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Evaluation of bacterial infection of split-thickness skin grafts at the Korle Bu Teaching Hospital

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ABSTRACT

Background: Split skin grafts are frequently employed to provide biological cover for extensive wounds. The clinical outcome of skin grafts depends on a variety of factors of which infection is one of the most important. The intent of this study was to define the micro-organisms causing skin graft infections and failures at the National Reconstructive Plastic Surgery and Burns Centre (NRPSBC) at the Korle Bu Teaching Hospital (KBTH).

Aim: The study assessed the extent to which bacterial infection of grafted wounds resulted in graft failure and subsequent re- grafting. Materials and Methods: The study was a longitudinal study conducted on the wards of the NRPSBC at the KBTH on patients with wounds who received split skin grafts. Wound swabs of discharging grafted wounds were inoculated into a Stuarts' transport medium to prevent desiccation and transported immediately to the microbiology laboratory for further processing.

Results: Fifteen (20.8%) of the grafts failed to take. The incidence of infected grafted wounds was 79.2% (57). Infected grafted wounds that resulted in graft failure were 14 out of 57 infected wounds (24.6%). *Pseudomonas aeruginosa* and Other *Pseudomonas* Species were identified as the bacteria frequently involved in graft failure at the NRPSBC.

Conclusion: In this study, we found a graft failure rate of 20.8%. This was influenced by the bacterial load present in the graft bed.

Key words: Split skin Graft, Take of graft, Graft Failure, Infection, Prophylactic antibiotics.

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INTRODUCTION

Skin grafts can be applied in a variety of conditions, such as traumatic wounds, defects after tumour resection, burn reconstruction, scar contracture release etc.[1-3] The clinical outcome of skin grafting depends on the variety of factors. The complication rate was varied between 2% - 30%.[4-8] The second most common cause of graft loss is infection; with hematoma formation is the most common.[9] Henderson et al.,[10] reported 15% graft failure rate due to infection. Similarly, seroma formation may prevent graft adherence to the underlying wound bed, avoiding the graft from receiving the necessary nourishment, as detailed above. Movement of the graft or shear forces may also lead to graft failure through disruption of the fragile attachment of the graft to the wound bed.[10,11] Another common source of failure is the poor recipient site. The wound may have poor vascularity, or the surface contamination may have been too significant to allow graft survival. Technical errors such as applying the graft dermis side in the superficial will result in complete graft loss. Using excess pressure, stretching the graft too tightly, or handling the graft in other traumatic ways may lead to partial or complete graft failure. Teh studied 21 patients with stasis ulcers in an attempt to pinpoint the causes of graft failure by assayed the wound exudates for fibrin degradation products, fibrinogen, available plasminogen, and active plasmin.[12] All wounds showed granulation tissue and were classified as clean or dirty. Fresh wounds had low bacterial counts and showed no detectable plasmin activity. Nasty wounds had high bacterial counts and increased levels of active plasmin. High plasmin and proteolytic enzyme activity were generally seen in wounds contaminated with beta-hemolytic Streptococci and various species of Pseudomonas. The presence of fibrin under auto-grafts was associated with success in 17 of 21 ulcers and the absence of fibrin was associated with graft failure. This finding suggests that dissolution of fibrin by plasmin and proteolytic enzymes is the probable mechanism in graft failure secondary to microorganisms. This study was designed to assess the extent to which bacterial infection of grafted wounds results in graft failure and subsequent re-grafting.

METHOD

Study Design

The study was a longitudinal analytical study. All patients with burn wounds and acute ulcers admitted to the NRPSBC at the KBTH during the period of the study who required split skin grafting as part of their treatment. This data was collected

over a period of nine months from May 2016 to January 2017. We excluded patients with chronic ulcers that shows no tendency to heal after three months of appropriate treatment or is still not fully recovered at 12 months.[13] Weight-bearing plantar ulcers and patients with previously failed skin grafts were also excluded. The sample has been calculated by the sample size formula for longitudinal study plus 10% drop out and estimated about 72 samples.[14] We measured the primary outcomes such as graft failure, graft infection, and re-grafting; then secondary outcomes such as type of bacteria, drug susceptibility, and length of stay after split-thickness skin grafting. Graft failure was defined as loss of split skin graft that will require re-grafting of the wound bed. A split-thickness graft wound was declared infected if there was Purulent discharge with or without laboratory confirmation from the wound bed; organisms isolated from an aseptically obtained culture of fluid or tissue from the wound bed in the presence of systemic manifestations of infection; and localized pain or tenderness; localized swelling and localized warmness to touch. University of Ghana Ethic committee has approved this study with number CHS-Et/M.3 – P 3.6/2015-2016.

