after confirming patient eligibility using inclusion and exclusion criteria. Randomization was done by random allocation which is carried out based on the sequence of patient admission. The treatment group consisted of patients with stage III-IV locally advanced breast cancer who were given FAC chemotherapy and MFW wound management with ciprofloxacin 200 mg/100 ml and metronidazole 500 mg/100 ml, while the non-treatment group was patients with stage III-IV locally advanced breast cancer who were given FAC chemotherapy and MFW wound management with 0.9% NaCl solution.

In this study, neither the subject nor the investigators were aware of the identity of the treatment group until after the data were collected. The independent variable was the administration of ciprofloxacin 200 mg/100 ml and metronidazole 500 mg/100 ml. The dependent variable was the number of bacterial colonies and the degree of fibrosis. While Body Mass Index (BMI), Hb levels, and age were confounding variables.

The experiment was approved by the Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: 03/EC/KEPK/FK-UNDIP/I/2022) and we used the CONSORT reporting guidelines for reporting parallel group randomized trials.

Analysis of the number of bacterial colonies

The number of bacterial colonies was grown on a Petri dish germ culture with a standard size of 9 cm diameter. The medium was blood agar. Samples from group 1 (pre-test) were taken on day 0 by swabbing the area of cancer wounds that were still vital with a sterile cotton swab. Samples from group 2 (post-test) were taken on the 7th day after wound care, carried out by swabbing the area of cancer wounds that were still vital with a sterile cotton swab. The material from the wound swab is then stored in the transport medium, labeled, and processed in the laboratory.

Before inoculation, forty samples from the wound swab were stored in the transport medium before inoculation were diluted with sterile 0.9% NaCl up to 10 cc (10 x dilution). Inoculation was carried out manually using a 10 µl (0.01 ml) ose needle with ten lines in a zig-zag direction so that it was evenly distributed on the blood agar medium in the Petri dish [16].

Colonies of bacteria of 30–300 colonies were expected to be obtained after inoculation and incubation process at 37°C (using the standard for *E coli*). Counting was done manually with a “click-counter” tool and a “sliding line” pen. A “counting zone” tool is used if the number of colonies is plenty [17].

The number of colonies obtained was then calculated by the amount of dilution and the size of the ose needle. The calculation data output is in the form of CFU/ml. The value of this variable is the delta or the difference between pre and post-treatment [18].

Fibrosis Analysis

These analyses are the process of hardening, strengthening, and contraction of tissue, which can be represented by the calculation of the number of fibroblasts. Group 1 sampling process (pre-test) was carried out on day 0, performed through wedge-shaped incisional biopsy, at the transitional boundary between healthy skin and cancerous wounds, using topical anesthetic then the sample was put into a container filled with 10% formalin buffer, then labeled and processed in the laboratory. The sampling process of group 2 (post-test) was carried out on the 7th day of post-wound treatment, carried out through wedge-shaped incisional biopsy, using topical anesthesia, at the transitional boundary between healthy skin and cancerous wounds, after that the sample was put into a container filled with 10% formalin buffer, then labeled and processed in the laboratory.

The number of fibroblasts was calculated by calculating the average number of fibroblasts per large field of view (400x) on the Hematoxylin-eosin (HE) slide, which was taken randomly in the border area between healthy tissue and cancerous wound tissue. An average of 10 fields of view are representative of the entire area on the HE slide (top side, bottom side, right side, left side, and center). The assessment was carried out by clinical agreement > 95% [19].

Statistical Analysis

All data were evaluated using SPSS 23.0 for Mac Software. The results were expressed as mean ± standard deviation. The Shapiro-Wilk test was used for the data normality test. Confounding analysis was done with Independent T-Test [20].

The Mann-Whitney test was carried out to examine the difference between the initial and final observations on the variable number of bacterial colonies and the degree of fibrosis. The relationship between the variable number of bacterial colonies and the fibrosis degree was tested using Kendall's tau b correlation test. Statistical significance was defined as $p < 0.05$ [20].

Results

Patients

The CONSORT diagram is shown in Figure 1. Patients participating in this study were recruited from the Dr. Kariadi Hospital in Semarang, Central Java, from October 2018 through November 2020, and sample recruitment was stopped when the number of samples for each group was fulfilled. Total of 123 patients aged 35-70 years old with locally advanced Breast Cancer stage III–IV and MFW who received FAC regiment chemotherapy were enrolled. 87 patients (70%) were excluded (2 patients underwent corticosteroid therapy, 9 patients had a history of allergies, 13 patients underwent radiotherapy, 3 patients had a history of heart disease, and 56 patients refused to be included in the study), and 40 patients eligible for further analysis.

