Official Journal of the Brazilian Association of Infection Control and Hospital Epidemiology

Journal of INFECTION CONTROL

ISSN 2316-5324 | Ano IV . Volume 4 . Número 3 . 2015 . Jul/Set.

> ACESSE AQUI A REVISTA ONLINE

ORIGINAL ARTICLE

A one-year prospective evaluation of infections in a brazilian reference center for burn care

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Recebido em: 20/11/14
Aceito em: 02/03/15
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RESUMO

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ABSTRACT

Introduction: Burned individuals have lost the skin mechanical barrier after burn damage, favoring colonization and blood stream infections. The objective this study is contributed with the knowledge on clinical and microbiological aspects and prognostic evolution of burned patients. **Methods:** A one-year prospective study was carried out in a Brazilian reference center for burn care. We were able to enroll 30 patients who accomplished the following requisites. Biopsies were conditioned in 10% buffered formalin recipients and sent to the Pathology Laboratory where they were processed according to standard routines. From Epiderme to Cellular Subcutaneous Tissue all observation fields were examined in order to search and describe present microbiota. McNemar's test was used to determine whether there was a significant difference in correlation analysis involving tissue culture and histopathology. **Results:** During study 228 samples were collected and an identical proportion of 57 samples were obtained between pathology biopsies, tissue culture biopsies, urine and blood cultures. A total of 32 positive results (7.35%) were encountered concerning microbiological analysis. Majority of histopathology punch's showed microorganisms presence (71,07%) in each collecting period. Gram negative bacilli were found in 61.44% of these samples with gram positive cocci encountered in only 9.63% of these biopsies. Microorganism absence was observed in 27.71% of histopathology samples. The comparison between histopathology and tissue culture was statistically significant (MacNemar Test, $p<0.0001$), and histopathology proved to be more sensitive. **Conclusions:** That the association of diagnostic methods seems to be the best way for the diagnosis of invasive infections in burn patients.

Keywords: *burn, microorganisms, infections, diagnosis.*

INTRODUCTION

Burns are one of the most dramatic types of trauma, accounting for more than 320.000 deaths in a two-year period (2000 to 2002), with the vast majority occurring in developing countries.[1] Approximately 500,000 patients with burn injuries seek medical attention in the US each year, with 40,000 of these require hospitalization and 60% intensive care in specialized centers.[2] Recently, with the onset of burn unit centers, management of these patients faced tremendous improvements.[3] However, in despite of several advances in the healthcare of burned patients, infections remain the main cause of mortality.[4] Indeed, due to a complex association of risk factors, these patients are approached under high suspicion for invasive infections.[5]

Burned individuals have lost the skin mechanical barrier after burn damage, favoring colonization, wound invasive and blood stream infections. The pathofisiollogy of burn infections is associated with progressive and changing colonization of burn scars and medical devices.[6] In this scenario, endogenous sources of microorganisms are well recognized. Environmental agents get to the infection sites through handy cross transmission and directly from contamined solutions, conditioning air flow and facilities surfaces.[7]

In addition, because of their usually critical condition, patients who have experienced severe burn are submitted to invasive procedures like central venous and urinary catheterization, as well as orotraqueal intubation and surgical interventions, thus enhancing exposition to device colonization and infection.[8] Finally, thermal injury leads to a severe systemic inflammatory activity, associated with cellular and humoral impaired immune response.[9]

The microorganisms involved in burn infection are composed, in the early onset of the disease by gram positive cocci mainly *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *difteroids*, since some of these bacteria are able to tolerate thermal injury,[10] taking advantage of disrupted skin to reach deep tissues. Afterwards, during the first week period, a progressively increase of gram negative colonization is observed, usually predominating *Enterobacteriaceae* followed by multi-resistant gram negative rods, like *Pseudomonas aeruginosa* and *Acinetobacter baummani*.[8,11] Nowadays, considered as emergent microorganisms, fungi also appear to be important in the etiology of late onset infections. Candida species and *Aspergillus spp.* are the most common isolates.[12]

