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Bacterial load in tissues and its predictive value for infection in open fractures

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ABSTRACT

The role of quantitative bacteriology is considered controversial for the prediction of infection in open fractures. A study was done in 20 patients with open fractures. Post debridement pieces of skin, muscle and periosteal tissue were obtained for quantitative bacterial counts. Among a total of 50 samples from all of the tissues, 29 showed positive bacterial counts (16 of 20 skin, 11 of 20 muscle and 2 of 10 periosteum samples). By quantitative estimation, the bacterial load was $>10^5$ per gram in 10 skin and 3 muscle tissue samples. Infection developed in 9 of the 20 cases within one month, and eight of these patients had contamination of $>10^5$ per gram in 8 of the skin but only 3 muscle samples. It was concluded that with tissue specific bacterial load estimation, prediction of subsequent infection can be made if skin tissue contains $>10^5$ per gram, or if muscle tissue carries any level of bacterial presence.

INTRODUCTION

Sepsis, occurring in between 2% and 25% of all open fractures, leads to significant morbidity.[7] General factors such as age, shock, marked obesity, immunological status and metabolic disease, such as diabetes mellitus, influences the incidence of infection.[1,11] The risk of sepsis in open fractures is also increased by local factors which include the amount of devitalization, the type and site of fracture, the time lapse between injury and debridement, the mode of fracture fixation, and the timing of antibiotic administration[8]. Infection can be reduced to a large extent by early stabilization of fractures and the provision of soft tissue coverage.[10] The success of soft tissue coverage correlates better with low bacterial counts in the tissues than with any other single factor.[10] It has been observed that wounds with tissue contamination of more than 10^5 bacteria per gram developed infection. In predicting subsequent infection, in spite of some favourable reports,[6,10] the role of quantitative bacteriology of open contaminated wounds is still disputed.[2] The lack of consensus on this issue stimulated us to perform more studies in this field. We also set out to determine the specific tissue skin, muscles or periosteum responsible for the bacterial load in causing infection in the wound.

MATERIALS AND METHODS

The study was done on 20 patients having open fractures in the extremities following motor vehicle accidents. There were four cases of polytrauma. Ten patients sustained Gustilo-Anderson Grade III open

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fractures. The average time between injury and surgical debridement was 11.5 hours (8–18 hours). The delay in the surgical debridement was either due to late presentation in the hospital, unstable general conditions or associated problems.

At the time of surgery, after adequate cleansing and near total debridement of the open wound, tissues were obtained for quantitative bacterial counts. Samples of skin, muscle and raised periosteal tissue (in G.A. grade III fractures only) were taken and kept separately in pre-sterilized weighted containers filled with normal saline. Within 24 hours the tissue samples were processed for quantitative bacteriological estimation. Each tissue sample was homogenised in 1 ml of normal saline using a mechanical tissue grinder with stepwise six ten fold dilutions in the method used by Merritt.[6] Viable counts were then taken at each dilution.

After wound debridement, the fractures were stabilized depending upon the overall wound condition, fracture pattern and the associated injuries. In six patients, wounds were left open.

All patients were given intravenous second or third generation cephalosporins, in combination with aminoglycosides. The debridement was repeated on the third or fourth day in four cases.

At the end of four weeks, each patient was examined for any evidence of infection. In cases with infected wounds, swabs were taken for culture and sensitivity of the pathogens involved. These patients were subsequently managed for the control of infection.

Statistical Analysis

The Fisher's Exact test was used to assess the relationship of the quantitative load of organisms with the subsequent development of infection. Comparison was made within groups having different bacterial load for relating to prediction of infection.

RESULTS

Among a total of 50 samples from all tissues, 29 showed positive bacterial counts (16 of 20 skin, 11 of 20 muscle and 2 of 10 periosteum samples) (Table 1). The skin tissue was more contaminated than the muscle tissue ($p < 0.02$) and the periostem($p < 0.001$). In two patients having periosteal contamination, the muscle and skin tissue also showed bacterial positiveness. Likewise in all wounds with bacteria in muscle tissue, there were positive bacterial counts in skin samples. The quantitative estimation showed bacterial population to be $>10^5$ per gram in 10 skin and 3 muscle tissue samples.

The bacteria grown were non-specific mixed flora in 17, Acenobacter in 6 and other organisms (2 Staph aureus, 2 Klebsiella, 1 Staph epidermidis, 1 Pseudomonas) in 6 of the 29 tissue samples. The wounds with simultaneous contamination of skin and muscle tissue had similar bacteria, except two cases where the skin had a mixed bacterial population while muscle tissue showed Klebsiella in one and aerobic spores in the other.

Infection was observed in 9 of the 20 cases at one month follow-up (Table 2). Eight of these patients had skin contamination of $>10^5$ per gram tissue (Group I), whereas only one of the 6 patients with $<10^5$ bacteria per gram (Group II) and none of the 4 patients with zero contamination (Group III) became infected. Infection preponderance was significantly more in Group I ($p < 0.05$), when compared to Group II or III either alone or combined. This showed that the skin tissue was predictive of subsequent infection if bacterial contamination was $>10^5$ per gram of tissue.

