Original Article

Temporal Changes in Bacterial Profile of Burn Wound Infections in a Tertiary Care Hospital and Risk Factors for Invasion: A Prospective Cohort Study

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ABSTRACT

Microbiology Section

Introduction: Burn wound infections remain a significant cause of morbidity and mortality in patients admitted to burn units. Burn wounds serve as a susceptible site for opportunistic colonisation by endogenous and exogenous organisms due to local and systemic immunosuppression. The bacterial infection profile changes over time in hospitalised patients which increases the risk of systemic invasion.

Aim: To determine the temporal changes in the bacterial profile of burn wound infections and identify the risk factors associated with invasive infections.

Materials and Methods: A prospective cohort study of patients with burn injuries was conducted over 15 months in the burns unit of a tertiary care hospital in Mumbai, Maharashtra, India. Since, it was a time bound prospective cohort study, samples from all patients admitted with burn wounds between June, 2018 and September, 2019 were considered as study participants (n=131). Wound swabs were obtained from patients upon admission and subsequently at regular intervals. Bacterial isolates were identified and their antibiotic susceptibility patterns were determined. Data were analysed to identify temporal changes in the bacterial profile to determine the risk factors associated with invasive infections.

Results: A total, of 131 patients with burn injuries were included. A total of 503 swabs were collected, of which 373 (74.2%) showed bacterial growth; five (1.34%) were contaminants and were excluded from analysis. Gram-negative bacteria were the predominant isolates in the first week, whereas gram-positive organisms predominated from the second week onwards. The most common organism isolated was Pseudomonas aeruginosa (n=100, 27.17%) followed by Staphylococcus aureus (n=84, 22.83%), Acinetobacter baumannii (n=74, 20.11%), and Klebsiella pneumoniae (n=65, 17.66%). A total of 74 blood culture specimens were collected from 47 patients suspected of having invasive infections based on clinical signs and symptoms of systemic infection clinically. Of these, 37 patients (78.7%) were culture positive. Mortality was noted in 28 patients (28/31, 90.32%) in the group with invasive infections. Upon calculating the Odds Ratio (OR), risk factors found to be associated with invasive infections included a higher degree and depth of burns and a longer hospital stay.

Conclusion: The shift from gram-negative to gram-positive bacteria and the increasing resistance over time highlight the importance of ongoing surveillance and appropriate antimicrobial stewardship measures in the management of burn wound infections.

Keywords: Antibiotic susceptibility, Invasive infection, Opportunistic infections, Total burnt surface area

INTRODUCTION

Burn wound infections are a global health problem in both developing and underdeveloped countries [1]. In India, more than 6 to 7 million people are exposed to moderate or severe burns annually [2]. Seventy-five percent of all deaths in these patients are due to infectious complications such as sepsis and bacteremia [1]. Burn wound surfaces are sterile immediately following thermal injury, but within 48 hours, they become colonised with the patient's own skin commensals. After approximately seven days, the wound becomes colonised with endogenous organisms from the patient's gastrointestinal or respiratory tract, or exogenously from the hospital environment [1]. Exogenous organisms from the hospital environment [1]. Exogenous organisms from the potential environment [1]. Exogenous organisms from the preventive strategies can be designed accordingly.

Temporal changes in microbial communities reflect constant fluctuations and recurrent variations over time in the community structure, composition, or function of microbes and are governed by both intrinsic and extrinsic factors [1,3]. Burn wounds serve as a susceptible site for opportunistic colonisation by endogenous and exogenous organisms due to local and systemic immunosuppression

[4]. This leads to changes in the bacterial infection profile over time depending on which organisms the wounds are exposed to at any given time in hospitalised patients [4,5]. Colonisation during prolonged hospitalisation increases the risk of invasion, systemic complications and death. The characteristics of different organisms also play major roles in their invasiveness. Invasive infection is indicated by inflammation of surrounding uninjured tissue, positive blood cultures with a pathogen isolated and systemic signs of sepsis with no other identifiable source of infection [1]. Staphylococcus aureus is the most common cause of early burn wound infection [1,6]. Other organisms that may cause infection during the course of admission to the ward include Pseudomonas aeruginosa, Klebsiella species, Proteus species, Acinetobacter species and Enterococcus species [1,5,7,8]. The aim of the present study was to determine the temporal changes in the bacterial profile of burn wound infections. The primary objective was to identify the frequency of association of various species of bacteria with burn wounds, to determine their susceptibility patterns, and to identify any temporal associations. The secondary objective of the study was to identify the risk factors for invasion.

