

Comparison of Surface Swab and Quantitative Biopsy Cultures Dependent on Isolated Microorganisms from Burn Wounds

Yanık Yaralarından İzole Edilen Mikroorganizmalara Göre Yüzeysel Sürüntü ve Kantitatif Biyopsi Kültürlerinin Karşılaştırılması

Mete Koray Vural¹, Ulku Altoparlak², Demet Celebi³, Mufide Nuran Akca⁴

¹Department of Microbiology and Clinical Microbiology Erzurum State Research and Training Hospital, Erzurum, Turkey.

²Department of Microbiology and Clinical Microbiology, Ataturk University, School of Medicine, Erzurum, Turkey.

³Department of Microbiology, Ataturk University, School of Veterinary, Erzurum, Turkey

⁴Department of General Surgery, Burn Unit, Ataturk University, School of Medicine, Erzurum, Turkey.

Abstract

Objective: Infections are one of the most important and potentially serious complications of burn wounds. Quantitative biopsy cultures are useful for showing that a burn wound infection is not present, but these cultures are not useful for showing that an infection is present. Swab cultures are used to diagnosis surface colonization and may not accurately reflect the organism(s) causing the wound infection.

Materials and Methods: To perform a comparison of surface swab and biopsy cultures for isolated microorganisms from burn wounds, 160 biopsy/surface swab pairs were collected from 160 patients.

Results: Seventy-seven patients (48.1%) showed positive microorganism cultures from the burn wounds; 19.3 percent had both positive surface cultures and significant bacterial counts from the biopsy cultures, whereas 28.8 percent of the patients had only positive surface cultures. There was moderate agreement (41%) between the results of surface swab and quantitative biopsy cultures used to diagnose burn wound infections. *Pseudomonas aeruginosa* (45.5%) was the most predominantly isolated bacteria from the wounds, and there was good concordance between the results of the surface swab and quantitative biopsy culture samples (78%). Coagulase-negative *Staphylococci* (CNS) and *Escherichia coli* strains were only isolated from the surface swab samples because they are colonizing bacteria. The univariate analysis revealed that there were significant associations between the results of the positive biopsy cultures and the total surface body area, open flame burns, prolonged hospitalization and female gender ($p<0.05$).

Conclusion: Surface swab and quantitative biopsy cultures have a high rate of concordance in predicting *P. aeruginosa* invasion and the colonization of *E. coli* and CNS strains in burn wounds.

Key Words: Burn wound, Surface swab culture, Quantitative biopsy culture

Özet

Amaç: Yanık yarası enfeksiyonları; yanığın çok önemli ve ciddi komplikasyonlarından birisidir. Kantitatif biyopsi kültürleri yanık alanında bir yara enfeksiyonu bulunmadığını desteklemek için yararlıdır, ancak bir enfeksiyon olup olmadığını göstermek için kullanışlı değildir. Sürüntü kültürleri yüzeysel kolonizasyonun tanısı amacıyla kullanılır ve gerçekten yara enfeksiyonuna neden mikroorganizma(lar)ı yansıtmayabilir.

Gereç ve Yöntem: Yanık yarısından izole edilen mikroorganizmalar baz alınarak yüzeysel sürüntü ve kantitatif biyopsi kültür sonuçlarını karşılaştırmak amacıyla, 160 hastadan eş zamanlı olarak sürüntü ve biyopsi örnekleri alındı.

Bulgular: Yetmişyedi (%48.1) hastanın yanık yaralarından mikroorganizma izole edildi. Bu hastaların %19.3'ünün hem biyopsi kültürlerinde anlamlı sayılarda mikroorganizma, hem de sürüntü kültürü pozitifliği saptanırken, %28.8'inde sadece sürüntü örneği kültürlerinde üreme tespit edildi. Yanık yarasının mikroorganizmalarca invazyonunun gösterilmesinde, yüzeysel sürüntü ve kantitatif biyopsi kültürleri arasında ki uyum orta derecede idi (%41). *Pseudomonas aeruginosa* en sık izole edilen mikroorganizma olup (%45.5), bu mikroorganizmanın tespiti açısından hem kantitatif biyopsi, hem de yüzeysel swab kültürleri arasında iyi bir uyum söz konusu idi (%78). Koagülaz negatif stafilokoklar (KNS) ve *Escherichia coli* suşları kolonizasyon etkeni olarak sadece yüzeysel sürüntü örneklerinden izole edildi. Univariate analizde, pozitif biyopsi kültürü sonuçları ile; yanık alanı, alev yanıkları, uzamış hastanede kalma süresi ve kadın cinsiyet arasında anlamlı bir ilişki tespit edildi ($p<0.05$).