Variables of age, hemoglobin, and BMI

Different data test was performed using Independent T-test on the variable data of age, Hb, and BMI with p-value >0.05, 95% CI, for all groups (age p = 0.216; Hb p = 0.505; BMI p = 0.404). It can be concluded that the variables of age, Hb, and BMI are not significantly different (homogeneous) and are not confounding variables (Table 1).

Number Germ of Colonies

The average value obtained on the CFU of the non-treatment group before wound care management is 201,800 CFU/ml and 191,450 CFU/ml after wound care.

Table 1. Baseline Characteristics of the Data Set

Characteristic	Groups		p
	Treatment (n=20)	Non-Treatment (n=20)	
Age	51.35 ± 7.65	54.00 ± 5.49	0.216*
Hb	12.76 ± 0.54	12.62 ± 0.80	0.505*
BMI	22.56 ± 2.62	23.27 ± 2.71	0.404*

Different data test was performed using Independent T-test on the variable data of age, hemoglobin, and BMI with p-value >0.05, 95% CI, for all groups (age p = 0.216; hemoglobin p = 0.505; BMI p = 0.404). It can be concluded that the variables of age, hemoglobin, and BMI are not significantly different (homogeneous) and are not confounding variables (Table 1).

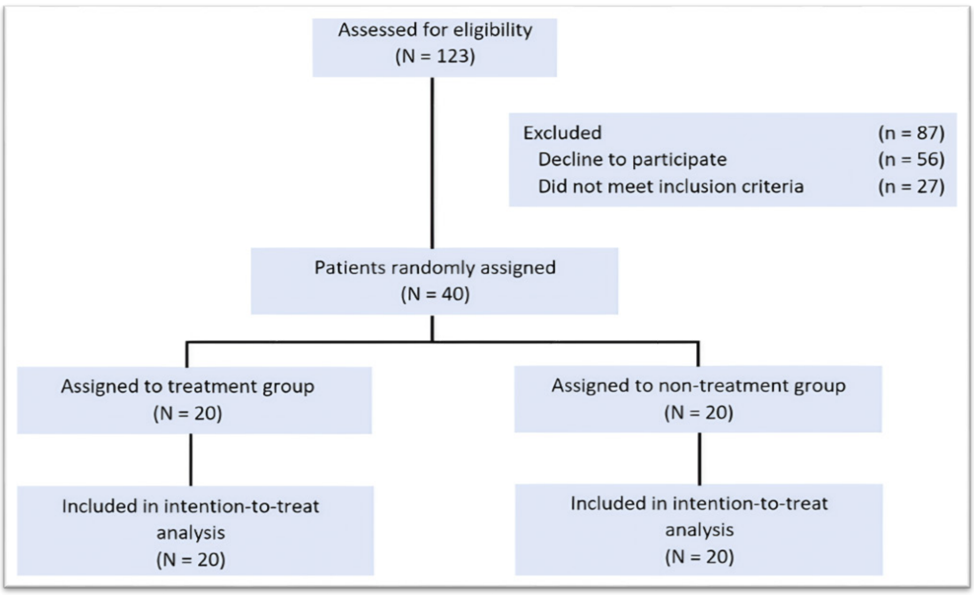

Figure 1. CONSORT Diagram. Patients were randomly assigned to receive MFH management either with Ciprofloxacin and Metronidazole solution (treatment group) or with 0,9% NaCl (non-treatment group)

Table 2. Descriptive and Normality of CFU Pre-Test and Post-Test

CFU	Group	Mean ± SD	Median (min-max)	p
Pretest	Non-treatment	201.80±75.65	191.5 (71 – 337)	0.302*
	Treatment	204.55 ± 55.82	198 (120 – 348)	0.541*
Post-test	Non-treatment	191.45±69.36	184.5 (68 – 330)	0.704*
	Treatment	101.05±14.07	103.5 (78 – 127)	0.522*

The average value obtained on the CFU of the non-treatment group before wound care management is 201,800 CFU/ml and 191,450 CFU/ml after wound care. While the average value of the treatment group before wound care management was 204,550 FCU/ml and after wound care was 101,050 CFU/ml. The data obtained is tested for normality with Shapiro-Wilk, the value of p >0.05, 95% CI, were obtained for all groups, so it can be concluded that the data is normally distributed (Table 2).