MATERIALS AND METHODS

This prospective cohort study was conducted over a period of 15 months, from June 2018 to September 2019. Since this was a

time-bound prospective cohort study, samples from all patients admitted for burn wound infections during the aforementioned period were considered as study participants (n=131). Permission was obtained from the Institutional Ethics Committee (IEC) before commencing this study (EC/113/2017 dated 28th November 2017). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and the Helsinki Declaration of 1975, as revised in 2000.

Inclusion criteria: All patients admitted to the burns unit in this hospital during this period, including those referred from outside, were enrolled after receiving written informed consent from adult participants and assent from children aged over seven years, along with parental consent were included in the study.

Exclusion criteria: Patients who did not consent to the study were excluded from the study.

A detailed history and associated risk factors, as per the available literature such as age, days of hospitalisation, percentage of Total Burned Surface Area (TBSA), depth of burns, type of referral, diabetes, hypertension and pregnancy, were recorded [7-9].

Specimen collection: Two swab specimens were aseptically collected from the burn wound using sterile cotton swabs moistened with sterile normal saline. Swabs were collected periodically on days 0, 4, and 8 and thereafter every week until the completion of the hospital stay or until the death of the patient. Specimens were processed according to the standard protocol and isolates were identified up to the species level by routine phenotypic tests. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method [9-13]. Growth of three or more colony morphologies with no polymorphonuclear cells on Gram's stain was excluded. A repeat specimen was used in these cases.

For reporting Coagulase-Negative Staphylococci (CoNS) from the burn wound, a detailed history, including clinical signs of infection and associated risk factors, was taken, along with repeat testing with a freshly collected sample. If a patient developed an invasive infection, systemic specimens, such as blood, sputum, tracheal aspirate, urine, or tissue, as appropriate, were processed for culture according to the standard protocol [8,10,11]. Specimens that showed no growth on wound swabs were considered non infected, whereas those that showed growth only in wound swabs were considered localised infections/colonisation. Those that showed growth in both wound swabs and blood cultures were considered invasive infections.

Depending on the depth of injury, burns were classified into superficial (involving only the epidermal layer of skin), partialthickness (involving the epidermis and portions of the dermis) and full-thickness (extending through and destroying all layers of the dermis and often injuring the underlying subcutaneous tissue) [14].

For estimating the percentage of TBSA burned for risk factor analysis, the 'Rule of Nines' was followed: head - 9%, each arm - 9%, anterior chest and abdomen - 18%, posterior chest and back - 18%, each leg - 18%, and perineum - 1% [15].

STATISTICAL ANALYSIS

Data were analysed using Statistical Package for the Social Sciences (SPSS) software version 20.0. The organisms isolated and their susceptibility were expressed as percentages, which were analysed by linear regression analysis, and the p-value was calculated. For risk factor analysis, participants were categorised into those with invasive infections and those with non invasive infections, and the p-value and Odds Ratio (OR) were calculated. The risk factors, i.e., age, days of hospitalisation, percentage of TBSA, depth of burns, type of referral, diabetes, hypertension, and pregnancy, were analysed using the Chi-square test. Statistical significance was set at p-value <0.05. The strength of the association between the risk factors was studied by calculating the OR. A value of >1 indicates a positive association.

RESULTS

A total of 131 patients were enrolled in this study. As the burns ward in this hospital is only for adult female patients and children (\leq 18 years) of both sexes, 95 (72.5%) female patients were included, of which 74 (78%) were adults and 21 (22%) were children. Of the 36 (27.5%) male patients enrolled, 33 (91.7%) were from the pediatric age group, and only three were adult males who were admitted to the surgical ward.

