Joint Diseases and Related Surgery

Jt Dis Relat Surg
2025;36(1):155-163

ORIGINAL ARTICLE

Sepsis predictors in earthquake survivors: A comparative analysis of amputation and fasciotomy patients

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On February 6, 2023, two major earthquakes, with magnitudes of 7.7 and 7.6 Mw on the Richter scale, occurred in Kahramanmaraş, resulting in more than 50,000 fatalities and injuries of over 100,000 individuals.[1,2] The spectrum of injuries following major earthquakes ranges from simple soft tissue trauma to open and closed fractures, compartment syndrome, and severe, life-threatening conditions.[3-6]

In the aftermath of earthquakes, extremity injuries are the most prevalent.[7,8] The compartmentalized nature of extremities, enclosed within a rigid system, makes compartment syndrome a significant concern. In such cases, early fasciotomy in the first 24 h is crucial to preserving tissue perfusion and preventing irreversible damage.[9,10] Although early fasciotomy is limb-saving, it carries specific risks,

Received: September 09, 2024
Accepted: November 21, 2024
Published online: December 10, 2024

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Citation: Comoglu M, Özdemir G, Sarıkaya B, Acehan F, Bingöl O, Kilic E, et al. Sepsis predictors in earthquake survivors: A comparative analysis of amputation and fasciotomy patients. Jt Dis Relat Surg 2025;36(1):155-163. doi: 10.52312/jdrs.2025.1971.

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ABSTRACT

Objectives: This study aimed to identify predictors of sepsis in earthquake survivors who underwent either amputation or fasciotomy and to compare these two patient groups across many clinical outcomes, including sepsis.

Patients and methods: In this retrospective study, a total of 1,608 patients who presented to our hospital following the February 6, 2023, Kahramanmaraş earthquakes were evaluated between February 2023 and March 2023. Of these, 138 patients (65 males, 73 females; median age: 35 years; range, 18 to 74 years) who underwent either fasciotomy or amputation were included in the study. Patients managed solely with fasciotomy were included in the fasciotomy group, while those who underwent amputation in at least one area were included in the amputation group. Sepsis predictors were investigated in this population. Additionally, the two groups were compared in terms of clinical outcomes, including bacteremia, sepsis, septic shock and mortality.

Results: Of the 138 earthquake survivors, 59 (42.8%) were in the amputation group, and 79 (57.2%) were in the fasciotomy group. There were no significant differences between the groups in terms of the development of crush syndrome or the need for renal replacement therapy (p=0.781 and p=0.411, respectively). The duration of entrapment under rubble was higher in the amputation group (p=0.030). While bacteremia was more common in the amputation group, there was no significant difference in relation to sepsis (p=0.002 and p=0.106, respectively). Septic shock and mortality rates were higher in the amputation group (p=0.001 and p=0.009, respectively). Multivariate analysis revealed that the number of traumatized sites (odds ratio [OR]=3.68, 95% confidence interval [CI]: 2.13-6.33, p<0.001), amputation at more than two sites (OR=4.27, 95% CI: 1.15-15.9, p=0.022), and fasciotomy at more than two sites (OR=2.71, 95% CI: 1.10-6.69, p=0.021) were significant predictors of sepsis.

Conclusion: Patients undergoing fasciotomy and amputation should be comprehensively evaluated for potential benefits and risks. Close monitoring for sepsis is particularly recommended for patients who have amputation or fasciotomy procedures at more than two sites.

Keywords: Amputation, earthquake, fasciotomy, sepsis.

such as sepsis.[11] Amputation is generally reserved for cases where the circulatory disturbance in the tissue has reached advanced stages, and recovery is no longer possible. Some clinicians consider that fasciotomy improves circulation and prevents the progression to muscle necrosis, while other experts recommend avoiding it in non-essential cases due to the risks of infection, sepsis, and death, particularly in late fasciotomies.[12,13] Proponents of amputation argue for its necessity in eliminating necrotic tissue, which can release potentially harmful substances, such as myoglobin and potassium, acting as a potential source of infection.[14]

Although the decision to perform amputation or fasciotomy is based on the patient's clinical condition following trauma, understanding the poor prognostic factors, including sepsis, that may arise after these procedures is crucial for patient management. Identifying the factors that predict sepsis, which is a major concern in patients undergoing these surgical interventions, is necessary. This study aimed to compare the clinical outcomes, including sepsis, between amputation and fasciotomy groups and to investigate factors that predict the development of sepsis.