Table 3. Differences between CFU on Pre-test and Post-Test

Group (95% CI)	CFU	P value	
	Pre-Test	Post-test	
Non-treatment	201.80±75.65	191.45±69,36	<0.001*
Treatment	204.55 ± 55.82	101.05±14.07	<0.001*

In different paired T-tests conducted in the CFU pre-test and post-test, there are significant differences in both the non-treatment group (p <0.001) and the treatment group (p <0.001). Further calculation of the difference (delta) of the CFU pre-test and post-test in the non-treatment group and the treatment group was calculated (Table 3).

Table 4. Differences in Delta CFU of the Non-Treatment Group and the Treatment Group

Group	Mann Whitney-U	
	Delta CFU	p
Non-treatment	10.35±10.01	<0.001*
Treatment	103.50±10.01	

The Mann Whitney-U difference test is used to test the difference in the degree of reduction in CFU between the non-treatment group and the treatment group and showed a significant difference (p<0.001) (Table 4).

Table 5. The Descriptive and Normality of Fibrosis Pretest and Post-Test

Fibrosis	Group	Mean ± SD	Median (min-max)	p
Pretest	Non-treatment	40.77 ± 28.02	34.5 (1.3 – 85.83)	0.158*
	Treatment	25.01 ± 18.09	21.5 (5 – 68.33)	0.134*
Post-test	Non-treatment	60.25 ± 23.34	60.42(17.5–90.83)	0.200*
	Treatment	51.44 ± 19.79	49.42 (20 – 78.33)	0.200*

The data normality test was carried out with Shapiro-Wilk and normally distributed data were obtained with p >0.05, 95% CI, for all groups, both pre-test, and post-test groups (Table 5)

While the average value of the treatment group before wound care management was 204,550 FCU/ml and after wound care was 101,050 CFU/ml. The data obtained is tested for normality with Shapiro-Wilk, the value of p >0.05, 95% CI, were obtained for all groups, so it can be concluded that the data is normally distributed (Table 2). In different paired T-tests conducted in the CFU pre-test and post-test, there are significant differences in both the non-treatment group (p <0.001) and the treatment group (p <0.001). Further calculation of the difference (delta) of the CFU pre-test and post-test in the non-treatment group and the treatment group was calculated (Table 3). The Mann Whitney-U difference test is used to test the difference in the degree of reduction in CFU between the non-treatment group and the treatment group and showed a significant difference (p<0.001) (Table 4).

Fibrosis

The data normality test was carried out with Shapiro-Wilk and normally distributed data were obtained with p >0.05, 95% CI, for all groups, both pre-test, and post-test groups (Table 5). Furthermore, the difference (delta) of fibrosis pre-test and post-test in the non-treatment and treatment groups were calculated (Table 6). The difference Mann Whitney-U test was used to test the difference in the degree of fibrosis reduction between the non-treatment group and the treatment group and showed a significant difference (p=0.046) (Table 7).

Table 6. Differences in Fibrosis Pretest and Post-Test in the Control and Treatment Groups.

Group	Fibrosis	p	
	Pre-Test	Post-test	
Non-treatment	40.76± 28.02	60.25 ± 24.34	<0.001*
Treatment	25.01± 18.08	51.44 ± 19.79	<0.001*

Furthermore, the difference (delta) of fibrosis pre-test and post-test in the non-treatment and treatment groups were calculated (Table 6).

Table 7. Delta Fibrosis Difference between Control and Treatment Groups

Group	Mann Whitney-U	
	Delta Fibrosis	p
Non-treatment	19.48 ± 9.12	0.046*
Treatment	26.43±11.96	

The difference Mann Whitney-U test was used to test the difference in the degree of fibrosis reduction between the non-treatment group and the treatment group and showed a significant difference (p=0.046) (Table 7).

Table 8. Kendall's Tau-b Correlation Test Results between Delta CFU and Delta Fibrosis in the Treatment Group

Variable	Mean ± SD	p	r	Information
Delta CFU	-103.50 ± 46.13	0.027*	- 0.360	Significant,
Delta fibrosis	26.43±11.96			Negative correlation

The average decrease in the number of bacterial colonies was 103,500 CFU/ml and the average increase in fibrosis was 26.43%. The relationship between the decrease (delta) of the CFU and the increase (delta) of fibrosis was shown from Kendall's tau-b correlation test in the treatment group with p = 0.027 with a correlation coefficient of r = -0.360, so it can be concluded that there is a relationship/correlation with a weak negative direction (Table 8).