A total of 131 patients with burns were included in the present study. From all patients, swabs were collected on days 0, 4, 8, and weekly until discharge or death. In total, 503 swabs were collected from 131 patients at periodic intervals, and 373 culture positives were obtained over the entire study period. Five cultures showed polymicrobial growth and were excluded from analysis, so 368 isolates were considered as infections. The results of the swabs collected are mentioned in [Table/Fig-1].

Organism	Distribution of isolates (n=368) n (%)
P. aeruginosa	100 (27.17)
S. aureus	84 (22.83)
A. baumannii	74 (20.11)
K. pneumoniae	65 (17.66)
Enterobacter species	15 (4.08)
Proteus species	15 (4.08)
Enterococcus species	7 (1.90)
Coagulase negative Staphylococcus species (CoNS)	5 (1.36)
E. coli	3 (0.82)
Table/Fig-1]: Microbiological profile in burn wounds.	

Of the 131 swabs collected on day 0 from an equal number of patients, 32 showed growth, of which four were contaminants.

The most common organism isolated was *Klebsiella pneumoniae* (9, 32.14%), followed by *Pseudomonas aeruginosa* (8, 28.57%) and *Acinetobacter baumannii* (5, 17.86%). Gram-negative bacteria were the predominant isolates until day eight [Table/Fig-2a]. Samples collected from the end of the second week showed predominant growth of *Staphylococcus aureus* [Table/Fig-2b].

A total of 74 blood culture specimens were collected from 47 patients suspected of having invasive infection based on signs and symptoms of systemic infection clinically and no other identifiable source of infection. Of these, 37 specimens (78.7%) were culture positive. The most common isolate was *Klebsiella pneumoniae* (16, 43.2%), followed by *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (5, 13.5% each). Other bacteria isolated included *Enterococcus* species, *Proteus* species, and *Enterobacter* species (3, 8.1% each), as well as *Staphylococcus* aureus (2, 5.4%).

Flames were an important cause of burns in 47 patients with invasive infections (OR=4.2, p-value=0.0007) [Table/Fig-3]. A longer hospital stay of >2 weeks was associated with higher invasion rates (OR=3.07, p-value=0.006). Invasive infections were also more associated with a higher degree and depth of burns (burns with >50% TBSA: OR=2.23, p-value=0.074) (deep burns OR=8.25, p-value=<0.00001) [Table/Fig-3].

Twenty-four patients (18.32%) showed no growth on wound swabs and were considered non infected, whereas 76 patients (58.02%) showed growth only in the wound swab and were considered localised infections/colonisation. Thirty-one patients (23.66%) showed growth in both wound swabs and blood cultures and were classified as having invasive infections.

Mortality was higher among those with invasive infections (n=28; 90.32%) compared to those with localised infections (n=15; 19.74%) (OR=37.9, p-value <0.001) [Table/Fig-4]. The mortality rate was higher in culture-positive sepsis (28, 90.32%) compared

Wound swabs	0 day	4 th day	8 th day	2 nd week	3 rd week	4 th week	>5 th weeks	Total
Total no. of samples	131	101	99	61	47	29	35	503
No growth	99 (75.57%)	18 (17.82%)	33.03%	3 (4.92%)	4 (8.51%)	1 (3.45%)	2 (5.71%)	130 (25.85%)
Polymicrobial growth	4 (3.05%)	0	0	1 (1.64%)	0	0	0	5 (0.99%)
Total isolates	28 (21.4%)	83 (82.2%)	96 (96.9%)	57 (93.4%)	43 (91.5%)	28 (96.6%)	33 (94.3%)	368 (73.2%)
Total gram negative isolates	24	71	77	39	22	15	24	272
Non-fermenting Gram negative bacilli								
• A. baumannii	5 (17.86%)	23 (27.71%)	23 (23.96%)	14 (24.56%)	6 (13.95%)	1 (3.57%)	2 (6.06%)	74 (20.11%)
• P. aeruginosa	8 (28.57%)	21 (25.30%)	27 (28.13%)	16 (28.07%)	11 (25.58%)	6 (21.43%)	11 (33.33%)	100 (27.17%)
Enterobacterales								
• K. pneumoniae	9 (32.14%)	20 (24.10%)	20 (20.83%)	4 (7.02%)	2 (4.65%)	2 (7.14%)	8 (24.24%)	65 (17.66%)
Enterobacter species	2 (7.14%)	3 (3.61%)	4 (4.16%)	4 (7.02%)	0	2 (7.14%)	0	15 (4.08%)
• E. coli	0	2 (2.41%)	1 (1.04%)	0	0	0	0	3 (0.82%)
Proteus group	0	2 (2.41%)	2 (2.08%)	1 (1.75%)	3 (6.98%)	4 (14.29%)	3 (9.09%)	15 (4.08%)
[Table/Fig-2a]: Temporal changes of b	acterial profile in I	ourn wounds (Gra	am negative bact	eria).	·		·	