Nevertheless, etiological evaluation of burn wound infections can be complex, especially when swabs samples are taken. This procedure normally results in a polimicrobial burden of agents, making antibiotic treatment choice a true challenge. Besides, one of the possible consequences is to guide the treatment to colonizing bacteria, since this approach only gets to the skin superficial layers and, therefore, unable to reach deep tissues. Quantitative and histological analysis from punch biopsies are suggested as complementary tools to increase diagnostic specificity, but those methods and it's results interpretation still require further studies for standardization.[13]

In South America, only Peru and Argentine published burns frequency and their medical complications data.[1] So far, few studies were conducted in order to evaluate burned patient infections in Brazilian burn care units.[5,6,14,15] In order to contribute with the knowledge on clinical and microbiological aspects and prognostic evolution of burned patients, a one-year prospective study was carried out in a Brazilian reference center for burn care.

MATERIAL AND METHODS

The study was conducted from April 2009 to January 2010 in Hospital Burn Unit (PAHBU), by the Center for Microorganism Investigations (CIM) and Pathology Laboratory, Faculty of Medicine from São José do Rio Preto (FAMERP), São Paulo State, Brazil. Sample collection and clinical evaluation was performed by the assistant physicians of the PAHBU.

A total of 278 patients were admitted in the PAHBU for burn care treatment within this study period. However, not all were eligible according to the established inclusion criteria. We were able to enroll 30 patients who accomplished the following requisites: were over 18 years-old, were admitted with extensive third degree burns (>20% total body surface)[16] without a permanence longer than 48 hours in other ward and no previous use of systemic antimicrobials. This study protocol was approved by the Ethics Committee of Catanduva Medical School. Data on the epidemiology, microbial etiology, clinical aspects and prognostic evolution were stored in forms and later in Excel files (Microsoft Excel 2007).

Sample collection

Wound care routine and sample collecting protocols were performed by the burned care unit attending physicians of the plastic surgery service and by the nurses of the same unit. Sample collection and handling was conducted following the statements of the Brazilian Health Ministry Agency for Infection Control.[17] Accordingly to this study protocol, serial sample collection was performed as follows: up to 48 hours of admission and within the subsequent weeks, an once per week sample was obtained until patient discharge. Samples were constituted by skin from burn scars (two simultaneous 5 mm punch from sites with suspicion signs of colonization or infection between burned scar and viable tissue area), blood and urine, collected for all patients enrolled. Samples obtained were immediately sent to the microbiology and pathology laboratories and were processed by bacteriology and basic mycology routines, as well as histopathology preparation for further analysis

Specimen processing

Tissue biopsy, urine and blood samples were processed by standards techniques for microorganisms identification.[18-20] Moreover, bacterial and fungal quantitative analysis for tissue biopsy was processed as previously described by Shulman *et al.,*[21] with modifications. Briefly, samples were weighed, placed in 2mL of sterile physiologic saline solution in a caddish and macerated. A portion of the homogenate was plated on to blood agar, chocolate agar, Sabouraud (Himedia, XE147. Mumbai, India) and Mycosel agar (BBL Mycosel, 5082653. Le Pont de Claix,France). The colony forming units (CFU) was determined by the following formula: CFU = n x V x 2/ W, where ***n*** is the number of colonies counted on a plate of given dilution, ***V*** is the inoculated homogenate volume (0.1 or 0.01 ml), ***2*** is the diluent volume used for tissue homogenization, and ***W*** is the weight tissue in grams. It should be noted that the decision to use 0.1- and 0.01-mL volumes of homogenate was based on the weight of a sample to give a precise colony counts between 10^4 and 10^6 colonies per gram. This range was desired because of the clinical relevance of a 10^5 colony count per gram of tissue as a predictor of invasive infections.[22]

Histology

Biopsies were conditioned in 10% buffered formalin recipients and sent to the Pathology Laboratory of Hospital de Base where they were processed according to standard routines. Briefly, after paraffin inclusion all fragments were sliced in 5 micra for optic microscopy amplification (400x;1000x) and analysis. From Epiderme to Cellular Subcutaneous Tissue all observation fields were examined in order to search and describe present microbiota. The Modified Gram technique, Hematoxilin-Eosin

and Silver corants were used to evidentiate bacteria, yeasts and filamentous fungi. Analyses were made by experienced pathologists in infectious diseases of the Faculty of Medicine from São José do Rio Preto Pathology Department.