The relationship between the number of CFU and fibrosis

The average decrease in the number of bacterial colonies was 103,500 CFU/ml and the average increase in fibrosis was 26.43%. The relationship between the decrease (delta) of the CFU and the increase (delta) of fibrosis was shown from Kendall's tau-b correlation test in the treatment group with p = 0.027 with a correlation coefficient of r = -0.360, so it can be concluded that there is a relationship/correlation with a weak negative direction (Table 8).

All patients who receive MFW wound management using either ciprofloxacin-metronidazole or 0.9% NaCl solution had no allergic reactions in the wound area and surrounding areas. The swabbing procedure on the area of the cancer wound to analyze the number of bacterial colonies was a safe procedure. Similarly, the biopsy incision procedure at the transitional boundary between healthy skin and cancerous wound, using topical anesthesia is a safe and comfortable procedure for those with controlled bleeding. All procedures performed during the pre-test and post-test were passed safely without any complications in all patients.

Discussion

The variables of age, hemoglobin and BMI in this study are not confounding variables. It was found that the treatment of locally advanced malignant fungating wound of breast cancer wounds given chemotherapy using moist gauze Ciprofloxacin 200 mg / 100 ml and metronidazole 500 mg / 100 ml caused a decrease in CFU with an average value of 103,500 CFU / ml, which was significantly different (p <0.001) when compared to the average decrease in CFU in wound care using moist gauze NaCl 0.9% of 10,350 CFU / ml. This is in line with the recommendation of EONS (European Oncology Nursing Society) where due to tumor involvement/infiltration in vascular and lymph flow in malignant fungating wound causing doubtful effectiveness of systemic antimicrobials, metronidazole solution and ciprofloxacin solution are potent antimicrobials for aerobic and anaerobic bacteria through topical administration (wound care with moist gauze metronidazole solution and ciprofloxacin solution) [7, 9, 13, 21, 22, 23].

It is found that the wound treatment of the locally advanced stage of malignant fungating wound breast cancer with chemotherapy using moist gauze of Ciprofloxacin 200 mg/100 ml and metronidazole 500 mg/100 ml caused a decrease in CFU with an average value of 103,500 CFU/ml, which is significantly different (p <0.001) when compared to the average CFU reduction in wound care using 0.9% NaCl moist gauze by 10,350 CFU/ml. This is in line with the recommendations of EONS (European Oncology Nursing Society) where due to tumor involvement/infiltration in the vasculature and lymph flow in malignant fungating wounds, the effectiveness of systemic antimicrobials is doubtful. Metronidazole solution and ciprofloxacin solution are potent antimicrobial agents for aerobic and anaerobic bacteria with local administration (wound care management using moist gauze metronidazole and ciprofloxacin solutions) [21, 24, 25, 26].

In this study, it is found that there is a relationship between the decrease in the CFU and the increase in fibrosis formation in the treatment group of wound management in locally advanced-stage malignant fungating wound breast cancer. This shows that the fibrosis process in this study is represented by fibroblast proliferation, not only influenced by infectious factors, namely the source of fibroblasts from local mesenchymal cells, EMT (Epithelial–mesenchymal transition) processes from endothelial and epithelial cells, bone marrow sources, and other factors that affect the process, including the therapy given [22, 27, 28, 29].

The limitations of this study include recall bias in collecting data on medical history, history of allergies, history of drug use and traditional medicine, and not assess intervening variables such as PDGF, Bfgf, TGF-β, IL-1, IL-13, IL-21 TNF, PPAR, VEGF, and Renin-Angiotensin.

In conclusion, the treatment of locally advanced-stage malignant fungating wound breast cancer treated with chemotherapy and wound care management using a moist gauze solution of metronidazole and ciprofloxacin showed significant improvement in the repairment of the wound. It improved the rate of reducing the number of germs or CFU and also increased fibrosis. This present study shows that the wound treatment of the locally advanced stage of MFW breast cancer with chemotherapy using moist gauze of Ciprofloxacin and metronidazole could decrease the CFU significantly compared to the wound care using 0.9% NaCl.

This study also shows a relationship between a decrease in the number of bacterial colonies (CFU) and an increase in the degree of fibrosis in the treatment group, which may be due to the involvement of blood vessels and lymphatics. These findings may promise to improve patient quality of life.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

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Ethical Approval

The experiment was approved by the Research and Ethics Committee of the Faculty of Medicine Diponegoro University, Indonesia (protocol number: 03/EC/KEPK/FK-UNDIP/I/2022), and explains that there are no issues.

Conflict of Interest

All authors declare no conflicts of interest.

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