PATIENTS AND METHODS

This retrospective study was conducted at the Internal Medicine and Orthopedics and Traumatology Departments of the Ankara Bilkent City Hospital. After the Kahramanmaraş earthquakes on February 6, 2023, a total of 1,608 patients were evaluated at our hospital. A total of 875 patients who received outpatient treatment, seven patients who died within 24 h, 234 patients with missing data, and 354 patients who did not undergo amputation or fasciotomy were excluded. A total of 138 patients (65 males, 73 females; median age: 35 years; range, 18 to 74 years) who underwent either fasciotomy or amputation during their hospital stay were included in the study (Figure 1). The patients were divided into two groups: those who underwent fasciotomies and those who underwent amputations. The fasciotomy group comprised patients who were managed solely with fasciotomies during their hospitalization, while the amputation group consisted of those who underwent at least one amputation. Therefore, if a patient who previously underwent a fasciotomy required amputation during follow-up, they were included in the amputation group. The two groups were compared in terms of clinical characteristics such as age, sex, comorbidities, time under debris (hours), traumatized areas, presence of bone fractures, laboratory parameters at admission, urine alkalization, hyperbaric oxygen therapy, and mannitol therapy. Clinical outcomes, including length of hospital stay, intensive care unit stay, prolonged hospitalization, crush syndrome, need for renal replacement therapy, bacteremia, sepsis, septic shock, and mortality, were also compared. Additionally, factors predicting the development of sepsis in the overall patient population were investigated. The study protocol was approved by the Ankara Bilkent City Hospital Ethics Committee (date: 24.07.2024, no: 2-24-368). The study was conducted in accordance with the principles of the Declaration of Helsinki.

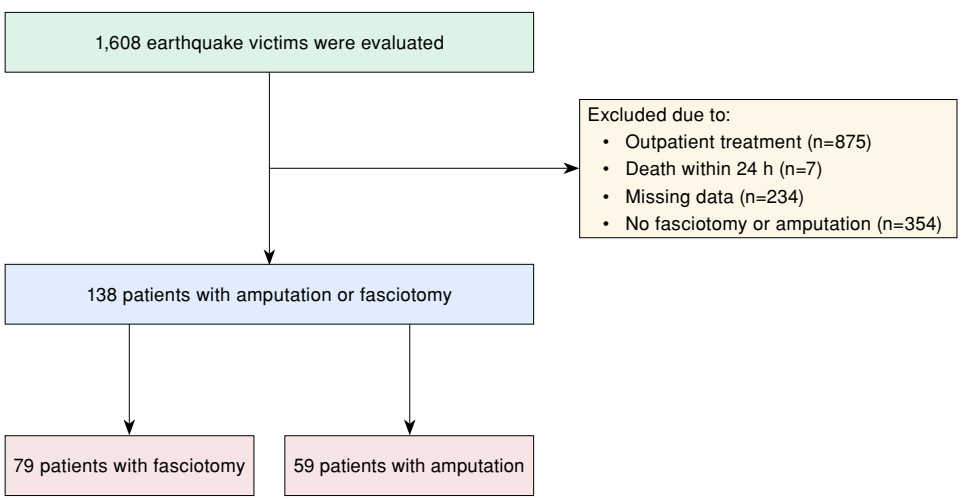


FIGURE 1. Flowchart of the study.

All patients presenting to our hospital with crush injuries received appropriate intravenous fluid therapy, urine alkalization, and mannitol treatments when indicated. Decisions regarding the necessity and levels of fasciotomy and amputation were made by the orthopedics and traumatology, plastic surgery, and cardiovascular surgery departments based on the clinical follow-up of each patient, taking into consideration factors such as vascular status, tissue viability, risk of severe infection, outcomes of previous fasciotomies, and the overall stability of the patient. In patients who underwent fasciotomy, wound care involved the use of wound and skin antiseptics, topical antibiotic creams, surgical and enzymatic debridement, as well as hyperbaric oxygen therapy and vacuum-assisted closure treatment, depending on patient suitability. Crush syndrome was defined as a crush injury involving a large mass of skeletal muscle, accompanied by systemic manifestations, such as acute kidney injury), electrolyte disturbances, and a peak creatine kinase level >1,000 U/L at admission.[15] Patients developing signs of infection were administered appropriate antibiotic therapy, with follow-up and management overseen by the infectious diseases clinic. Sepsis was defined according to the international consensus criteria as life-threatening organ dysfunction caused by a dysregulated host response to infection.[16] The SOFA (Sequential Organ Failure Assessment) score was used to determine the presence and severity of organ dysfunction, with patients scoring two or more points classified as having sepsis.[16] Septic shock was identified in patients who experienced hypotension and required vasopressor support despite adequate fluid resuscitation.[16] Prolonged hospitalization was defined as a duration exceeding the 75th percentile of the overall study population's hospital stay. For analytical purposes and ease of use, trauma sites were categorized into seven regions: the four extremities, head and neck, thorax, and abdomen. The total number of traumatized sites was also included in the analysis.

