

## Assessment of burn wound infection by swab and biopsy culture: a comparative study

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### Abstract

**Background:** Wound infection is a frequent and severe complication in burn patients accounting for a high number of mortalities. There are some reports comparing swab and biopsy cultures of wounds of various etiologies or chronic wounds.

**Objectives:** The aim of the study was to evaluate burn wound infection by different bacteriologic methods of surface swab and tissue biopsy culture and see if they can predict the outcome.

**Methods:** A prospective study was conducted in Shahid Motahari Burn Center, Tehran, on patients suffering from burns with more than 20% of TBSA admitted in 2001-2002. Wound swab and biopsy specimens were collected within the second week. Patients were followed to the point of their discharge or death.

**Results:** Bacterial growth was reported in 100% of swabs, in 89.3% of the biopsies in differential culture media, and in 18.7% of the biopsies in selective culture media. These different cultures had similar pathogen reports. Of the 75 studied patients, 78.7% died. The frequency rates of positive tissue cultures and bacterial load were not different between the dead and those who survived.

**Conclusion:** Bacteriologic methods do not have enough predictive value to assess clinical outcome.

**Keyword:** burn, infection, swab, biopsy.

### Introduction

It is estimated that 50-75% of mortality in burn patients after the initial resuscitation pertains to infection [1,3]. Patient factors such as age, extent of injury, and depth of burn coupled

with microbial factors such as type and number, enzyme and toxin production and motility of organisms determine the likelihood of invasive burn wound infection [4]. Exposed burned body surface is readily available for bacterial colonization [5]. The most important route for development of a burn wound sepsis is superfi-

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cial bacterial contamination of the wound changing to become invasive [6]. It has been recognized that the degree of bacterial wound contamination has a direct correlation with the risk of wound sepsis [5]. Based on quantitative tissue biopsy cultures, varying bacterial concentrations have been reported to be necessary for the establishment of burn wound sepsis. In most studies, a bacterial level of  $1 \times 10^5$  bacteria per gram of tissue has been used as diagnostic criteria for invasive infection and septicemia [7,11].

Nowadays, the most widespread methods used for microbial monitoring of burn wounds are swab culture and biopsy culture. The biopsy method reveals the bacterial load of the full thickness of the wound. It also creates a full thickness skin defect [6]. The swab culture on the other hand, is a non-invasive and less expensive method than the serial dilution culture in differential and selective media for burn wound biopsies, but it gives no information on deeper layers of the wound [5,6,12]. There are some reports comparing swab and biopsy cultures of wounds of various etiologies or chronic wounds [12,14]. This study was undertaken to determine the extent to which qualitative swab culture of the burn wound is consistent with tissue biopsy cultures and also to assess if they can predict the outcome.

## Methods

### *Patients*

This is a cohort study, carried out on burn patients admitted in the Infectious Disease Ward in Shahid Motahari Burn Center, Iran University of Medical Science (IUMS), Tehran, during the period between 2001 and 2002. All patients suffering from full-thickness burns with more than 20% of total body surface area (TBSA) who signed an informed consent were studied. They were followed to the point of their discharge or death.

### *Collection and analysis of cultures*

Wound culture samples were collected after serial clinical assessment within the second week, the most probable time for burn infection. Wound swab and biopsy specimens were collected from the leading edge of the wound sites showing signs of infection such as discoloration, bad odor and rapid separation of the eschar or presence of pus. Topical agents were removed from the site with sterile gauze, but the surface was not cleaned prior to swabbing or biopsy.

### *Surface swab collection*

The surface samples of wounds were collected by swabs and put onto sterile buffer or normal saline to make the organisms in suspension.

### *Biopsy collection*

Tissue biopsies were obtained by a scalpel approximately 1 gram in weight. One segment of the aliquots taken from each patient was transported in sterile normal saline to the pathology laboratory. The segment left (equal in size) was cut into small particles then homogenized in 1 cc of sterile normal saline using a homogenizer (BioMaster-Stomacher, Sewerd, England). The suspension was serially diluted and 0.1 cc sample from each dilution was prepared for inoculation. Blood samples were also cultured after collection.

