Does the clinical status of an ulcer correlate with the bacterial count of the ulcer biopsy

To identify the clinical appearance of the ulcer that may be predicted as being infected through the correlation of bacterial count with the clinical state of ulcer. Thirty four consecutive chronic lower limb ulcers were prospectively studied in a nine month period. The bacterial count was determined on the biopsy specimen of each ulcer. A correlation between this count and each of two methods of assessment of chronic ulceration was sought. In 27 cases, one single organism was cultured from the biopsy specimen. The most commonly cultured organisms were Klebsiella in 15, and pseudomonas in 14 cases. Although the abdefs' method of assessment of ulcers correlates better (than clinical status method of assessment) with the organism count, the results were statistically insignificant in both instances. The correlation coefficient, r, however fell within the 95% confidence interval in both instances.

Keywords: ***chronic ulcer, correlation,bacterial count, biopsy,***

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Histologic examination of a wound biopsy is the only reliable means of making the diagnosis of invasive wound infection. Facilities for such histologic examinations are not available in peripheral and some general hospitals in Nigeria. Whereas peripheral wounds particularly chronic ulcerations in the lower limbs are prevailent in the tropics, treatment of infection of such ulcers to forestall complications like underlying bone infection and septicaemia will be unsatisfactory unless prompt diagnosis of wound infection is carried out.

Since quantitative cultures of the wound appear to be helpful in confirming absence of wound infection[1], an attempt at the correlation of organism count with the state of the wound should help in determining wounds without invasive infection. The aim of this study therefore is to identify the clinical appearance of the ulcer predicted as being infected.

Patients and Methods

Thirty four consecutive cases of chronic ulcers situated in the lower limb were entered into the study. These were found in patients attending the surgical and general outpatient clinics of University College Hospital, Ibadan. The study period was nine months from January to September 1996. Only ulcers due to trauma and non specific infection were considered.

The ulcers were assessed using two different methods, each method being utilised by one and the same examiner. In one method, the abdefs' scoring method, consisting of scoring the ulcer based on certain features of an ulcer that begin with the first few English alphabets (excluding letter c), points were alloted as follow:

A) Aetiology– 1. local e.g. trauma, infection
2. controlled systemic disease
3. systemic disease, uncontrolled
4. malignancy

B) Base– 1. soft, mobile
2. hard, fixed

D) Discharge– 1. slight to moderate
2. copious, purulent, etc.

E) Edge– 1. flat, shelving, punched out
2. undermined, raised

F) Floor– 1. predominantly granulation
2. predominantly sloughy

S) Size– 1. less than or equal to 2.5 cm in one dimension
2. greater than 2.5 cm in one dimension.

Thus the highest score obtainable by any ulcer is 4+2+2+2+2+2=14 denoting the poorest state of ulcer.

In the other method, termed clinical status scoring method, points were alloted as follows:

0. ulcer healed.
1. ulcer very healthy, will heal with conservative management
2. ulcer with healthy granulation, ready for skin grafting
3. slightly uneven or slightly unhealthy granulation
4. predominantly granulation but with small area of slough
5. moderately sloughy, but interspersed with some granulation
6. very sloughy and very little granulation seen at the surface
7. very sloughy and discharging moderately
8. dirty ulcer (slough all over and discharging copiously) but no local or systemic spread
9. dirty ulcer with local or systemic spread

A wound biopsy was taken after injecting biopsy site with xylocaine/adrenaline local anaesthetic. The site of biopsy was at 12 o'clock, that is, the superior pole of the ulcer. An ellipse of ulcer base was taken and transported in normal saline within a sterile universal container to the microbiology laboratory. Bacterial count was determined on each specimen, so also routine aerobic culture as follows: Pieces of tissue were weighed in a sterile universal container. One ml. of Ringers' solution was added to the tissue and homogenised in a sterile tissue grinder. Using commercially prepared standard wire loops, each homogenate was inocculated in well dried blood agar and MacConkey agar plates. Similarly, ten fold dilutions were also made in Ringer's solution for surface count using the Miles and Misra technique[2,3]. All plates were incubated aerobically at 37°C. All bacterial isolates were identified by standard bacteriological methods[4]. From the result, the number of colony forming units per gram of tissue was determined. Antibiotic sensitivity tests were carried out by agar diffusion method using commercially prepared antibiotic discs[2,5]. Histologic examination was not performed.