Sonuç: Yüzeysel sürüntü ve kantitatif biyopsi kültürleri; *P. aeruginosa* invazyonu ile *E. coli* ve KNS kolonizasyonunun tespitinde çok iyi bir uyum gösterdiler.

Anahtar Kelimeler: Yanık yarası, Yüzeysel sürüntü kültürü, Kantitatif biyopsi kültürü

Received: September 18, 2012 / **Accepted:** November 23, 2012

Correspondence to: Ulku Altoparlak, Department of Microbiology and Clinical Microbiology, Ataturk University, School of Medicine, 25100, Erzurum, Turkey Phone: +90 442 233 11 11 /6787 e-mail: ulkuca@hotmail.com

doi:10.5152/eajm.2013.05



Introduction

There is a high risk of colonization and infection in burn wounds. This susceptibility results from the combined effect of the disruption of the normal skin barrier, the presence of coagulated proteins and other microbial nutrients in the wound, and the avascularity of the eschar, which prevents the delivery of immunologically active cells, humoral factors, and blood-borne antibiotics to the eschar [1]. The flora of the burn wound also influence the risk of infection and the invasive potential of infections that occur [2]. Early contaminants of the wound surface immediately after burning are most likely skin flora, particularly gram-positive organisms. As time passes, gram-negative organisms become more prevalent inhabitants of the burn wound, but the gram-positive organisms remain the predominant flora [3, 4].

Surface cultures are useful for identifying the organisms present within the burn wound and for identifying the predominant members of the burn wound flora, but these samples are incapable of differentiating burn wound colonization from burn wound infection [5]. It is now accepted that quantitative cultures of biopsy samples from burn wounds, histopathological examination of burn wound specimens and clinical signs and symptoms of infection are required to differentiate between colonization and actual infection. A low quantitative bacterial count is a good indication that a burn wound infection is not present, but a quantitative count of 10^5 (or greater) organisms per gram of tissue correlates with histologic evidence of invasive infection in fewer than 50% of paired tissue samples [6].

In this study, we aimed to compare the surface swab and quantitative biopsy samples from burn wounds and to evaluate the infective agents that were present.

Materials and Methods

Patients and study design: This prospective study was conducted in Ataturk University Aziziye Hospital, a 1200-bed tertiary teaching hospital that has a Burn Unit with 14 beds. A total of 160 patients admitted to the Burn Unit were included in the present study. All patients sign informed consent forms before examination and their names are anonymized for this study. Microbiological assessment of the burn wounds was carried out following the collection of surface swab and full-thickness biopsy specimens.

Procedure for wound sampling: Both swab and biopsy samples were taken simultaneously from the wound of each patient. To obtain a culture of the burn surface, topical agents (if any) were first removed with a gauze soaked in sterile saline. An area measuring 4 cm² was swabbed using two sterile swab sticks. For dry wounds, the swab was moistened with

sterile saline before swabbing. After the collection, the swabs were inoculated on blood and MacConkey Agar [7].

For quantitative cultures of biopsy samples, approximately 20-50 mg of tissue from the wound along with the underlying fat was removed using 5 mm punch biopsy forceps. The tissue was then weighed, suspended in 2 ml of physiological saline and homogenized. After making serial dilutions, 0.1 ml of undiluted or diluted sample was inoculated on blood and MacConkey Agar [5,7]. Surface viable counts were determined, and the number of organisms per gram of tissue was calculated. Bacterial isolates were identified by conventional bacteriologic methods or by using commercial identification kits. No growth up to 48 h was considered negative [8].

Statistical analysis: All statistical analyses were performed using SPSS Version 13.0 with $p < 0.05$ accepted as statistically significant. Univariate analysis was used to compare the relationship between age, gender, burn depth, total surface burn area, hospital duration, burn etiology and positive results of quantitative biopsy versus surface swab cultures. The Chi-square test was used for categorical variables, and Student's t-test was used for continuous variables. To evaluate concordance, the Kappa index was used (Table 1) [6].