Procedure

Patients' wounds were swabbed pre-operatively, in theatre before the operation site was prepared. All operations performed under either general or regional anaesthesia with prophylactic antibiotics (Intravenous Cefuroxime- Child 1 month -18years: 50mg/kg, Adult: 1.5gm). Skin grafts performed by standard operating techniques and split-thickness skin grafts harvested using a Graft knife or a Dermatome. We used the graft knife with the distance between the roller and the blade was kept constant with the wheel locked between calibrations 1 and 2; the Dermatome was with calibration 0.10 inches, both to ensure the graft thickness was similar. The grafts were secured to the wound beds with sutures or staples and with immobilizing devices if applied onto free surfaces. All patients routinely used intravenous antibiotics Cefuroxime (Child 1 month to 18years: 20mg/kg every 8 hours, Adult: 750mg every 8 hours). A dose was given intra-operatively and regular doses were given post-operatively for five days and then on oral Cefuroxime (Child 3 months-12 years: 30 mg/kg/day suspension PO in 2 divided doses, or Adult: 500mg 12 hourly for) from POD 6 to POD 14. Subsequently, the grafted sites were swabbed on postoperative day 5. The 'take' of the graft was assessed by any of the three specialists with blinded to the results of the cultures on postoperative days 5, 8 and 14. The graft 'take' recorded as a percentage. On a postoperative day 14, patients classified as Good Graft Take or Graft Failure. If the result was graft failure, wound dressing continued and antibiotics adjusted as per the results of the wound swabs and sensitivity.

Microbiology Analysis

Microbiological analysis was conducted at the Medical Microbiology Department of the School of Biomedical and Allied Health Sciences of the University of Ghana Medical School. Two specimens collected using cotton swab sticks and transported immediately to the laboratory. One wound swab was inoculated onto sheep blood agar, chocolate agar and MacConkey agar for aerobic culture and all of them incubated at 37°C for 24–48h with 5% carbon dioxide enrichment for chocolate agar plates to allow for the growth of fastidious organisms. Bacterial identification by Gram-stain microscopy and by conventional biochemical methods (Simmonds Citrate, Triple sugar iron, catalase and oxidase test, etc.). The Minibact-E (Rosco Diagnostica) used for Enterobacteriaceae which could not be identified by conventional methods. The second swab stick was used to determine the bacterial load in the sampled wound. The tip of the cotton swab was cut into a falcon tube. One ml of physiological saline was pipetted onto the cut tip and vortexed to get a neat solution. Three serial dilutions were performed from a smart solution. One ml of the three dilutions, including the intelligent answer, were inoculated individually onto trypticase soy agar and swirled around to cover the surface of the plate. The plates were incubated at 37 degrees Celsius. Colony counts were determined for plates with colonies numbering between 30-300 colony-forming units. Colonies were higher than 300 were considered as too numerous to count.

$$\text{Bacterial Load} = \frac{\text{Colony count (CFUs)on an agar plate}}{\text{Total dilution of tube (used to make plate for colony count)} \times \text{volume plated}}$$

Antimicrobial susceptibility testing for aerobic bacterial isolates was done by Kirby-Bauer disc diffusion method following the Clinical Laboratory Standards Institute (CLSI) guidelines.

The use of ImageJ® in Graft take measurements

ImageJ® image analysis program created at the National Institutes of Health[15] was used to measure the area of the wound covered by the graft as well as the raw area(s) (i.e. the area not covered by the graft).