The methods of assessment of ulcers were compared using linear regression. The bacterial count was correlated with each of the methods of assessment. The analyses were perfomed using epistat and epiinfo statistical programmes. Level of statistical significance was taken to be ≤ 0.05

Results

The mean (s.d.) clinical status score of the ulcers was 4.1 (1.6), table i. This means that they consisted predominantly of granulation with a small area of slough. Mean abdefs' score was 8.1 (1.3). In

Table i: *Linear regression of abdefs' score on clinical status score*

Case	Abdefs' score	Clinical score
1	8	4
2	9	5
3	7	2
4	8	3
5	8	3
6	9	4
7	8	2
8	8	6
9	7	3
10	8	3
11	9	4
12	7	4
13	7	6
14	8	6
15	8	3
16	9	6
17	7	4
18	11	6
19	11	6
20	8	4
21	8	9
22	9	4
23	6	2
24	6	3
25	7	1
26	8	4
27	8	3
28	8	3
29	8	3
30	7	4
31	8	5
32	7	4
33	11	5
34	11	5
No	34	34
Mean	8.15	4.09

regression equation: $y = -0.34 + 0.54x$
where x = abdefs' score
y = clinical score
T = 2.83
dt = 32
p = 7.9 x 10–3 that is $p < 0.01$

27 cases one single organism was cultured from the biopsy specimen, table ii, while in each of five cases, two organisms were cultured. Culture yielded no growth in two cases. The most commonly cultured organisms were Klebsiella in 15, and pseudomonas in 14 cases. Proteus was cultured in five cases while in three others, Staphylococcus albus was cultured.

Linear regression and correlation results are expressed in table i, and addenda to table 1. It will be noticed that abdefs' method of assessment of ulcers associates well with clinical status scoring method, $T = 2.83$; $df = 32$; $p < 0.01$. Thus the equation required to calculate the clinical status score when an abdefs' score is known and vice visa is found in table i. Better correlation of the organism count exists for abdefs' scoring method $p = 0.18$, than for clinical status scoring method $p = 0.81$ (addendums to table i). However in both instances the results were not statistically significant. The 95% confidence interval obtained at the correlation of organism count with

Table ii: *Bacteriology of 34 cases with chronic leg ulcer*

CASE	BACTERIA isolated	SENSITIVITY (see key below)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	proteus mirabilis						s			s	s	s	r			
2	klebsiella spp						s			r	s	s	r			
3	no growth															
4	staph albus															
5	Proteus mirabilis					s	r	s				s	r	s		
	pseudomonas aeru					s	s					s	r	s		
6	proteus mirabilis					s	s	s			r	r	s			
	klebsiella spp					s	s	s			r	r	s			
7	proteus mirabilis	r		r		s	s	r		r		s				s
8	pseudomonas aeru					s	s					s				
9	staph albus															
10	pseudomonas aeru					s	s					s			s	s
11	klebsiella oxytocum	r				s	s	r			s	s				
12	pseudomonas aeru					s	s					s			s	s
13	staph albus															
14	klebsiella oxytocum	r		r		r		r			s	s	s			
15	klebsiella oxytocum	r		r		s		r		s		s	s			
16	pseudomonas aeru					s						s			s	s
17	proteus spp	r		r		r		r			r	s	r		s	
18	klebsiella spp	r		r		r		r			r	s	r		s	
	pseudomonas aeru					r					r	s	r			
19	pseudomcnas aeru					s	s					s			s	s
20	pseudomonas aeru					s	s				s	s			s	s
21	pseudononas aeru					r	s					s			s	s
22	pseudomonas aeru					s	s					s			s	s
23	klebsiella spp	r		r		r		r			s					
24	klebsiella oxytocum	r		r		r		r			s	s				
25	no growth															
26	klebsiella spp	r		r		r		r		r		s	s			
27	klebsiella spp	r		r		r		r			s	s	s		s	s
	pseudomonas aeru					r						s			s	s
28	klebsiella spp	r		r		s		r		s		s	s			
29	pseudomonas aeru					s					r	s			s	s
	klebsiella spp	r		s		s		r				s				s
30	klebsiella rhinosc	r		r				ı			s	s	s			
31	klebsiella spp	r		r		r		r			r	s			s	s
32	pseudomonas aeru					r					r	s			s	s
33	klebsiella spp	r		r		s	s	r		r		s				
34	pseudomonas aeru					s	s					s			s	s