Results

In this study, there were 160 cases, with a mean age of 10.5 years; the range was 1-71 years, including 106 patients (66.3%) aged ≤ 5 years, 40 patients aged 6 to 35 (25%), and 14 patients (8.7%) aged ≥ 36 years. Eighty-six of the patients were males (53.8%), and 74 patients were females (46.2%). A total of 122 patients (76.3%) arrived with less than 30% total surface body area (TBSA) burns, 19 patients (11.9%) had 30-40% TBSA burns, 3 patients (1.8%) had 40-50% TBSA burns, and 16 patients (10%) had greater than 50% TBSA burns. The mean burn size of the population was $19.4 \pm 15.6\%$ TBSA. Among the patients, 112 (70%) had suffered second-degree burns, while the remaining 48 patients (30%) had third-degree burns. Of the cases, 73 (45.6%) spent less than 10 days in the hospital, followed by those who spent 11-20 days (39 patients, 24.4%), 21-30 days (23 patients, 14.4%), 31-40 days (7 patients, 4.4%)

Table 1. Interpretation of Kappa index

Kappa	Concordance
<0	None
0-20%	Insignificant
20-40%	Low
40-60%	Moderate
60-80%	Good
80-100%	Very good

or more than 40 days (18 patients, 11.2%) at the hospital. Scalds (hot water, hot soup and hot oil burns) were the most frequent cause of burns (55%), followed by open flames (38.8%) and electricity (6.2%).

Of the 160 burn patients, 77 patients (48.1%) showed positive microorganism cultures from the burn wounds, 19.3 percent of the patients had significant bacterial counts from quantitative biopsy cultures, and 28.8 percent of the patients had only positive surface cultures. The results of the univariate analysis are shown in Table 2. In the univariate analysis, burn depth and age group showed no significant association with the results of the quantitative biopsy cultures. As for gender, there was a significant association between the positive biopsy results and the female gender (28.4%) ($p < 0.05$). In patients hospitalized for less than 10 days, only 5.4% of the biopsy cultures contained 10^5 colony forming units per gram of tissue (CFUs/g). This rate increased to 44.4% among those hospitalized for more than 40 days. Significant bacterial counts clearly contributed to prolonged hospitalization ($p < 0.05$). Open flame cases were the etiology most common-

ly associated with positive biopsy cultures in burn patients (32.3%) ($p < 0.05$). Electric current did not cause any of these cases. There was a significantly lower level of TBSA in patients with positive surface swab cultures (18.8 ± 14.0) compared with patients who had biopsy cultures with significant bacterial surface counts (37.5 ± 10.7) ($p < 0.05$).

In the positive biopsy culture samples, 29 cultures showed a single bacterial species, and 2 of the cultures showed two bacterial species present. According to the surface swab culture results, 29 of the cultures showed a single species, 16 cultures showed 2 isolates, and 1 of the cultures showed a polymicrobial community consisting of three species. A total of 97 cultivable isolates belonging to 15 different microbial species were recovered from the burn wounds (Figure 1). The concordance between culture results is shown in Table 3. In our study, the concordance between surface swab and quantitative biopsy cultures in diagnosing burn wound invasions was moderate (41%). For *Pseudomonas aeruginosa*, there was good concordance in estimating the invasion of deep tissues between the results of the surface swab and quan-

Table 2. Characteristics of surface and biopsy culture positive burn patients

Characteristic	Frequency or mean \pm SD		Univariate analysis P value
	Surface culture positive cases	Biopsy culture positive cases	
Age			
≤ 5 years	35	18	
6-35 years	9	10	0.24
≥ 36 years	2	3	
Sex			
Male	28	10	0.02
Female	18	21	
Burn depth			
II	35	20	0.31
III	11	11	
Total surface burn area	18.8 \pm 14.0	37.5 \pm 10.7	0.00
Hospital duration (days)			
≥ 10	24	4	
11-20	9	7	
21-30	5	8	0.008
31-40	2	4	
41 \leq	6	8	
Aetiology of burns			
Scalds	30	11	
Open flame	11	20	0.001
Electricity	5	0	

