THE EFFECT OF BURN WOUND EXCISION ON BACTERIAL COLONIZATION AND INVASION

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ABSTRACT

Survival following thermal injury has improved in the last two decades. Incidence of wound infection and sepsis has declined in the same interval. Early excision has been advocated as one of the major impacts, but its safety and efficacy, and the exact timing of burn excision are still in debate. We hypothesize that acute burn wound excision (in the first 24 hours post-burn) is superior to conservative treatment and delayed excision to prevent bacterial colonization and invasion. Twenty consecutive patients affected of thermal injuries were studied. Twelve patients had acute burn wound excision and eight patients conservative treatment and delayed excision. This second group of patients received topical treatment in another facility and delayed excision on transfer to our service on post-burn day 6. Quantitative bacteriology was performed on the excised wound and on a biopsy of the wound bed before auto and/or homografting. Time effect on bacterial count, differences between superficial and deep biopsies and early versus late debridement effects were studied. Patients admitted early presented bacterial counts under $10^5$ bacteria per gram of tissue. Patients in this group did not present with infection or graft loss. Patients admitted late presented counts over $10^5$ bacteria ($p=0.001$ vs. early admission). Three patients presented infection and graft loss in the late group ($p<0.05$ vs. early group). Burn wound excision significantly decreased bacterial colonization in all patients ($p<0.001$). High bacterial colonization and infection correlated with topical treatment and late excision ($p<0.001$). We conclude that burn wound excision significantly reduces bacterial colonization. Patients undergoing topical treatment and delayed burn wound excision present with higher bacterial colonization and increased incidence of infection. Acute burn wound excision should be considered in all full thickness burns.
INTRODUCTION

Survival following thermal injury has improved over the last several decades. The incidence of burn wound sepsis has been related to burn size, depth and age of the patient. The combined effect of coagulated proteins and the avascularity of the burn eschar results in a high risk for infection. Microorganisms invade the burn eschar over time leading to burn wound infection, and, eventually, progressing to burn wound sepsis. The development and use of systemic antibiotics, followed by the availability of effective topical treatments, and, ultimately, the use of early burn wound excision and closure have decreased the incidence of burn wound sepsis to less than 10%. Topical antimicrobial therapy, by itself, can not protect from invasive burn wound infections, but the benefits of early burn wound excision vs. conservative treatment and serial excision and the exact timing of such excision, however, is still in question.

Although a change in the epidemiology of burn wound infections has been evidenced in the last two decades, the exact timing of the burn wound excision is still in debate. Early excision and closure is regarded as the main impact in such decline in invasive burn infection. The lack of precise data to support it, and the question regarding the safety and efficacy of acute excision and grafting are some of the arguments exposed by many physicians to treat severe burns with conservative treatment and delayed serial excision.

The present study compares the results of quantitative cultures in the burn eschar and in the excised burn to assess the efficacy of burn wound excision in decreasing burn colonization and to assess the effect of time in burn wound colonization.

PATIENTS AND METHODS

Twenty consecutive pediatric patients affected of thermal injuries were studied. Approval by the Internal Review Board of the University of Texas Medical Branch was obtained and all proceedings were performed according to its guidelines.

The patients were divided in two groups, depending on the time of burn excision and the type of treatment (acute excision vs. conservative treatment and delayed excision). On admission all full-thickness burns were excised and covered with autografts and/or cryopreserved homografts when donor site availability was limited. Resuscitation was started on admission according to the Galveston formula and continued through the operative session. After admission and trauma assessment, fluid requirements are estimated and resuscitation is started through a big lumen catheter. Further monitoring includes double or triple lumen central venous catheter, arterial catheter through femoral artery, pulseoximeter, Foley catheter, and temperature probe. During surgery, resuscitation is continued and monitored through urine production and the rest of the physiologic parameters. Blood loss is estimated at 0.5 ml per cm² to be excised, and given to the patient during surgery to maintain
normal haemodynamic parameters. During the whole operative session, resuscitation is monitored in the same way as if the patient were in his/her room. Fluids are titrated to maintain a urine output of 1 ml/kg/hour. The estimated blood requirements are given to the patient through the entire procedure and the blood levels of hemoglobin and haematocrit are determined every thirty minutes. Therefore, the patient receives during surgery his/her resuscitation requirements and the estimated blood requirements in the form of reconstituted whole blood. No attempts are made to correct the blood loss with crystalloids.