Key:

1	tetracycline	2	chloramphenial	3	streptomycin	4	kanamycin	5	gentamycin
6	amikacin	7	cotrimoxazole	8	nalidixic acid	9	sulfamicllin	10	recephine
11	ceftazidime	12	zithromax	13	oflozacin	14	ciproxin	15	tarvid

s: sensitive
r: resistant

abdefs' and clinical status scoring methods are also indicated in the addendums to table i. The correlation coefficient, r, fell within the 95% confidence interval in both instances. Utilizing the linear regression equation, an abdefs' score of >8 should arouse a suspicion of local infection.

Addendum to Table I

Correlation of Abdefs' score on organism count

Correlation coefficient: r = –0.20
T = 1.37
df = 32
p = 0.18
95% confidence interval: –0.50 < r < 0.15

Linear regression equation: y = 8.34 – (1.97 x 10–7x)
where y: abdefs' score
x: organism count

Correlation coefficient: r = –0.20
T = 1.34
df = 32
p = 0.18
95% confidence interval: – 0.50 < r < 0.15

Addendum to Table I

Correlation of Clinical status score on organism count

Correlation coefficient: r = –0.08
T = 0.24
df = 32
p = 0.81
95% confidence interval: –0.26 < r < 0.40

Discussion

The abdefs' scoring method is a reliable means of evaluating ulcers. This subject is addressed in a paper that has been submitted for publication. It takes different aspects of the wound into consideration. For the purpose of this study an additional assessment of surrounding skin colour to reflect the colour change of inflamation might have been beneficial except for the fact that such colour changes might be difficult to decipher in blacks. Similarly, pain which is a symptomatic component of infection is a subjective feature and has therefore been excluded from the clinical evaluation.

Microscopy, culture and sensitivity of a swab of the surface of a wound or ulcer is an unreliable means of detecting the organisms that are responsible for infection in the wound. Often mixed surface contaminants are cultured. When part of the tissue is sent for examination, after, an incisional biopsy, as was in this study, single organisms are often cultured. Methods for quantitating surface flora have been described, but comparisons with biopsy specimens have been contradictory[6]. Quantitative bacteriology from burn wound biopsies confirms burn wound infection and improves patient management. Histological and microscopic examination of biopsy material from a burn wound permits early and accurate diagnosis of wound infection and enables the surgeon to alter care to control such infection[7].

Bacterial counts of less than 10^6 organisms per gram of tissue has been found to correlate well with absence of infection on histological examination. However, less than 50% of quantitative culture that shows a growth of greater than 10^6 per gram of tissue are associated with histological evidence of infection[8].

The "p" value is a measure of the strength of evidence against the "null" hypothesis. It is not the probability of the truth of the null hypothesis. Thus in this study, it can be said that abdefs' method of assessment correlates (with organism count) to a greater degree than clinical status scoring method. Frequently, statistical and clinical significance do not go together. This limitation has led to current encouragement in literature to move away from significance testing to using confidence interval[9,10]. This enables one to quote as has been done here, an estimated correlation along with a measure of the precision or reliability of that estimate[10].

Since histological and microbiological count facilities are not available in some centres, it is desirable to be able to predict the clinical state of an ulcer that may be considered infected. An abdefs' score of >8 should arouse a suspicion of local infection. Since wound infection may progress to cellulitis, and septicaemia, such ulcers may benefit from appropriate antimicrobial therapy. Cost considerations precluded a possible confirmation of our optimal abdefs' score by histology. This would have constituted a beneficial additional adjunct to this study.

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