RESULTS

Patient Demography

In total, 72 patients were included in the study. The median age of the patients was 30 years, (range of three months – 67 years) with a mean of 32±19 years old. Patients aged 18-29 years formed almost a third of the study population (30.6%). Men outnumbered women (54.2% vs 45.8%) while 53.5% of the patients above 18 years were found to be obese or overweight (Table 1). The duration of ulcer pre-surgery ranged between 8 days and 90 days. Over half of the wounds (54.2%) were grafted from day 22 to day 60 of ulcer existence. No patient had pre-operative hemoglobin below 8g/dl however, 2.8% had post-operative hemoglobin below 8g/dl. Twenty-two patients (30.6%) required blood transfusion postoperatively. Eight patients (11.1%) had diabetes. Seven patients were Hypertensive (9.3%) and one patient (1.4%) had sickle cell disease. More than half (51.4%) of the patients stayed in the hospital for 22 – 60 days post-SSG.

Wound Outcome and Bacteriology

The overall SSG failure rate was 20.8%. (Table 2). Analysis of the wound swabs taken immediately before grafting showed that 64 (88.9%) had been contaminated, 8 (11.1%) being sterile. The graft failure rate amongst the wounds with positive wound swabs was 23% while the failure rate among those with negative cultures was 12.5%.

Table 1 Demographic and clinical characteristics of study participants

Characteristic		Proportion n, %
Age range (years) (N=72)		3/12-67.0
Median age [Interquartile Range] (years)		30 [19-47.5]
Mean age (years)[Standard Deviation](N=72)		32 [19]
Age group (N=72)	< 18 years	15 (20.8)
	18-29 years	22 (30.6)
	30-39 years	9 (12.5)
	40-49 years	9 (12.5)
	50-59 years	12 (16.7)
	> 59 years	5 (6.9)
Gender (N=72)	Male	39 (54.2)
	Female	33 (45.8)
BMI category (N=58)	Normal	27 (46.5)
	Overweight/Obese	31 (53.5)
Ulcer aetiology (N=72)	Trauma	28 (38.9)
	Burns	26 (36.1)
	Cellulitis	6 (8.3)
	Post ex tumour	5 (6.9)
	Flap site	3 (4.2)
	Fasciitis	2 (2.8)
	SSG donor site	2 (2.8)
Ulcer duration (N=72)	8-14 days	9 (12.5)
	15-21 days	8 (11.1)
	22-28 days	19 (26.4)
	29-60 days	20 (27.8)
	61-90 days	16 (22.2)
Pre-operative haemoglobin category (N=72)	8-10 g/dL	12 (16.7)
	>10 g/dL	60 (83.3)
Post-operative haemoglobin category (N=72)	< 8 g/dL	2 (2.8)
	8-10 g/dL	31 (43.1)
	>10 g/dL	39 (54.2)

Table 1 *Continue*

Characteristic		Proportion n, %
Diabetes (N=72)	Present	8 (11.1)
	Absent	64 (88.9)
Hypertension (N=72)	Present	7 (9.3}
	Absent	65 (98.6)
Sickle cell disease (N=72)	Present	1 (1.4)
	Absent	71 (98.2)
Length of hospital stay (N=72)	14-21 days	13 (18.1)
	22-28 days	22 (30.6)
	29-60 days	15 (20.8)
	61-90 days	9 (12.5)
	> 90 days	13 (18.1)
Blood transfusion given (N=72)	Yes	22 (30.6)
	No	50 (69.4)

Table 2 **SSG Outcome**

Graft Outcome	Number of Patient, n (%)
Failure	15 (20.8)
Successful	57 (79.2)

Table 3 **Pre-operative wound culture results in relation to graft outcome**

Characteristic	Graft Outcome			
	Failure	Successful		
Pre-operative wound swab	**n (%)**	**n (%)**	**Odds Ratio [95% CI]**	**p-value**
Positive Cultures	14 (21.9)	50 (78.1)	2.14 [0.24-102.91]	0.483
Negative Cultures	1 (12.5)	7 (87.5)	1.00	

Table 4 **Post-operative wound culture results in relation to graft outcome**

Characteristic	Graft Outcome			
	Failure	Successful		
Post-operative wound swab	**n (%)**	**n (%)**	**Odds Ratio [95% CI]**	**p-value**
Positive Cultures	14 (24.6)	43 (75.4)	4.56 [0.58-206.26]	0.129
Negative Cultures	1 (6.7)	14 (93.7)	1.00	

