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Use of burn wound biopsies in the diagnosis and treatment of burn wound infection

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Improvements in both general care and wound care have favorably influenced the outcome of burn patients. Principal among these improvements have been the use of effective topical antimicrobial chemotherapy and the early postburn removal of ischemic nonviable burned tissue by excision, which have reduced the incidence of invasive burn wound infection as a cause of death [6]. Even so, current burn wound care is imperfect and certain patients, usually those with extensive burns that involve more than 50% of the total body surface (particularly children and the elderly), escape from microbial control and develop invasive burn wound infection [10].

The status of the burn wound must be monitored on a scheduled basis utilizing an integrated program of clinical, microbiologic, and pathologic examinations. The similarity of the systemic responses and the changes in laboratory values that accompany uncomplicated burn injury per se to those that accompany infection necessitates that reliance be placed on identifying changes in appearance of the wound that are produced by infection [9]. The wound must be examined at regularly scheduled (at least daily) intervals to identify changes in its appearance that are indicative of infection at a time when the process can be arrested and the patient salvaged.

The most frequent change in wound appearance due to invasive wound infection is focal, dark red, brown, or black discoloration of the eschar [12] (Table 1). Unfortunately, such color changes are nonspecific and can be caused by intraeschar hemorrhage due to minor local trauma. The most reliable sign of invasive burn wound infection is conversion of an area of partial-thickness injury to full-thickness skin necrosis. The alarming velocity with which those changes can occur is exemplified by the rapid centrifugal expansion of ischemic necrosis that is characteristic of invasive phycomycosis and the unexpectedly rapid separation of the eschar associated with fungal and yeast infections [7]. Unfortunately, such rapid eschar separation may also occur in the absence of invasive infection when the burn injury has been deep enough to cause liquifaction of the underlying subcutaneous fat. Certain clinical findings are characteristic of infections caused by specific microorganisms. Green discoloration of subcutaneous fat due to the metabolic product pyocyanin, and the presence of erythematous nodules that evolve into focal areas of eschar formation (ecthyma gangrenosum) are typical of pseudomonas infections. Violaceous discoloration of edematous unburned skin at the margins of the burn wound is also characteristic of invasive pseudomonas burn wound infection, but edema and erythematous discoloration of the unburned skin at the wound margin, producing an exaggerated "step-

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Table 1. Clinical signs of invasive burn wound infection

I.	Dark brown, or violaceous discoloration of the burn wound: can be focal, multifocal or generalized.
II.	Conversion of partial-thickness injury to full-thickness necrosis.
III.	Hemorrhagic discoloration of subeschar tissue.
IV.	Green pigment visible in subcutaneous fat.
V.	Erythematous nodular lesions (ecthyma gangrenosum) in unburned skin.
VI.	Edema and or violaceous discoloration of unburned skin at wound margins.
VII.	Unexpectedly rapid separation of eschar: most commonly due to fungal infection.
VIII.	Rapid centrifugal advance of subcutaneous edema with central ischemic necrosis: typical of *Phycomycotic* infection.
IX.	Vesicular lesions in healing or healed second-degree burns *
X.	Crusted serrated margins of partial-thickness burns of face *

* Characteristic of herpetic infection

off" between intact skin surface and burn wound bed are generic changes indicative of nonspecific infection.

Other organism-specific changes include the dusky discoloration of saponified subcutaneous fat often associated with invasive fungal infection, and the vesicular lesions in healing or healed partial-thickness burns and the crusted serrated margins of partial-thickness burns, particularly those in the nasolabial area of the face that are virtually pathognomonic of herpes simplex virus infections [1].

The unreliability and lack of specificity of local signs and symptoms require that other methods be used to assess the microbial status of the burn wound and diagnose burn wound infections. Surface culture techniques may be used for epidemiologic monitoring and to determine the predominant flora of the burn wounds of individual patients, but the frequent surface microbial colonization of burn wounds and the failure of such cultures to sample the subeschar space make them ineffectual in diagnosing burn wound infection. Cultures of burn wound tissue can provide a more precise assessment of burn wound microbial ecology and dynamics.