Statistical analyses

Epi Info version 6.04b (CDC, Atlanta, US) was used for data storage and statistical analyses. McNemar's test was used to determine whether there was a significant difference in correlation analysis involving tissue culture and histopathology.

RESULTS

Most patients were male (70%), with a median age of 38.9 years old (SD= ±12.3 years) ranging from 21 to 68 years-old. The median admittance standing time was 13.5 days varying between 10 and 30 days. The most common burn source was flame exposition (55.17%), followed by electricity and scalds (20.69% each) and chemical contact (3.45%). Housework-related activities (48.3%) and labor-related exposition (37.9%) accounted for the great majority of the accident settings while leisure activities occurred in 13.8%. A mortality rate of 26.66% was observed, most of the deceived patients were male and half of the burn injury was labor related.

Regarding to the risk factors for infections, urinary catheterization was introduced in 90% of our population, while central venous catheters were placed in 60% of them. Surgical wound procedures, composed by deep surgical debridement or surgical grafts, were indicated in 46.66% of our study patients. Mechanical ventilation was initiated in 26.66% of all patients. Antimicrobial therapy was prescribed for 83.33% of our subjects. Potential imunossupressive comorbidities were verified in only four patients of this study group, including heart failure, diabetes mellitus and a HIV positive patient.

During the study period, 228 samples were collected and an identical proportion of 57 samples were obtained between pathology biopsies, tissue culture biopsies, urine and blood samples for culture. An average rate of 7.6 samples by patient was collected. A total of 32 positive results (7.35%) were encountered concerning microbiological analysis. Urine culture showed the highest rate of positive results (21.05%), when compared with tissue (19.29%) and blood cultures (15.78%). Bacteremia was present in 37.5% of the deceived patients.

Microorganism's distribution by methods and clinical specimens is presented in Table 1. Gram negative bacilii were the most prevalent isolated group (71.87%), followed by fungi (18.75%) and gram positive cocci (9.37%). In the gram negative group, *Pseudomonas aeruginosa* was the most common pathogen accounting for 59.09% of these agents, while *Acinetobacter baumanii* was recovered in 31.81%, half of them from blood cultures (bacteremia episodes). *Staphylococcus aureus* was found in 66.66% of the gram positive group, and it was the determined cause of one bacteremia episode, after seven days of this patient PABHU admission. In biopsies cultures results, gram positive cocci were firstly found with 48 hours of hospital admittance.

Table 1 – Microorganism's distribution by methods and clinical specimens in burned patients hospitalized at the PABHU, a reference burn unit, São Paulo State, 2011.