Wound swabs	0 day	4 th day	8 th day	2 nd week	3 rd week	4 th week	>5 th weeks	Total
Total no. of samples	131	101	99	61	47	29	35	503
Total gram positive isolates	4	12	19	18	21	13	9	96
S. aureus (Total)	1 (3.57%)	9 (10.84%)	16 (16.67%)	17 (29.82%)	20 (46.51%)	13 (46.43%)	8 (24.24%)	84 (22.83%)
1. MRSA*	0	5 (55.56%)	11 (68.75%)	14 (82.35%)	17 (85%)	9 (69.23%)	4 (50%)	60 (71.43%)
2. MSSA [†]	1 (100%)	4 (44.44%)	5 (31.25%)	3 (17.65%)	3 (15%)	4 (30.77%)	4 (50%)	24 (28.57%)
Coagulase negative <i>Staphylococcus</i> species (CONS)	2 (7.14%)	2 (2.41%)	1 (1.04%)	0	0	0	0	5 (1.36%)
Enterococcus species	1 (3.57%)	1 (1.20%)	2 (2.08%)	1 (1.75%)	1 (2.33%)	0	1 (3.03%)	7 (1.90%)
[Table/Fig-2b]: Temporal changes of bacterial profile in burn wounds (Gram positive bacteria).								

*MRSA: Methicillin resistant Staphylococcus aureus; †MSSA: Methicillin sensitive Staphylococcus aureus

Patient characteristics	(Invasive infections) n=47	(Non-invasive infections) n=84	Chi-square	p-value	Odds Ratio (OR)	95% CI
Cause of burns • Flame/blast/Self-infliction (n=76) • Scalding/electric/chemical/miscellaneous (n=55)	37 (48.7%) 10 (18.2%)	39 (51.3%) 45 (81.8%)	11.613	0.0007	4.2 0.234	1.88-9.69 0.10-0.53
Days of hospitalisation • <2 weeks (n=88) • >2 weeks (n=43)	24 (27.3%) 23 (53.5%)	64 (72.7%) 20 (46.5%)	7.527	0.006	0.32 3.07	0.15-0.69 1.43-6.56
Total body surface area (TBSA) % • <50% (n=97) • >50% (n=34)	30 (30.9%) 17 (50%)	67 (69.1%) 17 (50%)	3.195	0.074	0.45 2.23	0.20-0.99 1.00-4.96
Depth of burns Partial thickness burn (n=68) • Deep partial/Full thickness burn (n=63)	10 (14.7%) 37 (58.7%)	58 (85.3%) 26 (41.3%)	25.670	<0.00001	0.12 8.25	0.05-0.28 3.57-19.08
Type of referral • Direct walk-in (n=70) • Referred (n=61)	21 (30%) 26 (42.6%)	49 (70%) 35 (57.4%)	1.742	0.187	0.58 1.7	0.28-1.18 0.84-3.56
Diabetes (n=8) Non diabetic (n=123)	5 (62.5%) 42 (34.15%)	3 (37.5%) 81 (65.85%)	1.537	0.215	3.21 0.31	0.73-14.11 0.07-1.36
Hypertension (n=12) Non hypertensive (n=119)	6 (50%) 41 (34.45%)	6 (50%) 78 (65.54%)	0.569	0.4506	1.90 0.5256	0.57-6.27 0.15-1.73
Pregnancy (n=3)	2 (66.7%)	1 (33.3%)	0.266	0.6059	-	-

to those who were suspected of having an invasive infection but were culture negative (2, 12.5%). This difference was statistically significant (p-value <0.0001) [Table/Fig-4].