During the period 1 January 1987 through 27 December 1991, cultures were performed on 2158 burn wound tissue samples (biopsies plus surgical specimens) removed from 501 of the 1080 burn patients admitted during that period. Six-hundred and sixty-five, or 31 %, of the biopsies that were removed from 210, or 42 %, of those patients showed microbial growth. Isolates recovered from 144 patients were positive for bacteria while isolates recovered from 44 patients were positive for yeasts, and isolates recovered from 130 patients were positive for filamentous fungi. Recovery of bacteria, in order of decreasing frequency, consisted of: *Staphylococcus aureus* from 45 patients, *Pseudomonas aeruginosa* from 36 patients, *E. coli* from 28 patients, *Enterococci* from 20 patients, *Klebsiella pneumoniae* from 11 patients, *Bacillus* species from 18 patients, and *Enterobacter cloacae* from 14 patients. Recovery of yeasts consisted of: *Candida albicans* from 29 patients, *Candida rugosa* from seven patients, and *Candida tropicalis* from three patients. The recovery of filamentous fungi consisted of *Aspergillus* species from 59 patients, *Fusarium* species from 11 patients, *Cladosporium* species from nine patients, *Penicillium* species from seven patients, and *Phycomycetes* (mucor species and rhizopus species) from three pa-

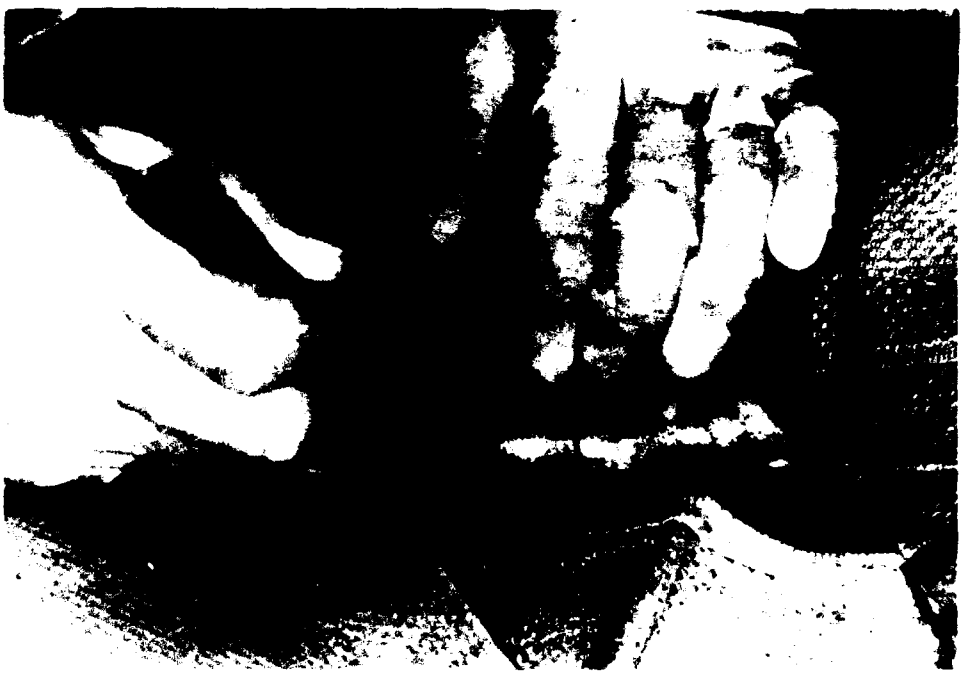

Fig. 1. The scalpel is used to biopsy the burn wound at a site selected on the basis of dark hemorrhagic discoloration as shown here. Note the depth to which the scalpel blade has been inserted to insure that unburned subcutaneous tissue will be included in the biopsy

tients. Such surveillance-type culture data are of assistance in monitoring the ecological changes that occur in the indigenous burn wound flora of a burn unit across time and in selecting agents for initial systemic antimicrobial therapy in patients who do develop an invasive infection. Systemic therapy is subsequently altered as necessary based upon culture and sensitivity of the organisms causing a specific infection. Such epidemiologic information is also useful in identifying the need to modify infection control measures to eliminate environmental or other vectors responsible for cross contamination and prevent the establishment of resistant endemic strains in a treatment facility.