### *Study of the organism*

Processed specimens were spread onto differential media (EMB and MacConkey for gram negative organisms, blood and chocolate agar for gram positive organisms) for 24-48 hours following selective media (Urea, SH2 Indole Motility (SIM), Triple Sugar Iron Agar (TSI), Methyl Red Voges Proskover (MRVP), Simon Citrate, Phenyl Alanine). After 24 and 48 hours of incubation at 37°C colonies were counted in both media and considered as wound colonizing bacteria.

Pathogens	Swab culture	Tissue culture	Blood culture
<i>Pseudomonas aeruginosa</i>	44	42	11
<i>Pseudomonas</i> spp	16	13	2
<i>Acinetobacter baumannii</i>	18	9	0
<i>Acinetobacter</i> spp	9	2	0
<i>Citrobacter diversus</i>	3	1	0
<i>Citrobacter</i> spp	1	0	0
<i>Klebsiella pneumoniae</i>	2	1	0
<i>Klebsiella</i> spp	2	0	0
<i>Staphylococcus aureus</i>	1	3	1
<i>Staphylococcus epidermidis</i>	1	0	0
E-coli	11	1	0
<i>Serratia marcescens</i>	1	1	0
<i>Serratia</i> spp	1	0	0
<i>Bacillus difteroid</i>	1	1	0
<i>Enterobacter</i> SPP	2	0	0
<i>Proteus</i> SPP	1	0	0

Table 1. Different pathogens in different cultures.

### Statistics

Statistical analysis was done by using the software program SPSS 13.5. Data were reported using mean±SD for descriptive results. The analytic results were made using t-test and Mann-Whitney U test for quantitative variables and Fisher exact test for the qualitative ones. P less than 0.05 was considered significant.

### Ethical consideration

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Patients or their parents who signed an informed consent were enrolled.

### Results

There were 75 biopsy/swab pairs which were collected from patients over a one year period. Twelve males (16%) and 63 females (84%) were included in this study. The mean±SD of

age was 22.8±15.7 years for males and 22.9±14.4 years for females. The most common cause of burn was attributed to explosions due to kerosene 40 (53.3%), gas 14 (18.7%), gasoline 9 (12%), open fire 6 (8%), boiled water 3 (4%), thinner 2 (2.7%), and gasoline+gas 1 (1.3%), respectively. The mean body surface area of the burn was 56.33±13.5 percent.

Bacterial growth was registered in all of 75 surface swabs. Growth was observed in 89.3% (67/75) of wound biopsy specimens after 24-48 hours. In these cultures 85.3% (64/75) had more than 100,000 colony forming units. Bacterial growth was recorded in 18.9% (14/74) of the blood cultures. The most common pathogen in all 3 cultures (surface swab, tissue biopsy and blood cultures) was *Pseudomonas aeruginosa*. The frequency of different pathogens in different cultures is listed in Table 1.

These different cultures had similar pathogen

Agreement percent	Blood culture & Swab culture (%)	Blood culture & Tissue culture (%)	Tissue culture & Swab culture (%)
<i>Pseudomonas aeruginosa</i>	76	80	82.7
<i>Pseudomonas</i> SPP	53.3	45.3	68
<i>Acinetobacter</i>	88	97.3	88
<i>Acinetobacter</i> SPP	86.7	96	85.3
<i>Staphylococcus aureus</i>	97.3	93.3	93.3

Table 2. Percentage of identical bacterial growth in different cultures.

reports. The correlation of different combinations of them is shown in Table 2.

Patients were followed till they were discharged. The mean hospitalization duration was  $23.55 \pm 23.57$  days (the minimum duration was 2 days and the maximum was 100 days). Within this period, 59 patients (78.7%) died and the 16 remaining (21.3%) were discharged after treatment. The mean duration of hospitalization was  $18.5 \pm 19.4$  days among dead patients and  $42.13 \pm 28.5$  days among those who survived which was significantly different ( $P = 0.001$ ).

Cause of death was electrolyte disturbances in 18 (30.5%) and infection in 41 (69.5%) cases. The mean age was  $23.47 \pm 13.9$  years among the dead and  $28 \pm 16.6$  years among those who survived which was not significantly different. The mean burn size was  $58.75 \pm 12.8\%$  of TBSA among dead patients and  $47.44 \pm 12.9\%$  of TBSA among surviving patients which was significantly different ( $P = 0.002$ ).