Antibiotic susceptibility testing was performed for routinely used antibiotics according to CLSI standards [10,12,13]. The resistance pattern is mentioned in [Table/Fig-5a,b]. In the present cohort, *Acinetobacter* species demonstrated higher resistance to any antibiotic tested as compared to *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* exhibited the least resistance to piperacillin and aztreonam. Meropenem resistance was lower than that of imipenem in both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. For

Type of infection	Death	Discharge	Chi-square test	p-value	Odd's ratio	95% CI	
Non infected (n=24)	3 (12.5%)	21 (87.5%)	-	-	-	-	
Localised infections (n=76)	15 (19.74%)	61 (80.26%)	42.751	<0.001	0.0263	0.01-0.09	
Invasive infection infections (n=31)	28 (90.32%)	3 (9.68%)			37.9	10.16-141.78	
Total (n=131) 46 (35.11%) 85 (64.89%)							
[Table/Fig-4]: Outcome of patients with localised infection/colonisation and invasive infection and its association with mortality.							

Antibiotic agent	Klebsiella pneumoniae (n=65)	Pseudomonas aeruginosa (n=100)	Acinetobacter baumannii (n=74)
Amikacin	22 (33.85%)	37 (37%)	63 (85.14%)
Gentamicin	29 (44.6%)	43 (43%)	67 (90.54%)
Netilmycin	-	-	61 (82.44%)
Cefepime	37 (56.93%)	37 (37%)	67 (90.54%)
Ceftriaxone	45 (69.23%)	-	72 (97.30%)
Ceftazidime	-	37 (37%)	67 (90.54%)
Ciprofloxacin	39 (60%)	43 (43%)	67 (90.54%)
Piperacillin	-	10 (10%)	-
Piperacillin-tazobactam	20 (30.77%)	9 (9%)	66 (89.19%)
Ampicillin-sulbactam	-	-	0
Trimethoprim-sulfamethoxazole	-	-	0
Aztreonam	-	19 (19%)	-
Imipenem	27 (41.54%)	38 (38%)	66 (89.19%)
Meropenem	22 (33.85%)	33 (33%)	66 (89.19%)
Colistin	0	0	0

Antibiotic agent	Resistance in MSSA (n=24)	Resistance in MRSA (n=60)	Resistance in Staphylococcus aureus (MSSA+MRSA) (n=84)
Gentamicin	10 (41.7%)	31 (51.67%)	41 (48.81%)
Ciprofloxacin	22 (91.67%)	39 (65%)	61 (72.62%)
Trimethoprim-sulfamethoxazole	19 (79.17%)	51 (85%)	70 (83.33%)
Clindamycin	2 (8.33%)	23 (38.33%)	25 (29.76%)
Erythromycin	4 (16.67%)	32 (53.33%)	36 (42.86%)
Tetracycline	2 (8.33%)	15 (25%)	17 (20.24%)
Penicillin	24 (100%)	60 (100%)	84 (100%)
Linezolid	0	0	0
Teicoplanin	0	0	0
Vancomycin	0	0	0

the other antimicrobial testing agents, the resistance was greater than 30% and in *Acinetobacter* species, it was more than 80%.

DISCUSSION

Burn injuries are a common global health problem that is also seen in India [2]. Burn wounds not only lower immunity but also provide a good nidus for bacteria to grow as these are exposed surfaces [10,16]. It is important to distinguish burn wound colonisation from infection [1,17]. Unnecessary usage of antibiotics in burn wounds that are colonised and not infected may cause more harm by affecting patient's protective normal skin flora thus exposing the wound to opportunistic as well as multidrug resistant pathogens [1,17].