Quantitative Bacteriology was performed on the burn wound eschar and on the burn wound bed after excision on the same specific area in order to perform comparisons between superficial and deep microbial flora. Quantitative counts were performed using the Robson-Heggers technique. Briefly, all burn wound specimens taken at the time of surgical intervention were inoculated to a variety of solid and liquid media. The liquid medium employed was an enriched thioglycolate medium or brain heart infusion broth, supplemented to facilitate the recovery of anaerobes as well as the aerobic pathogens. Aliquots of the serial diluted specimens were subsequently inoculated to sheep blood agar plate with phenethyl alcohol, and MacConkey agar.

Data collected included patient demographics, hospital course, with a special emphasis on burn wound infection, graft loss, sepsis, and quantitative cultures.

In order to determine the effect of time of burn wound excision, patients were categorized in two groups. The first group included all patients admitted in our burn center in the first 24 hours after the burn injury (early excision group). The second group included all the patients that were initially treated in another facility and subsequently transferred to our center for definitive treatment (late excision group). All patients in the two groups were operated on admission and total burn wound excision was performed.

To determine the effect of burn wound excision on bacterial colonization and its efficiency to leave an sterile wound bed, the results of quantitative wound cultures in the excised burn eschar and in the excised burn wound bed were compared in every patient. The results in the immediate burn wound excision group were compared to the results in the delayed excision group, so as to determine the effect of time of excision in burn wound colonization and invasion.

Statistical analysis was performed with paired and unpaired t-test when appropriate, Fisher exact test and linear regression. Significance was accepted at p<0.05. Data are shown as mean +/- SEM.

RESULTS

Twenty consecutive patients were prospectively studied. In general, patients were young (7.4 ± 1 years old), and were burned to a total body surface area (TBSA) of 34 % ± 5, with a full thickness component of 30 % ± 4.
The effect of burn wound excision on bacterial colonization and invasion

Twelve patients were admitted in the first 24 hours after the injury, and immediate burn wound excision and auto/homografting was performed (Early excision group), whereas 8 patients were treated topically with silver sulphadiazine twice daily in another facility for 7 ± 2 days and subsequently transferred to our center, where total burn wound excision on admission was performed (Late excision group). The reason for transfer was financial in all cases. Demographics of both groups are shown in Table 1. All patients who were admitted immediately after the injury were resuscitated according to the Galveston formula and were taken to the operating theater while being still resuscitated. There were no peroperative or postoperative complications, and all patients remained stable during the whole surgical procedure.

All patients in the early excision group had bacterial counts in the burn eschar below the range of 10^4 colonies per gram of tissue (9400 ± 6200 colonies /g of tissue). Surgical excision significantly decreased the colonization of the wound. Bacterial counts in the excised wound bed were below the range of 10^2 colonies per gram of tissue (98 ± 58 colonies/g of tissue) (p<0.05, paired t-test, Figure 1). None of the patients included in the early excision group had burn wound infections or graft loss. Microorganisms isolated included Staphylococcus sp. in 5 patients, Staphylococcus epidermidis in 5 patients, and Staphylococcus aureus in 2 patients. Two patients had more than one isolate. Microbiology data is depicted in Table 2.

Patients included in the late excision group had bacterial counts in the burn eschar over the range of 10^5 colonies /g, reaching in some cases 10^6 colonies per gram of tissue (6x10^5 ± 1.7x 10^4 colonies/g of tissue). Surgical excision significantly decreased the colonization of the wound. Bacterial counts in the excised wound bed were below the range of 10^4 colonies per gram of tissue (6500 ± 4100 colonies /g of tissue) (p<0.001, paired t-test, Figure 2). Three patients presented with infection and graft loss, and two patients presented with sepsis after the surgical excision of the burn wound. Patients who presented with infection and graft loss had burn eschar cultures of 10^6 organisms/g tissue and 10^4 organisms/g tissue to the excised wound.

### TABLE 1
Demographics of patients included in the early and late excision groups

<table>
<thead>
<tr>
<th></th>
<th>Early Excision (n=12)</th>
<th>Late Excision (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.5 ± 1.5</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Sex (male/female;%)</td>
<td>64/34</td>
<td>53/47</td>
</tr>
<tr>
<td>TBSA burned (%)</td>
<td>34 ± 3</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>TBSA full thickness burns (%)</td>
<td>30 ± 3</td>
<td>38 ± 5</td>
</tr>
<tr>
<td>Day of excision</td>
<td>1 ± 0.5</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

Data shown as mean ± SEM

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### TABLE 2
Micobiology Results in Early and Late Excision Groups