	Microbiological Samples by Specimens			
Patient	**Tissue Culture**	**Histopathology**	**Blood Culture**	**Urine Culture**
1	*S. aureus*	Gram Positive Cocci	Negative	Negative
2	*P. aeruginosa*	Gram Positive Cocci / Gram Negative Bacilii	Negative	Negative
3	*P. aeruginosa*	Absent	Negative	*P. aeruginosa*
4	Negative	Absent	Negative	Negative
5	Negative	Gram Positive Cocci	Negative	*P. aeruginosa*
6	*A. baumanii*	Absent	Negative	Negative
7	*K. pneumonie*	Gram Negative Bacilii	Negative	Negative
8	Negative	Gram Negative Bacilii	Negative	Negative
9	Negative	Gram Negative Bacilii	Negative	Negative
10	Negative	Gram Negative Bacilii	*C. albicans*	*C. albicans*
11	Negative	Gram Negative Bacilii	Negative	Negative
12	*A. baumanii*	Gram Negative	Negative	Negative
13	Negative	Absent	Negative	Negative
14	Negative	Gram Negative Bacilii	Negative	Negative
15	Negative	Gram Positive Cocci	Negative	Negative
16	Negative	Gram Negative Bacilii	Negative	Negative
17	*A. baumanii / P. aeruginosa*	Gram Negative Bacilii	*A.baumanii / P. aeruginosa*	Negative
18	Negative	Gram Negative Bacilii	Negative	*Paecilomyces sp.*
19	Negative	Gram Negative Bacilii	Negative	Negative
20	Negative	Gram Negative Bacilii	Negative	Negative
21	*S. viridans*	Gram Negative Bacilii	Negative	Negative
22	Negative	Gram Negative Bacilii	Negative	Negative
23	Negative	Gram Negative Bacilii	Negative	Negative
24	Negative	Gram Negative Bacilii	Negative	Negative
25	Negative	Gram negative bacilii	*A. baumanii*	Negative
26	Negative	Gram Negative Bacilii	Negative	Negative
27	Negative	Gram Negative Bacilii	Negative	*A. baumanii / P. aeruginosa*
28	Negative	Gram Negative Bacilii	Negative	Negative
29	Negative	Gram Negative Bacilii	*P. aeruginosa*	*C. glabrata*
30	Negative	Absent	Negative	*E. aerogenes*

However, gram negative bacilii were recovered since the very day of admission until the third week of the patients' hospitalization, in all types of clinical specimens.

Although quantitative tissue culture analysis showed only two biopsies with colony units forming per tissue gram >10^5 ufc/g, the same qualitative results were obtained in blood cultures, at the same time. *Pseudomonas aeruginosa* and *Acineotobacter baumanii* were isolated after tissue culture (>10^5) and blood samples in the second and third collecting period timings, from the same patient. *Candida spp.* was the most prevalent isolated fungi and the only candidemia episode was due to *Candida albicans*. *Candida glabrata* positive urine samples were obtained from one of the patients in three consecutive episodes. *Paecilomyces* sp was the only filamentous fungi encountered in our study, from one urine sample. No yeast was isolated from biopsy tissue culture. Candiduria was observed as soon as 48 hours of admission and a second week candiduria result was accompanied by a candidemia episode by the same agent (*C.albicans*).

The vast majority of histopathology analyzed punch's showed microorganisms presence (71.07%) in all collecting periods. Gram negative bacilii were found in 61.44% of these samples with gram positive cocci encountered in only 9.63% of these biopsies. Microorganism absence was observed in 27.71% of histopathology samples. The comparison between histophatology and tissue culture was statistically significant (MacNemar Test, p<0.0001), with the first method proving to be more sensitive. In seven episodes, a timing concordant result was observed for an agent observed in histopathology and blood cultures, against only three similar occurrences with tissue versus blood cultures. Also, there was a positive correspondence between histopathology results and the recovery of agents in those two positive Shulman results above described (Table 1).

DISCUSSION

Most common burn victims in developing countries generally are describe as middle aged men exposed to flame in work-related settings. Despite of house-related activities has been found with a slight superior frequency than work-related accidents, our findings were very similar to the worldwide literature reports in terms of gender, aging and burn source exposition.[23] A similar relation involving petroleum processing industries has already been studied.[24]

Critically ill patients admitted in intensive care units are vulnerable to some well know infections syndromes as urinary tract infections and blood stream infections. Patients with deep and large burns are considered as critical ill subjects and usually show these same kinds of infections.[5] Urinary catheter devices are associated to a fast and intense biofilm production.[25] Considering that 90% of our patients were submitted to a urinary catheter, the highest positivity rate founded in urine culture can be explained. Gram negative were the most common isolated agent, a similar finding already described in other two published urinary tract infections in burned patients.[26,27] Recovering of the same agent in urine sample and blood was found in 16.66%, a slight superior rate comparing with was noted in general literature, where around 15% of complicated urinary tract infections were accompanied by blood stream invasion.[28]