In the muscle tissue samples, all 3 cases with a contamination level $>10^5$(Group I), half of the eight patients with $<10^5$ (Group II) and 2 of the 9 patients with zero contamination (Group III) developed infection. The difference in the predictive value was statistically significant only in Group III patients when compared with Group I or II either alone or combined. This meant that in muscle tissue any level of contamination was linked with subsequent infection and the level of 10^5 was not important.

The periosteal tissue samples in two patients showed bacterial contamination $<10^5$ per gram and both also had subsequent infection. The number was, however, insufficient for any statistical evaluation.

All nine cases developing infection were Gustilo Anderson grade III open injuries, except one which was grade II and subsequently developed pseudomonas infection. This was one of the 2 diabetic patients in the study group of 20.

In the infected wounds the pathogens cultured were mixed flora in 4, Klebsiella in 3, and Pseudomonas and Staphylococcus aureus in one each. In 4 patients the bacteria initially found was the final infective organism. In 2 patients initial Acenobacter contamination ended in mixed bacterial infection, while out of 2 having initial mixed bacterial contamination, infection occurred due to single bacteria. Only in one case was the infective bacteria different from the initially grown.

Table 1
Distribution of the patients according to the nature of the injury, bacterial population and subsequent infection

1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	47	–	BB leg	IIIB	12	E..F.	Mixed	$>10^5$	Mixed	$<10^5$	–	–	Mixed
2	60	Diab.mellitus	BB leg	IIIB	14	E.F.	Mixed	$<10^5$	Mixed	$<10^5$	–	–	Pseudomonas
3	25	–	Elb.Inj.	IIIA	18	E.F.	Acenobacter	$>10^5$	–	–	–	–	Mixed
4	19	–	BB farm	II	8	I.M.F.	Mixed	$<10^5$	–	–	–	–	–
5	45	Poly trauma	BB leg	IIIC	18	Amput.	Staph.aureus	$>10^5$	Staph.aureus	$>10^5$	Staph.aur	$<10^5$	Staph.aureus
6	23	–	BB leg	IIIA	8	E.F.	Acenobacter	$>10^5$	Acenobacter	$<10^5$	–	–	Mixed
7	45	–	BB leg	IIIB	9	E.F.	Mixed	$<10^5$	Mixed	$<10^5$	–	–	–
8	60	–	BB leg	II	8	POPcast	–	–	–	–	–	–	–
9	25	Poly trauma	BB leg	IIIA	18	E.F.	Acenobacter	$>10^5$	Klebsiella	–	–	–	–
10	30	Poly trauma	BB leg	IIIB	14	E.F.	Mixed	$>10^5$	Klebsiella	$>10^5$	—	–	Klebsiella
11	38	–	BB leg	II	8	POPcast	Mixed	$>10^5$	–	–	–	–	–
12	28	–	BB f.arm	II	8	I.M.F.	–	–	–	–	–	–	–
13	34	–	BB leg	II	18	PO	Pcast	Mixed	$>10^5$	Mixed	$<10^5$	–	–
14	45	Diab.mellitus	BB leg	II	15	I.M.F.	–	–	–	–	–	–	–
15	60	–	BB leg	IIIA	17	E.F.	Mixed	$>10^5$	Mixed	$>10^5$	Mixed	$<10^5$	Mixed
16	28	–	BB leg	II	9	POPcast	–	–	–	–	–	–	–
17	42	–	BB f.arm	II	14	I.M.F.	Acenobacter	$<10^5$	Acenobacter	$<10^5$	–	–	–
18	30	–	BB leg	II	12	I.M.F.	Staph.epid.	$<10^5$	–	–	–	–	–
19	38	Polytrauma	BB leg	II	14	E.F.	Mixed	$>10^5$	Mixed	$<10^{5'}$	–	–	Klebsiella
20	36	–	BB leg	IIIA	18	E.F.	Mixed	$<10^5$	–	–	–	–	–

1, Serial number 2, patient's age in years 3, Associated problem 4, fracture area 5, Gustilo Anderson Classification of the wound 6, Delay in surgical debridement (in hrs.) 7, Mode of fracture fixation (E.F.–External fixation, I.M.F.–Intramedullary fixation) 8, Skin tissue bacteria 9, Bacterial population in skin tissue 10, Muscle tissue bacteria 11, Bacterial population in muscle tissue 12, Periosteal bacteria 13, Quantitative bacterial population in periosteal tissue 14, Organism in the infected wounds

Table 2
Infection rate in patients with open fractures related to bacterial contamination in the skin tissue

	Bacterial count in tissue samples (per gram tissue)			
		$>10^5$	$<10^5$	nil
	Number	Group I	Group II	Group III
A, Skin tissue	20	10	6	4
Infection in	9	8*	1	0
B, Muscle tissue	20	3	8	9
Infection in	9	3	4	2*

The *observation is significantly different from the other two groups if combined ($p < 0.05$).