Burn wound biopsies can be used to obtain quantitative culture data and perform a histologic examination of both the eschar and the subeschar tissue. To prepare the wound for a biopsy, that area of the burn showing the most pronounced tinctorial and morphologic changes should be cleansed to remove debris and residual topical agent. Following cleansing, a lenticular 500-mg tissue sample that includes eschar and underlying unburned subcutaneous tissue is obtained by use of the scapel (Fig. 1). If local anesthesia is necessary, the anesthetic agent should be injected at the periphery of the planned sampling site to avoid distortion of the architecture of the specimen. The specimen is bisected and one-half is processed in the microbiology laboratory to identify the organisms present and to characterize the predominant members of the microbial flora. Although recommended by some, quantitative cultures are unreliable in diagnosing burn wound infection [8]. Falsely high quantitative counts can result from culturing pooled secretions or exudates, culturing an

eschar at the time of slough that occurs as a result of bacterial proliferation at the nonviable tissue interface, or from delay in specimen transport that permits in vitro microbial proliferation. Conversely, falsely low quantitative culture counts can result from culturing a non-representative desiccated area of the wound, culturing residual topical agent, and storage or transport conditions that allow the specimen to desiccate.

Studies by McManus et al. have shown that quantitative cultures are clinically useful only to confirm the absence of invasive infection, i.e., low microbial counts are seldom associated with histologic evidence of invasive infection. The natural history of a full-thickness burn wound entails a progressive increase in microbial density, particularly at the subeschar interface between viable and nonviable tissue (where the organisms contribute to collagenolysis and sloughing of the nonviable tissue) with proliferation enhanced by maceration and pooling of wound exudate. Consequently, quantitative counts of more than 10^5 organisms per gram of tissue in a biopsy specimen (the density claimed by some to be diagnostic of invasive infection) are actually unreliable and correlate with histologic evidence of invasive burn wound infection in less than 50% of biopsies [5]. In short, the histologic examination of a biopsy specimen harvested from an area of the burn wound suspected of harboring infection has a shorter turnaround time than culture techniques; it is the only accurate means of differentiating colonization of nonviable eschar (always present to some degree) from invasion of viable tissue and making a reliable diagnosis of invasive burn wound infection, and can readily identify infections caused by fungi and viruses for which cultures are of little clinical usefulness.

Accordingly, the other half of the biopsy specimen is processed by either a rapid section technique that requires 3 to 4 hours for slide preparation or by a frozen-section technique that requires only 30 min for slide preparation [3, 4]. Since the frozen-section technique is associated with a falsely negative rate of 3.6%, the biopsy sample should be processed by standard techniques to confirm frozen section diagnosis and exclude falsely negative frozen section readings. The pathologist examines the sections looking for the histologic signs of burn wound infection listed in Table 2. The identification of bacteria, fungi, or viruses in viable tissue in the histologic section confirms the diagnosis of invasive burn wound infection. The other histologic criteria indicative of burn wound infection may be found in association with inflammation, but their presence should heighten one's index of suspicion and prompt a careful search for microorganisms. Both falsely negative and falsely positive biopsy readings may occur. A falsely positive reading may be caused by artifacts produced

Table 2. Histopathologic signs of burn wound infection

I.	Microorganisms present in unburned tissue.
II.	Heightened inflammatory reaction in unburned tissue.
III.	Hemorrhage in unburned tissue.
IV.	Small vessel thrombosis and ischemic necrosis of unburned tissue.
V.	Dense microbial growth in subeschar space and along hair follicles and sweat glands.
VI.	Intracellular viral inclusions
	A. Type-A Cowdry bodies
	B. Intracellular virions

Table 3. Histologic staging of burn wound microbial status

Stage		Characteristics
I.	Colonization	
	A. Superficial	Microorganisms present on burn wound surface
	B. Penetration	Microorganisms present in variable thickness of the eschar
	C. Proliferation	Variable density of microorganisms in subeschar space
II.	Invasion	
	A. Microinvasion	Microscopic foci of microorganisms in viable tissue adjacent to subeschar space
	B. Generalized	Multifocal or wide-spread penetration of microorganisms deep into viable subcutaneous tissue
	C. Microvascular	Involvement of small blood vessels and lymphatics

by tissue processing or adherence of foreign bodies or as the result of misinterpretation by an inexperienced pathologist. Falsely negative readings can occur as a result of inadequate tissue dehydration, excessive thickness of the histologic sections, inadequate sampling (biopsy of an uninfected site or sampling of only the eschar without attached unburned tissue), and misinterpretation of histologic findings by an inexperienced pathologist.