In dead patients, 91.5% (54/59) and in the discharged ones 81.2% (13/16) had positive tissue cultures, but it was not significantly different. In dead patients 81.4% (48/59) had more than 100,000 colony forming units in tissue culture and in the discharged patients it was 100% (16/16), but it was not significantly different. In dead patients, 19% (11/58) and in the discharged patients 18.8% (3/16) had positive blood culture, but it was again not significantly different.

The mean burn size was  $54.54 \pm 13.4\%$  of TBSA among patients with negative result for tissue culture and  $64.14 \pm 11.6\%$  of TBSA among patients with positive result which was significantly different ( $P = 0.016$ ).

### Discussion

Burn wound infection is one of the frequent and severe complications in patients who have sustained burns [15]. In this study we compared the surface swab with tissue biopsy cultures of burn wound specimens. We followed patients until they were discharged or died. Out of stud-

ied patients 59 (78.7%) died, which is a high mortality rate. In an Indian study [16] the mortality rate of the burned patients was reported to be 38%. It was also 21% in the Pakistan setting [3]. Sjoberg et al [17] in a study in Zimbabwe reported the mortality as 59%. In the above studies, patients with more than 70-80% of TBSA as burn size were excluded due to their poor prognosis and high probability of mortality within 72 hours. Moreover, our hospital is a referral burn center in Tehran in which the severest and the most complicated cases are admitted.

The burn size was greater in the dead patients compared with those who survived which is similar to data in the literature [3,16]. The mean age of dead patients did not differ from those who survived showing similarity to the outcome of the study conducted in India [16]. However, in a study in Pakistan it was shown that old age is a mortality risk factor [3]. Duration of hospital stay in surviving patients was more than dead cases. Patient suffering from more critical disorders died within 72 hours.

The most frequent pathogens in tissue biopsy and blood culture were *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*. In swab cultures *Pseudomonas aeruginosa* followed by *Acinetobacter* were more frequent.

According to the study in Pakistan, *Pseudomonas aeruginosa* and *Klebsiella* were the most prevalent organisms during the second week whereas *Staphylococcus aureus* was more frequent during the first 5-7 days [3]. Mc Manus also reported *Pseudomonas aeruginosa* as the most common microorganism in burn wounds. The mean post burn duration at which biopsies were performed was 13 days (2 weeks) [18]. However, *Staphylococcus aureus* is reported as the most frequent in most of the studies [3,6, 16,19].

Comparison of swab and tissue biopsy culture showed that in 82.7% of cases, methods showed identical results for *Pseudomonas aeruginosa*. In 93.3% of cases, methods showed

identical results for *Staphylococcus aureus* growth. Microorganisms had the highest rate of variation between blood culture and tissue biopsy culture with congruity only in 45.3% of occasions with *Pseudomonas*. Bill et al reported a correlation of 79% between quantitative swab culture growing and tissue biopsy culture with chronic wounds of various etiologies [12]. Steer et al reported a correlation of 54% between biopsy and swab growing the same set of organisms [19]. According to Basak et al's study, swab cultures correlated with biopsy specimens in 72% of cases [14]. The investigators believe that there is a considerable similarity between the methods. It can be due to higher quality of swab sampling which was not contaminated or was exactly taken from the source of infection. Different situation of hospitals, laboratory devices, patients and technicians are also involved.

As logic predicts, wound infection and subsequent sepsis increase mortality rate but in our study the frequency of positive tissue cultures and bacterial load were not significantly different between dead patients and those who survived. The same results were obtained in Steer's study in the UK [19]. In the other study conducted by Steer et al with burns > 15 percent of TBSA, a relationship between bacterial counts and subsequent sepsis was not demonstrated [20]. In McManus' study, excluding inhalation injury as the major source of sepsis rather than wound infection, quantitative counts show no difference between those who were septic and those who were not [18]. A high mortality was noted in the first 72 hours of admission due to (other acute) electrolyte disorders rather than infection.

There was a lower frequency of positive blood cultures (18.9%) in the dead and those who survived compared to surface swab (100%) and tissue biopsy culture (89.3%). Both of them are attributed to prophylactic antibiotic administration upon patient admission.

Determining the specific pattern of burn

wound bacterial colonization is necessary for every setting to manage wound infection before microbiological culture results are available.

### Conclusion

This study suggests that there is a good correlation between surface swab and tissue biopsy for identifying the pathogens on and within the burn wounds, but they don't have enough predictive value to assess the clinical outcome. On the other hand, burn size positively correlates with wound infection and outcome.

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