<table>
<thead>
<tr>
<th>PNr</th>
<th>Group</th>
<th>Type of Injury</th>
<th>Superficial Biopsy</th>
<th>Deep Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Microbial identification</td>
<td>Quantit. Result</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Colonies /gram)</td>
</tr>
<tr>
<td>1</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Early Flame</td>
<td></td>
<td>No growth</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>10000</td>
</tr>
<tr>
<td>4</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus hominis-hominis</em></td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus hominis-hominis</em></td>
<td>1000</td>
</tr>
<tr>
<td>6</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>100000, 10000</td>
</tr>
<tr>
<td>7</td>
<td>Early Scald</td>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus warneri</em></td>
<td>1000</td>
</tr>
<tr>
<td>9</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus haemolyticus</em></td>
<td>1000</td>
</tr>
<tr>
<td>10</td>
<td>Early Flame</td>
<td></td>
<td>No growth</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus epidermidis</em></td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>Early Flame</td>
<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>10, 10</td>
</tr>
<tr>
<td>13</td>
<td>Late Flame</td>
<td></td>
<td><em>Staphylococcus aureus, Enterococcus faecium</em></td>
<td>10000000</td>
</tr>
<tr>
<td>14</td>
<td>Late Flame</td>
<td></td>
<td><em>Enterococcus faecium</em></td>
<td>1000</td>
</tr>
</tbody>
</table>
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The microorganisms responsible for infections included *Staphylococcus aureus* and *Escherichia coli* in one patient, *Enterococcus faecalis* and *Pseudomonas aeruginosa* in the second patient, and *Escherichia coli* and *Enterobacter cloacae* in the third patient. Bacteria isolated in the second and third patients were also responsible of the septic episodes. Patients included in the late excision group had a significant incidence of infection, graft loss, and sepsis than patients in the early excision group (p<0.05, Fisher test). When patients with bacterial counts over $10^6$ were analyzed separately, this group of patients had a 75% of infection rate vs. 0% in the rest of the patients (p<0.01, Fisher test). Microorganisms isolated in all patients included in the late excision group included *Staphylococcus aureus* in 3 patients, *Enterococcus faecalis* in 3 patients, *Enterococcus faecium* in 2 patients, *Escherichia coli* in 2 patients, *Enterobacter cloacae* in 2 patients, and *Pseudomonas aeruginosa* in 2 patients. Microbiology data is depicted in Table 2.

When the effect of time of excision was studied, patients who received topical treatment and delayed excision presented with significantly higher bacterial counts in the burn eschar than patients treated with early excision (p=0.001, late vs. early excision group, unpaired t-test, Figure 3). Moreover, high bacterial counts and infection correlated with delayed excision (R square=0.65, p<0.01).
FIGURE 1: Burn wound excision significantly reduced the bacterial colonization in patients included in the early excision group.

FIGURE 2: Burn wound excision significantly reduced the bacterial colonization. Bacterial load in the excised wound, however, was over the $10^4$ threshold.
DISCUSSION

Many advances in medical technology during the last decades have improved the overall survival of burn victims. For centuries, burn wounds were treated without surgical intervention. Serial debridement of the dead tissue and application of topical ointments was the treatment of choice. The list of topical ointments used in the treatment of burns is as large as human imagination. The use of plant gums or frogs warmed in oil are good examples. By the early 20th century all sort of topical treatments had been abandoned; eschar was permitted to slough spontaneously, and open wounds were left to granulate. The continuous improvement in burn resuscita-
tion led to the development of better and more effective topical treatments aimed to control infections in burn injury. The widespread use of effective topical treatments improved the prognosis among burn victims, which produced the introduction of tangential excision of the burn wound and the development of programs of early excision treatment.

(*) p< 0.05, Quantitative cultures delayed excision group vs. early excision group

**FIGURE 3:** Patients treated conservatively for one week presented with higher bacterial colonization than patients treated with acute excision
Currently, survival is being reported in patients with TBSA burned over 98%\(^9,10\). Burn patients are now considered as victims of major trauma, and, as such, managed with early surgical intervention to remove all devitalized tissue and achieve wound closure. Despite many reports of excellent outcomes with the utilization of programs of early burn wound excision and grafting, there are still many centers and authors who question the safety, utility, and efficacy of this approach. The continuous development of new topical treatments for full thickness wounds and the proclaiming of topical treatment with cerium nitrate sulphadiazine\(^11\) and delayed excision as an alternative to early excision and grafting are good examples.