A grading scheme has been developed to classify the microbial status of biopsy specimens on the basis of histologic evidence of microbial density and penetration (Table 3) [13]. As is immediately evident, the important differentiation is between Stage IC, colonization with penetration of the full-thickness of the eschar und proliferation at the viable/nonviable tissue interface, and Stage IIA, microinvasion of viable tissue which confirms the presence of an invasive burn wound infection (Fig. 2). Mortality increases as histologic staging designation increases, i.e., the risk of remote spread and mortality associated with a Stage II classification is greater than that associated with Stage I disease. The occurrence of mortality in patients with Stage I biopsy findings appears to be related to other complications in patients from whom the eschar cannot be excised and the wound closed prior to full-thickness penetration of the microorganisms. The striking difference in the mortality associated with Stages IC and IIA reflects the impact of invasive infection on burn patient outcome. A histologic staging of IIC with microvascular and lymphathic involvement is associated with a high incidence of microbial recovery from the blood, dissemination of the infection to remote tissues and organs, and an almost universal mortality (Fig. 3) [11].

The histologic findings in a burn wound biopsy must always be interpreted in light of the patient's clinical status and trajectory. A negative reading of a biopsy obtained from a patient with progressive expansion in the size or number of wound areas showing tinctorial or morphologic change consistent with infection demands additional biopsies of those areas. Those occasional patients who show clinical deterioration consistent with sepsis and whose wounds show rapid, dark discoloration consistent with accelerated maturation should have serial biopsies performed and, if progressive increase in the stage of the successive biopsies is noted, wound care should be altered to use only sulfamylon topical chemotherapy and effect surgical excision of the involved tissue as soon as the patient's general condition permits.

Fig. 2. This histologic section from a burn wound biopsy shows dark staining masses of bacillary organisms in the upper half of the section extending into unburned tissue: Stage IIA invasive infection

Fig. 3. This histologic section from a burn wound biopsy shows fungal elements and marked inflammatory changes within the lumen of an involved microvessel: Stage IIC invasive infection

During the period January 1987 through December 1991, the burn wounds of 178 patients treated at this Institute underwent biopsy. The biopsies from 67 patients were reported by the pathologist as being histologically negative, i.e., organisms may not be visible when the microbial density is less than 10^5 per gram of tissue. The biopsies from 85 patients showed only colonization, i.e., Stages IA - IC, and the biopsies from 26 patients were reported as showing invasive burn wound infection, i.e., Stages IIA - IIC. In 18 patients the infecting organisms were filamentous fungi, *Aspergillus* species in 16 and *Mucor* species in two, and in four patients *Candida* species were the causative organisms. In four patients bacteria were the infecting organisms, *Pseudomonas aeruginosa* in two, *Staphylococcus aureus* in one and group D *Enterococcus* in one. The current predominance of nonbacterial burn wound infections and the relative rarity of gram-negative bacillary burn wound infections emphasize the utility of histologic examination by which one can readily identify invading fungi. The mean age of the 26 patients in whom invasive burn wound infection was diagnosed in a biopsy specimen obtained on the basis of clinical suspicion was 41.3 years, and the mean total extent of burn was 56.6 % of the total body surface with 45.1 % being full-thickness burn. The invasive wound infection was diagnosed as early as the seventh postburn day and as late as the 56th postburn day, with the mean time of diagnosis being the 22nd postburn day. Eighteen, or 60 %, of those patients died.

The mean age of the 18 patients with wound infections caused by filamentous fungi was 38.2 years and the mean total extent of burn was 66 % of the total body surface. Twelve, or 75 %, of the 16 patients with *Aspergillus* wound infections died but, somewhat surprisingly, both of the patients with mucor species infection survived. The average extent of burn (67.3 %) in the patients who died with invasive fungal infection was not statistically different from the size of burn (64 %) in the patients who survived, but the effect of age on both burn and infection related mortality was evident; the average age of those patients with filamentous fungal infections who died was 44 years, vs. an average of 27 years for those patients who survived both the fungal infection and their burn. In four patients in whom an initial burn wound biopsy showed some gradation of Stage-I colonization, a repeat biopsy 2 to 11 days later at another site revealed invasive *Aspergillus* infection. The diagnosis of wound invasion prompted immediate excision of the infected tissue. The survival of two (50 %) of those patients emphasizes the importance of early diagnosis made possible by biopsy monitoring.