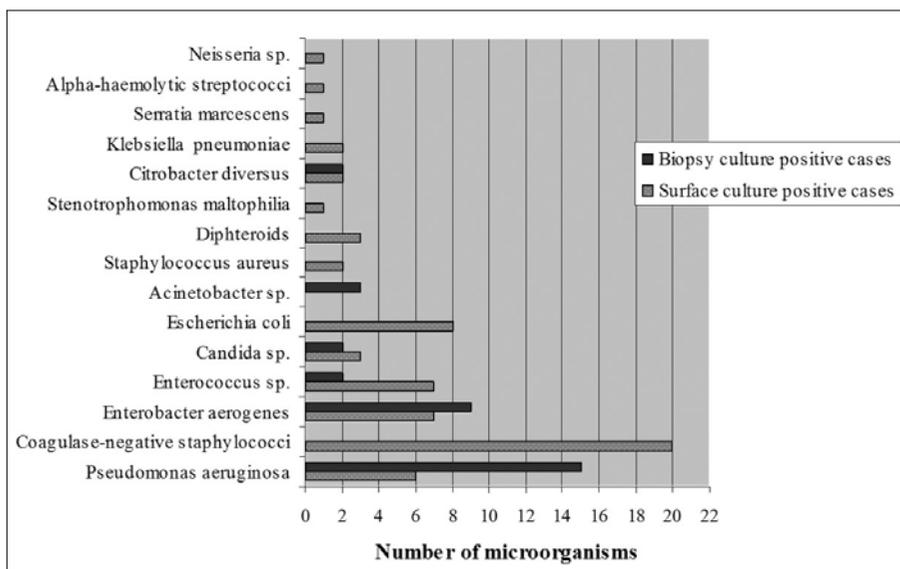


Figure 1. Microorganisms isolated from surface swab and quantitative biopsy cultures.

titative biopsy culture samples (78%). Coagulase-negative *Staphylococci* (CNS) and *Escherichia coli* strains were only isolated from the surface swab samples because they are colonizing bacteria.

Discussion

Although wound surface cultures are the simplest diagnosis method, they poorly indicate the organisms that invade burn wounds. Full thickness biopsy cultures with quantification of the number of bacteria in the burn wound is the best method for determining burn wound invasion [9]. This study was undertaken to compare the results of burn wound biopsy cultures with corresponding cultures of superficial swab samples by identifying the microorganisms isolated from burn patients.

Pseudomonas is generally the most frequently isolated gram-negative bacterium from burn patients, and it is the most serious cause of life-threatening infections. The devitalized tissue and moist environment of burns provide an ideal environment for colonization and infection with *P. aeruginosa*, which is exacerbated by compromised immune function in burn patients [10]. In our study, *P. aeruginosa* strains were isolated in significant counts from quantitative biopsy cultures from fifteen patients, but the remaining 6 patients only had positive surface swab sample results. There was good concordance between the surface swab and biopsy culture results. Our findings on the high frequency of *P. aeruginosa* in burn wounds coincide with many previous reports wherein this organism was identified to be responsible for the majority of invasive burn wound infections [10-13]. In other studies,

the concordance between the results of the surface swab and quantitative biopsy cultures was 52-54% [6, 14, 15].

Even though the pathogenicity of coagulase-negative *Staphylococci* is unclear, several studies have suggested that CNS should be considered a significant pathogen in both burn patients and critically ill surgical patients [14]. In our study, CNS was identified in the surface swab cultures of only 20 patients (28.6%), and they were considered to be commensal bacteria. Similarly, studies from other burn institutions revealed higher rates of recovery from infection caused by this organism in burn wounds because it is a colonizing agent [16-18].

E. coli is one of the major causes of infections in burn patients based on surface swab and biopsy cultures from burn wounds [2]. However, in this study, *E. coli* was determined to be a colonizing bacteria according to the results of

Table 3. Kapa index of frequently isolated microorganisms

Results	Kappa
Global	41%
<i>Pseudomonas aeruginosa</i>	78%
<i>Coagulase negative Staphylococci</i>	0%
<i>Enterobacter aerogenes</i>	64%
<i>Enterococcus sp.</i>	21%
<i>Candida sp.</i>	44%
<i>Escherichia coli</i>	0%
<i>Acinetobacter sp.</i>	38%
<i>Citrobacter diversus</i>	50%

the quantitative biopsy culture samples, which yielded less than 10^5 cfu/g of tissue.

In this study, there was moderate concordance between the results of the surface swab and quantitative biopsy cultures used to diagnose the invasion of burn wounds by microorganisms (41%). Similarly, Danilla et al [6] found that the global concordance between the two methods was moderate (52%); however, they also noted that only *S. aureus* and *A. baumannii* showed greater than 60% concordance. Uppal et al. [5] reported that both sampling methods yielded the same organisms in 95% of cases; in their study, biopsy was found to be a more valuable method because it demonstrates the critical load of an organism beyond which metastatic invasion of the organism takes place. Basak et al. [7] noted a 72 percent correlation between surface swab culture and quantitative wound biopsy culture results. Steer et al. [14, 15] showed that the concordance between the two methods was 54%. Sjöberg et al. [19] indicated that the results of the surface cultures and tissue cultures were identical in 29% of cases; that is, the samples either showed no growth or identical bacterial growth.

Significant associations were found between the results of the positive biopsy cultures and the total surface body area, open flame burns, prolonged hospitalization, and female gender in our study. However, there are no other studies examining these data that can be used for comparison.