In the present study on the temporal association of bacteria in burn wound infections, it was found that gram-negative bacteria predominated in the first week, whereas the association with *Staphylococcus aureus* increased from the third week onwards. Additionally, the bacterial species present in the same patient would differ over time. This observation contrasts with a few other studies where *S. aureus* was identified as the most common pathogen in early burn wound infections [1,5,18,19]. Typically, within the first 48 hours after a burn, gram-positive bacteria, such as *Staphylococcus* species present in the surrounding skin, heavily colonise the wound surface [20,21]. After 5-7 days, these wounds become colonised by other microbes, including gram-negative bacteria and yeasts, which are primarily derived from the patient's

own gastrointestinal tract and upper respiratory tract or from the hospital environment, or acquired from healthcare personnel [1,21]. Among the gram-negative bacteria, *Pseudomonas aeruginosa* was found to be the most common (100, 27.17%), followed by *Staphylococcus aureus* (84, 22.83%), *Acinetobacter baumannii* (74, 20.11%), and *Klebsiella pneumoniae* (65, 17.66%). Most cultures were negative on day zero; however, as hospital stays increased, the wounds became colonised with various opportunistic organisms present endogenously or in the environment [1]. In the present study, colonisation of burn wounds was detected as early as within 48 hours. This study also aimed to determine the bacterial spectrum of invasive burn wound infections. Of the 47 patients suspected of having invasive infections based on clinical signs and symptoms of systemic infection, 37 (78.7%) were culture positive. The most common isolate was *Klebsiella pneumoniae* (16, 43.2%).

Among the factors known to be associated with a higher risk of invasive infection, flame burns (OR=4.2), a length of hospitalisation greater than 2 weeks (OR=3.0), involvement of a larger body surface area (OR=2.23), and deep burns (OR=8.25) were associated with a higher risk. Similar findings have been reported in other studies [9,22,23]. Invasive infections were significantly associated with deep burns compared to superficial burns (OR=0.12). Deep burns expose blood vessels in wounds directly to the environment, facilitating the entry of organisms into the bloodstream. These findings are consistent with those from previous studies [16,22,24,25].

In the present study, no significant association was found between patients referred from outside and direct patients. In contrast, Dhopte A et al., reported higher mortality rates in outpatients compared to those who came in as direct walk-ins. Early referral to a tertiary care centre helps prevent microbial colonisation by maintaining wound sterility, ensuring early wound coverage, and providing adequate nutritional support [23].

Co-morbid conditions known to increase the risk of infection in burn wound patients include diabetes, hypertension and pregnancy [7,8]. In this study, diabetes and pregnancy were identified as associated factors for invasive infection. Hypertension was also evaluated as a possible risk factor, but the results were not found to be significant. Diabetes is a neurovascular disorder that increases the likelihood of developing any injury, including burns, due to decreased sensitivity [26].

In patients with diabetes, delayed wound healing may contribute to an increased risk of infection. However, in this study, only 8 (6.11%) burn patients had diabetes mellitus, and no significant association with invasive infections was found. Three (2.26%) patients were pregnant, but no significant association was found between invasive infections and pregnancy.

The antibiotic susceptibility pattern demonstrated high rates of resistance to most of the antibiotics tested for gram-negative bacteria. These results align with the trends of resistance observed in most pathogens from India [2,4,24,27]. Sixty (71.4%) isolates of *S. aureus* were methicillin-resistant. An interesting observation in this study was that drug resistance was more common in strains isolated after the first week of admission, which may be attributed to selection pressure resulting from prolonged broad-spectrum antibiotic use. For *Staphylococcus aureus*, ciprofloxacin, co-trimoxazole, erythromycin and gentamycin were found to be effective. Sharma S et al., reported similar findings [4].

Limitation(s)

Although tissue biopsy along with blood culture is a better predictor of invasive burn wound infections, it could not be performed in these patients to differentiate true pathogens from mere colonisers. Since very few cases demonstrated involvement of the urinary and respiratory systems, the data were insignificant for analysis.

CONCLUSION(S)

As seen in this study, the most common organisms leading to wound infections in burn patients were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, in that order, with gram-negative organisms predominating in the first week and *Staphylococcus aureus* in the second week. There was a changing trend in the bacterial profile of burn wound infections over time, with gram-negative bacteria predominating in the first week and *Staphylococcus aureus* in the second week. This study highlights the need for periodic monitoring of the bacterial profile in burn wounds, along with their antimicrobial susceptibility patterns over time, thus ensuring early and appropriate diagnosis and treatment in patients, thereby improving outcomes.

Author's contribution: All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by AAG and PSP. The first draft of the manuscript was written by AAG, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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