There were four patients in whom *Candida* was the cause of invasive burn wound infection and three (75 %) of those patients expired. The average extent of burn in those patients was only 27 % of the total body surface, but the average age was 72 years. The average time of death of the three patients with *Candida* infection who expired was the 58th postburn day. There were four additional patients in whom bacteria were the cause of invasive burn wound infection and three, or 75 %, of those patients expired. The mean age of the patients who developed bacterial burn wound infection was 24 years, the average extent of burn was 43 % of the total body surface, and the average time of death was the 22nd postburn day.

Twenty of the entire group of 26 patients with histologically confirmed invasive burn wound infection underwent excision of the infected wound as soon as they could be prepared for surgery: an average of 2.4 days after biopsy. The other six patients who had burn wound infections were so physiologically unstable that excision could not be performed and they expired on an average of 6 days after biopsy

Table 4. Biopsy microbial status and patient outcome

Biopsy stage	Survivors	Deaths	Mortality rate
IIA	7	13	65%
IIB and IIC	1	5	83%

(range day of biopsy to 15 days postbiopsy). In all of those six patients invasive burn wound infection was present and was considered to be a contributing factor to the patient's demise. In only two patients was the infection considered to be the principal cause of death. Even in this small group of patients, histologic stage of invasion was related to outcome. Six (35%) of the 20 patients with Stage IIA infections survived while only one (17%) of the patients with Stages IIB or IIC infections survived (Table 4).

There were an additional 16 patients in whom invasive infection was diagnosed as an incidental finding in surgical specimens. Six of these 16 patients expired. In five patients, burn wound infection was present at the time of death and was considered to be a contributing factor in the patient's demise. In the other patient, autopsy examination revealed no burn wound infection. The fact that only six, or 38%, of those 16 patients expired emphasizes the importance of early excision of infected burns before the infection produces wound changes or systemic signs.

Tissue biopsies can also be used to confirm depth of a burn wound, i.e., differentiate partial-thickness from full-thickness burns; assess adequacy of debridement of electric injury or an infected burn wound, i.e., differentiate viable from nonviable tissue; diagnose suppurative thrombophlebitis, i.e., differentiate thrombosis from intraluminal infection [14]; diagnose disseminated intravascular coagulation (DIC), i.e., differentiate hemorrhagic change due to local trauma from petechia due to coagulopathy; and diagnose exfoliative skin diseases, i.e., differentiate idiosyncratic or hypersensitivity related exfoliative dermatitides from *Staphylococcal* scalded skin syndrome [2]. During the time period covered by this review, tissue biopsies were used for all those purposes except for diagnosis of burn depth, a use obviated by early burn wound excision, and for diagnosis of DIC, a use obviated by availability of accurate laboratory tests.

There were 11 patients from whom segments of 13 previously cannulated veins were biopsied because of a suspicion of suppurative thrombophlebitis in the absence of gross intraluminal suppuration. In two patients fungal suppurative thrombophlebitis was histologically documented with *Aspergillus* identified in one saphenous vein and *Aspergillus* plus *Candida* in a forearm vein. Identification of the intraluminal infection prompted immediate excision of the infected vein in both patients. The excision controlled the septic process and both patients survived.

During the same time period, 85 wound biopsies were performed in 27 patients to characterize and diagnose exfoliative dermatitic processes. In 24 patients, toxic epidermal necrolysis (TEN) was confirmed and differentiated from *Staphylococcal* scalded skin syndrome, which was present in only two patients. There was one patient in whom the dermatitic change was due to an allergic reaction. In one of the ten patients, Stage IIA *Aspergillus* species wound invasion of the face was identified as a preterminal complication. Both of the patients with *Staphylococcal* scalded skin syndrome survived, and 18 of the 24 TEN patients survived.

The use of burn wound biopsies to assess the microbial status of the burn wound and differentiate invasive burn wound infection from microbial colonization of an eschar permits timely intervention to control the infection and increase the patient's likelihood of survival. The use of wound biopsies to differentiate burn depth, assess the adequacy of tissue debridement, and diagnose a variety of infectious and inflammatory conditions enables the attending surgeon to tailor therapeutic interventions, both surgical and pharmacologic, to meet the individual patient's needs and treat documented complications appropriately.

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