Pre-operative bacteria culture was not associated with graft outcome (p=0.483) (Table 3). Wound swabs taken on POD 5 showed that almost 80% of wound beds (n=57; 79.2%) still had bacteria. Of the wounds with positive wound swabs, the rate of graft failure was 24.6% while the failure rate was 6.7% in the scars with negative cultures. Post-operative day five bacteria culture was not associated with graft outcome (p=0.129) (Table 4). The most common bacterial species detected in the pre-operative wounds was Pseudomonas sp., which was isolated from 23 (35.9%) of 64 ulcers with bacteria isolated. Other common species found were *Pseudomonas aeruginosa* (34.4%) and *Staphyloccocus aureus* (10.9%). The most frequently cultured bacteria in the graft failure percentage were: Pseudomonas sp. (26.1%); Pseudomonas aeruginosa (27.3%) and Proteus mirabilis (30%) (Table 5). Overall, the most common bacterial species detected in the wounds on POD5 was *Pseudomonas* spp. Other common species found were Pseudomonas aeruginosa (26.3%) and *Staphyloccocus aureus* (10.6%).

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Table 5 Types of pre-operative bacteria cultured in relation to graft outcome

Organism	Wounds Contaminated by Organism N=64 n (%)	Graft outcome with organism present	
		Successful Graft N = 49 n (%)	Graft Failure N= 15 n (%)
Diptheroids	2(3.1)	2 (100)	0 (0)
Klebsiella pneumonia	2 (3.2)	2(100)	0 (0)
Proteus mirabilis	10 (15.6)	7 (70)	3 (30)
Proteus vulgaris	1 (1.6)	1 (100)	
Providentia stuarti	1 (1.6)	1 (100)	0 (0)
Pseudomonas aeruginosa	22 (34.4)	16 (72.7)	6(27.3)
Pseudomonas sp	23 (35.9)	17(73.9)	6(26.1)
Staphyloccocus aureus	7 (10.9)	6(85.7))	1 (14.3)
Unidentified GNR	1 (1.6)	1(100)	
Yersinia enterocolitica	1(1.6)	1 (100)	
Citrobacter sp	1 (1.6)	1 (100)	
Shigella sp.	1 (1.6)		1(100)

**1% are column percentages and may not add up to > 100 as one patient may have more than one bacteria cultured.*

Table 6 Types of post-operative bacteria cultured in relation to outcome

Organism	wounds contaminated by organism N= 57 n (%)	Graft outcome with organism present	
		Successful Graft N = 43 n (%)	Graft Failure N=14 n (%)
Diptheroids	3 (5.3)	3 (100)	0 (0)
Escherichia coli	1 (1.8)	1 (100)	0 (0)
Hafnia alvei	1 (1.8)	0 (0)	1 (100)
Klebsiella pneumonia	4 (7.0)	1(25)	3(75)
Proteus mirabilis	9 (14)	4 (44.4)	5 (55.6)
Providentia stuarti	2 (3.5)	1 (50)	1 (50)
Pseudomonas aeruginosa	15 (26.3)	11 (73.3)	4(26.7)
Pseudomonas spp	32 (56.1)	25 (78.1)	7(29.9)
Staphyloccocus aureus	6 (10.5)	4 (67)	2 (33)

**1% are column percentages and may not add up to > 100 as one patient may have more than one bacteria cultured.*

Injuries with isolated Pseudomonas spp. 30% resulted in graft failure while *Pseudomonas aeruginosa* just 26.7% resulted in graft failure. When *Staphyloccocus aureus* was isolated from the wound, 33% resulted in graft failure and *Klebsiella pneumonia* 75% resulted in graft failure. (Table 6)

DISCUSSION

A variety of factors are believed to influence skin graft take adversely; hematoma, shearing movements,[16] inadequate compliance, deficient blood supply,[17] are examples. Infection is said to be the second most frequent cause of Skin graft loss.[9] By using current best skin grafting techniques, we achieved results comparable with that recorded in the literature of 2-30 %.[4-8]

The most frequently encountered bacteria in the positive bacterial cultures for pre-operative swabs and POD5 swab was *Pseudomonas* spp followed by *Pseudomonas aeruginosa*. Together these two accounted for over 70 % and 80% of all the bacteria cultured pre-operatively and on POD 5 respectively. Gjødsbøl et al.,[18] also reported that more than half of the leg ulcers (52,2%) investigated in their study, concerning chronic venous leg ulcers, were colonized by *P. aeruginosa*, thus emphasizing the possible relevance of Pseudomonas in getting to the core of the problem. Wounds with positive cultures displayed a higher failure rate as compared with

injuries with negative perceptions. These results show that the majority of graft failures had positive bacterial cultures. This work showed that positive bacterial culture was not associated with a higher failure rate as compared to wounds with negative perceptions which is similar to work already published.[19]