Blood stream infections were diagnosed in nine episodes in our population during study period, most of them caused by gram negative agents (77,77%). High rates of gram negative bacteremia in burned patients has already been cited,[29] even ranking these agents as the most frequent isolated pathogen, especially by *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumonae.* Gut translocation and tissue lyses enzymes production were implicated.[26,30] Although there was no catheter-related blood stream infections in the present study, a previous published paper in our burn unit has show a 4.51% catheter-related infections, a two-folder rate compared with these infections in general intensive care units.[6] Gram negative bacilli were isolated in 77.3% of these episodes. Blood stream infections as a soft skin infections consequence are reported in 5% general subjects.[31] In our population, of 57 blood cultures collected, seven bacteremia episodes (12.28%) could be associated with burn wound infections, since the same organism was encountered in tissue culture or the same morphology was found in histopathology, at the same collecting period.

Histopathology is method able to evaluate burn wound depth and microorganism invasion. Evaluation grandings were proposed, where the most important considered finding is the presence of microorganism beneath burn scar and viable tissue transition area.[32] The need for another sensitive diagnostic tool is justified because tissue cultures can produce false-negative results when patients are under topical and systemic antimicrobial therapy, a very common occurrence in those subjects.[33] In our study population all patients received topical antimicrobials and 83.33% of them were submitted to systemic antimicrobial therapy. In addition, it has already been described in medical literature that gram negative bacteria, especially *Pseudomonas aeruginosa*, can promote fungal growth inhibition in burned tissues cultures.[34] Indeed, despite of the fungal absence in our overall biopsies results, histopathology showed a more sensitive rate comparing with tissue cultures, with a strong statistical significance (McNemar Test p<0.0001).

Quantitative microbiological burn wound biopsies started to be studied since 40 years ago.[35] However, medical literature are still controversial regarding to the ability of this method in improve burn wound infections diagnose specificity.[36-38] Out of 57 tissue cultures in our study, only two samples presented with colony units forming >10^5 (ufc/g). Surpriselly, it was found in the same patient, in consecutive collected samples and, most important, tissue recovered *Pseudomonas aeruginosa and Acinetobacter baumannii* were also founded in parallel blood cultures. For these two samples, gram negative morphology was either detected in histopathology. These findings suggests that a concordant result among morphology and high bacterial burden in burn wounds are associated with burn wound infections and seems to predict blood stream invasion. Additional studies with larger samples sizes are necessary to evaluate the real value of histopathology as a sensitive tool for burn wound infections diagnose, as well as the power of quantitative burn wound tissue analysis in enhance diagnose specificity.

A high proportion of *Pseudomonas aeruginosa and Acinetobacter baumannii* was found in all sorts of our collected specimens, two of the most virulent know bacteria. According to literature, gram negative high prevalence's are mainly explained by environmental colonization pressure and gut translocation.[7,39] On the other hand, modern use of topical and broad spectrum antimicrobials agents can booster this process. Another worrying observed aspect was the high rate of blood stream invasion in a young population without classics immunosuppressive comorbidities, denoting how burn injury are able to decrease immune system. These findings considered in combination could explain the higher mortality rate encountered in our study population, compared with other burn subjects analysis.[40] Thus, infection control measures and antimicrobial stewardship programs have to be implemented to efficiently face these problems.

Finally, a possible limitation of this study can be related to factors such as confusion, mainly because of incomplete forms and the small sample size that could limit or difficult our results and interpretation.

We conclude that the association of diagnostic methods

seems to be the best way for the diagnosis of invasive infections in burn patients. Furthermore, the data provide important clues for a future larger comprehension, prevention and control of burn injuries.

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