DISCUSSION

It has recently been observed[5] that pre-debridement and post-debridement bacterial cultures from open fracture wounds are essentially of no value in prediction of subsequent infection. For this purpose, the role of quantitative bacteriology is also considered controversial.[2,4,6] It has also been suggested[9] that the presence of higher colony counts and the presence of pathogens in the last piece of tissue at debridement may indicate inadequate debridement with the pathogens uncovered or it may indicate nosocomial pathogens.

In observing the results of the present study, both the initial contamination and the subsequent infection rate appeared to be quite high. One reason for such a high incidence could be the delay in surgical debridement[2]. Other factors included predominantly Gustilo Anderson grade III injuries and high velocity trauma, as all patients were involved in motor vehicle accidents. Such injuries are likely to have a higher level of tissue contamination and as such the requirement of more radical debridement cannot be under-estimated.

Out of 20 cases, infections occurred in 9. In 8 wounds, the bacterial counts were more than 10^5 per gram of skin tissue. Infected organisms differed only in one case from the initial contamination, while in four it was the same. In four cases either mixed bacterial population ended in single organism infections, or a single infected organism was joined by others to result in mixed infection.

This shows that the contamination of $>10^5$ per gram of skin tissue is related to subsequent infection. In the muscle tissue analysis, 7 of the 11 patients with bacterial contamination, irrespective of the level, developed infections compared with only 2 of the 9 patients having zero contamination. This suggests that any level of muscle contamination is predictive of future infection in the wound. It is likely that the presence of bacteria in the muscle tissue at the end of the debridement could be a subclinical phase in the development of subsequent infection.

The quantitative microbiological estimation using the method of Merritt[6] requires at least an interval of 24 hours. If it tests positive for the muscle tissue of a particular patient, the need for re-debridement at that stage becomes clear, even if the wound does not appear bad at that time. The delay in re-debridement of the wound can be reduced. To improve the clinical application of quantitative microbiology, a rapid slide technique for identifying the critical level of bacteria has been developed.[3] Using it for assessing skin contamination, the relevent information can be obtained in 30 minutes. In this case, a positive result can be taken as indicative of further debridement.

The predominantly polymicrobial growth in the initial contamination has been followed by either polymicrobial or Klebsiella infection in skin as well as in muscle tissue samples. This shows the uncertainity in the prediction of the infective bacteria from both the skin and muscle tissue samples. The effect of medical intervention in the course of management could be a factor in it. It has also been observed [5] that a significant percentage of late infections occur with hospital acquired organisms, suggesting that the inocculation of pathogens occurs subsequent to the initial injury.

The number of patients in this study was not enough to evaluate the importance of other factors such as antimicrobial agents or the irrigation fluid used. All cases had delayed surgical debridement, so this factor was not evaluated. Internal fixation was used in some cases and none of them became infected, though this cannot be suggestive in any way as internal fixation was used in cases where the wound was thought to be clean by the operating surgeon.

In conclusion, the present study although small, helps to demystify the controversial observations of the past.[2,4,6] It highlights the value of tissue specific bacterial load in the prediction of subsequent infection. While skin samples require bacterial contamination $>10^5$ per gram of tissue to be infective, the presence of any bacteria in the muscle tissue at the end of debridement should be taken as a predictor of subsequent infection. If available, the rapid slide technique can be used to decide the need for further debridement.

REFERENCES

1. **Goodson W, Hunt T.** Wound healing and the diabetic patient. *Surg Gynecol Obstet* 1979, 149:600.
2. **Grunuiger RP.** Diagnotic microbiology in bone and joint infections. In Gustilo,Grunuiger, Tsukayama Eds. *Orthopaedic Infection,* W B Saunders Co. 1989, 42–51.
3. **Heggers JP, Robson MC, Doran ET.** The quantitative assessment of bacterial contamination of open wounds by a slide technique. *Trans R Soc Trop Med Hyg* 1969, 63:532.
4. **Krizek TJ, Robson MC.** Evolution of quantitative bacteriology in wound management. *Am J Surg* 1975, 130:579–84.
5. **Lee J.** Efficacy of Cultures in the Management of Open Fractures. *Clin Ortho Rel Res* 1997, 339:71–5.
6. **Merritt K.** Factors increasing risk of infection in patients with open fractures. *J Trauma* 1988, 28:823–7.
7. **Monson TP, Nelson CL.** Microbiology for orthopaedic surgeons; Selected aspects. *Clin Ortho Rel Res* 1984, 190:14.
8. **Rittman W., Perren S.** *Cortical bone healing after Internal Fixation and Infection*. New York, Springer-Verlag 1974.
9. **Roth A, Fry DE, Polk HC.** Infectious morbidity in extremity fractures. *J Trauma1986,* 26:757–61.
10. **Thomas J, Charles M and Jerry B.** The use of quantitative bacterial counts in open fractures. *Clin Ortho Rel Res* 1989, 248:227–30.
11. **Trafton P.** Infected Fractures.In: Gossling H and Pillsbury, Eds. *Complications of Fracture Management*. Philadelphia: JB Lippincot 1984, 51–78.