The rationale of burn wound excision lies on the fact that, by nature, burns contain devitalized tissue. This necrotic tissue is surrounded by damaged and edematous tissue kept moist at 37 Celsius degrees, rendering excellent conditions to support the growth of microorganisms\(^13,14\). The microbial population of the burn wound immediately after burning is sparse and predominantly gram-positive. As time passes, gram-negative organisms colonize the burn eschar and by the end of the first postburn week they are the predominant microorganisms\(^15\). Colonization of the burn wound by gram-negative microorganisms occurs predominantly from endogenous bacteria. Due to local and general changes in the gut which are aggravated by the use of systemic antibiotics, the gastrointestinal system is colonized by pathogens which in turn will colonize the burn wound by postburn day 5\(^16\). It is believed that intraeschar proliferation and penetration can be retarded and even prevented by the use of topical antimicrobial agents\(^12,13,17\). The incidence of local burn infections and of burn wound sepsis, confirms, however, that the protection of topical antimicrobials is at best, partial\(^15\). The type of organisms present in the eschar strongly influences the incidence of invasive infection. Burn wound infections caused by gram-positive tend to be superficial and remain localized\(^18\). The enzymes and other metabolic products produced by gram-negative microorganisms enhance the invasive potential and the rapid spread of these infections\(^19\). The utilization of quantitative microbiology helps to perform burn wound surveillance\(^6,20\) and to perform a presumptive diagnosis of invasive infections when bacterial colonization is over \(10^5\) organisms/gram of tissue. Nevertheless, the only reliable way to make the diagnosis of invasive burn wound infection is histologic examination\(^20,21\). Quantitative cultures of a wound biopsy appear to be helpful in confirming the absence of wound infection, but when they grow more than \(10^5\) organisms/gram of tissue, burn wound infection can only be confirmed in 50% of histologic examinations\(^20,22\).

The data obtained in our series of patients corroborate the above mentioned findings. Patients in the late excision group presented with wound cultures significantly higher than the early excision group and the type of colonization followed a pattern of gram positive to gram negative colonization as time passed. There were no cases of local or systemic infection in patients who had early burn wound excision, whereas three patients had infections in the late excision group, despite receiving topical treatment with silver sulphadiazine. It confirms that conservative topical treat-
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ment renders a higher incidence of morbidity, and supports the rationale of early burn wound excision. When burn wounds are excised acutely after burning, the surgical intervention occurs when the bacterial colonization by gram-negative bacteria has not started yet. The colonization by gram-positive bacteria is sparse so graft loss and burn wound infection and sepsis do not occur. Surgical excision decreased an average of $10^2$ organisms per gram of tissue in both the early and delayed excision groups. A sterile wound, however, was never achieved. The lowest and highest bacterial counts were $10^1$ and $10^4$ organisms per gram of tissue. It is clear, therefore, that prompt eschar excision renders a low contamination rate on the burn wound, whereas bacterial contamination in delayed excision leaves still a high bacterial count. This fact, besides the type of microorganisms, makes burn wounds treated with delayed excision more prone to infection. All patients that had quantitative cultures below $10^5$ organisms/gram of tissue did not have infection or graft loss, whereas patients that had cultures over $10^6$ organisms/gram of tissue presented with a 50% incidence of burn wound infection and graft loss. Furthermore, when patients grew over $10^6$ organisms/gram of tissue, the incidence of burn wound infection and sepsis reached 75%. We believe that although quantitative cultures can not make the diagnosis of invasive infections, they show clearly the potential of a given wound to develop such infection. Burn wounds that grow bacteria over $10^5$ organisms per gram of tissue should be considered as a red flag for invasive burn wound infection.

Despite of all the former the exact timing of early excision or, in other words, when early is not longer early is still in debate. We believe that all severe burn injuries should be treated with immediate wound excision and grafting. Our group has previously shown the safety and efficacy of this approach \cite{2-5, 10, 23-24}. Burn excision and grafting can be started as soon as the initial assessment and stabilization have been completed and carried out while the resuscitation is continued. This approach does not increase complications and, in our hands, makes the management of patients easier than with a standard approach. This was also our experience in the present series of patients. Many authors have shown the benefits of early burn wound excision, and have proved it to be one of the major impacts in the improvement of burn survival \cite{25-28}. All of them, however, used a less aggressive approach, with a sub-acute excision, after the resuscitation has been completed. Based in the present data and in former studies \cite{2-5}, however, we believe that all severe burns should be excised within 48 hours in order to profit all its benefits.

In summary, conservative topical treatment has a higher incidence of infection and graft loss than early excision and grafting. Delaying burn wound excision increases bacterial load and increases gram-negative colonization. When such colonization reaches $10^6$ organisms/g tissue in the burn eschar, they should be considered at risk for developing invasive burn wound infection even when the wounds are excised. Burn wound excision decreases an average of $10^2$ organisms per gram of tissue; so burn wounds with high counts are at risk to develop burn wound sepsis both before and after surgery.
REFERENCES

14. Lawrence JC. Burn bacteriology during the last 50 years. Burns 1992; 18:s23-s29
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