In conclusion, surface swabs and quantitative biopsy cultures have a high concordance rate for predicting *P aeruginosa* invasion and the colonization of *E. coli* and coagulase-negative *Staphylococci* strains in burn wounds.

Conflict of interest statement: The authors declare that they have no conflict of interest to the publication of this article.

References

- Pruitt BA Jr, McManus AT, Kim SH, Goodwin CW. Burn wound infections: current status. *World J Surg* 1998; 22: 135-45. [\[CrossRef\]](#)
- Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. *Clin Microbiol Rev* 2006; 19: 403-34. [\[CrossRef\]](#)
- Rafia K, Tredget EE. Infection control in the burn unit. *Burns* 2011; 37: 5-15. [\[CrossRef\]](#)
- Erol S, Altoparlak U, Akcay MN, Celebi F, Parlak M. Changes of microbial flora and wound colonization in burned patients. *Burns* 2004; 30: 357-61. [\[CrossRef\]](#)
- Uppal SK, Ram S, Kwatra B, Garg S, Gupta R. Comparative evaluation of surface swab and quantitative full thickness wound biopsy culture in burn patients. *Burns* 2007; 33: 460-3. [\[CrossRef\]](#)
- Danilla S, Andrades P, Gómez ME, et al. Concordance between qualitative and quantitative cultures in burned patients. Analysis of 2886 cultures. *Burns* 2005; 31: 967-71. [\[CrossRef\]](#)
- Basak S, Dutta SK, Gupta S, Ganguly AC, De R. Bacteriology of wound infection: evaluation by surface swab and quantitative full thickness wound biopsy culture. *J Indian Med Assoc* 1992; 90: 33-4.
- Traditional cultivation and identification. In: Forbes BA, Sahm DF, Weissfeld AS, editors. *Bailey and Scott's Diagnostic Microbiology*. 12th ed. St. Louis, MO: Mosby; 2007. p. 93-119.
- Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. *Clin Microbiol Rev* 2001; 14: 244-69. [\[CrossRef\]](#)
- Tredget EE, Shankowsky HA, Joffe AM, et al. Epidemiology of infections with *Pseudomonas aeruginosa* in burn patients: the role of hydrotherapy. *Clin Infect Dis* 1992; 15: 941-9. [\[CrossRef\]](#)
- Taneja N, Emmanuel R, Chari PS, Sharma M. A prospective study of hospital-acquired infections in burn patients at a tertiary care referral centre in North India. *Burns* 2004; 30: 665-9. [\[CrossRef\]](#)
- Chai J, Sheng Z, Yang H, Diao L, Li L. Successful treatment of invasive burn wound infection with sepsis in patients with major burns. *Chin Med J (Engl)* 2000; 113: 1142-6.
- Essayagh T, Zohoun A, Essayagh M, et al. Bacterial epidemiology in the burns unit at military teaching hospital Mohamed V of Rabat. *Ann Biol Clin* 2011; 69: 71-6.
- Steer JA, Papini RP, Wilson AP, McGrouther DA, Parkhouse N. Quantitative microbiology in the management of burn patients. I. Correlation between quantitative and qualitative burn wound biopsy culture and surface alginate swab culture. *Burns* 1996; 22: 173-6. [\[CrossRef\]](#)
- Steer JA, Papini RP, Wilson AP, McGrouther DA, Parkhouse N. Quantitative microbiology in the management of burn patients. II. Relationship between bacterial counts obtained by burn wound biopsy culture and surface alginate swab culture, with clinical outcome following burn surgery and change of dressings. *Burns* 1996; 22: 177-81. [\[CrossRef\]](#)
- Nasser S, Mabrouk A, Maher A. Colonization of burn wounds in Ain Shams University Burn Unit. *Burns* 2003; 29: 229-33. [\[CrossRef\]](#)
- Guggenheim M, Zbinden R, Handschin AE, Gohritz A, Altintas MA, Giovanoli P. Changes in bacterial isolates from burn wounds and their antibiograms: a 20-year study (1986-2005). *Burns* 2009; 35: 553-60. [\[CrossRef\]](#)
- Li HM, Liang ZQ, Liu DE, Meng CY. An investigation of bacterial ecology and analysis of bacterial resistance to antibiotics in a burn ward in Nanning district. *Zhonghua Shao Shang Za Zhi* 2005; 21: 107-10.
- Sjöberg T, Mzezewa S, Jönsson K, Robertson V, Salemark L. Comparison of surface swab cultures and quantitative tissue biopsy cultures to predict sepsis in burn patients: a prospective study. *Burn Care Rehabil* 2003; 24: 365-70. [\[CrossRef\]](#)