The data collected in this work did not suggest that the presence of specific strains, such as *P. aeruginosa* or *S. aureus*, pre-operatively results in a suboptimal outcome. Wounds containing either *P. aeruginosa* or *S. aureus* did not appear to have a minor issue. This study is contrary to that of Jackson et al.,[20] which stated that the isolation of Pseudomonas from an ulcer immediately before skin grafting significantly impairs skin graft take. In most cases, the presence of bacteria did not appear to affect graft make significantly. However, in wounds with *Klebsiella pneumonia*, *Proteus mirabilis*, and *Providentia stuarti* when cultured on POD5 resulted in the graft failure rate of over 50%. *Staphylococcus aureus*, when present on an ulcer has a one out three resulted in graft failure (32%) this serves to confirm what has previously reported.[21]

Wounds that had graft failure had higher bacteria counts generally. Wounds grafted from 15–21 days had 100% success rates followed by wounds grafted on days 8 to 14. These were found to have the least bacteria counts of 3.75540×10^{5} and $9.10770\times10^{5,}$ respectively. Generally, it was noticed that for the ulcers of the various etiologies, the number of bacteria cultured had a correlation with the success or failure of the graft. The exceptions were with the flap site grafts and SSG donor site where bacteria were cultured in situations where the graft was successful while there was no culture of bacteria in the graft failure.

There has been a school of thought which insists that the number of organisms a wound bed has is critical.[11] Another analysis suggests that the type of bacteria present is more critical.[22] Pseudomonas, according to work done by Jackson et al.,[20] were reported to cause skin graft failure in burn patients. This work agrees with the former school of thought.

All patients for this study were placed on routinely used Intravenous antibiotics Cefuroxime intra-operatively and post-operatively for five days. Antibiotics continued with oral Cefuroxime from POD 6 to POD 14. *Pseudomonas* spp and *Pseudomonas aeruginosa* together they constituted over 70 % and over 80% of all the bacteria cultured pre-operatively and on POD 5 respectively. This study thus demonstrates that the practice of routine administration of Cefuroxime is not appropriate since cefuroxime does not have anti-pseudomonad activity. Findings from this study suggest that the choice of antibiotics for use in purposes of skin grafting in the unit must contain a combination of medicines with antipseudomonal properties. These include Quinolones, e.g. Ciprofloxacin, Levofloxacin) Aminoglycosides (e.g. Amikacin, Gentamycin) Carbapenems (e.g. Imipenem, Meropenem, Doripenem) tazobactam piperacillin, Colistin and Ceftazidime.[23] As was stated by Henderson et al.,[10] peri-operative antibiotics may be a significant factor in improving graft take and should be the subject of a definitive trial.

Limitations

We were unable to determine pre-operatively whether the organisms cultured from the wound swab indicated infection and not just contamination or colonization. Confirmatory wound biopsies that would have confirmed this were not performed. However, Lawrence et al.,[24] have shown that quantitative methods of bacterial isolation from a burn wound that has been dressed give no more useful information than surface swabbing. Additionally, Jones et al.[25] found that surface swabbing of leg ulcers in people with diabetes yielded the same bacteria as swabs taken from the ulcer base when any slough and exudate had been removed. Furthermore, studies were done by Steer et al.,[26] suggest that wound swabs can reliably establish the presence of *Pseudomonas, Staphylococcus* and *Streptococcus species* with accuracy in the range of 92–95% compared with tissue biopsy.

CONCLUSIONS

In this study, we found a graft failure rate of 20.8%. This study was not associated with sterility or otherwise of the pre-operative graft bed. However, it seemed to be influenced by the bacterial load present in the graft bed. *Pseudomonas* spp and *Pseudomonas aeruginosa* were the most common bacteria cultured in the pre and post-operative periods. This study suggests that the routine use of cefuroxime intra-operatively and post-operatively for SSGs, which is the protocol at the NRPSBC needs to re-evaluate.

AUTHOR CONTRIBUTION

All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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