Statistical analysis

Statistical analyses were performed using IBM SPSS, version 26.0 software (IBM Corp., Armonk, NY, USA). The normality of the data distribution was assessed using the Kolmogorov-Smirnov test. Normally distributed data were expressed as mean ± standard deviation (SD), and nonnormally distributed data were presented as median (interquartile range). Student's t-test or the Mann-Whitney U test was used for continuous variables, whereas the chi-square test and Fisher exact test were used to compare categorical variables. The univariate logistic regression analysis was performed with parameters that were likely to be associated with sepsis. Parameters found to be associated with sepsis at the $p<0.1$ level in the univariate analysis were included in the forward stepwise multivariate logistic regression analysis to determine the independent predictors of sepsis. The minimum sample size required for a multivariate logistic regression model was calculated with the formula $N=10\,k/p$, as suggested by Peduzzi et al.,[17] where N represents the minimum required sample size, k is the number of independent variables in the model, and p is the positive case rate. Since the approximate frequency of sepsis in the model consisting of three (k=3) independent variables was 34.8% (p=48), the minimum required sample size was found to be 86. Therefore, it was accepted that an adequate sample size had been reached with the inclusion of 138 patients in the study. A p value <0.05 was considered statistically significant in all analyses.

RESULTS

Of the 138 patients, 59 (42.8%) were in the amputation group, and 79 (57.2%) were in the fasciotomy group. The median age and sex were similar between the groups (p=0.545 and p=0.336, respectively). Although hyperbaric oxygen therapy was more frequently applied in the fasciotomy group, there was no significant difference between the groups (p=0.387). The median length of hospital stay was higher in the amputation group [59 (30-92) *vs.* 35 (19-56), p=0.001]. The rate of intensive care unit admission was similar between the groups (p=0.160). Bacteremia was more common in the amputation group (p=0.002). The most frequently isolated pathogen in tissue cultures was *Acinetobacter baumannii (A. baumannii)*. Although bacteremia was more frequently observed in the amputation group, there was no significant difference between the two groups in terms of sepsis [25 (42.4) *vs.* 23 (29.1), p=0.106]. Septic shock and mortality rates were higher in the amputation group (p=0.001 and p=0.009, respectively; Table I).

Albumin, calcium, and hemoglobin levels were lower in the amputation group (p=0.045, p=0.008, and p=0.003, respectively). Lactate and C-reactive protein levels were higher in the amputation group (p=0.002 and p<0.001, respectively). The two groups were similar with respect to the remaining laboratory parameters (Table II).

The duration of entrapment under rubble was higher in the amputation group [30 (6-48) *vs.* 12 (8-32), p=0.030]. Bone fractures, burn injuries, and the number of extremity traumas were similar

TABLE I
Comparison of clinical characteristics and outcomes between the two groups

	Amputation (n=59)				Fasciotomy (n=79)				
Parameters	n	%	Median	Q1-Q3	n	%	Median	Q1-Q3	*p*
Age (year)			37	27-46			34	24-45	0.545
Sex									
Female	34	57.6			39	49.4			0.336
Major comorbidity*	10	16.9			11	13.9			0.625
Treatment modalities									
Urine alkalization	36	61			47	59.5			0.857
Mannitol	11	18.6			13	16.5			0.737
Hyperbaric oxygen	14	23.7			24	30.4			0.387
Length of hospital stay (Day)			59	30-92			35	19-56	**0.001**
Prolonged hospital stay (>75th percentile)	25	42.4			11	13.9			**<0.001**
ICU admission	46	78			53	67.1			0.160
Length of ICU stay (Day)			14	5-25			8	4-15	**0.027**
Crush syndrome	24	40.7			34	43			0.781
Renal replacement therapy	17	28.8			28	35.4			0.411
Bacteremia	48	81.4			45	57			**0.002**
Acinetobacter baumannii	33	55.9			21	26.6			
Pseudomonas aeruginosa	12	20.3			10	12.7			
Klebsiella pneumoniae	12	20.3			8	10.1			
Enterococcus faecalis	7	11.3			11	13.9			
Candida species	3	5.1			5	6.3			
Other**	8	13.6			11	13.9			
Sepsis	25	42.4			23	29.1			0.106
Septic shock	16	27.1			5	6.3			**0.001**
Mortality	10	16.9			3	3.8			**0.009**

Q1: First quartile; Q3: Third quartile; ICU: Intensive care unit; * Hypertension, diabetes mellitus, and cardiovascular disease; ** *Staphylococcus spp.*, *Escherichia coli, Corynebacterium spp., Proteus spp.*,

TABLE II
Comparison of laboratory parameters between the two groups

	Amputation (n=59)			Fasciotomy (n=79)			
Parameters	Mean±SD	Median	Q1-Q3	Mean±SD	Median	Q1-Q3	*p*
Urea (mg/dL)		49	24-90		49	21-98	0.969
Creatinine (mg/dL)		0.76	0.52-2.38		0.74	0.51-3.39	0.648
Creatine kinase (U/L)		17,674	4,696-56,000		18,486	3,977-64,956	0.762
Uric acid (mg/dL)		4.7	3-7.6		4.4	2.9-7.7	0.630
Albumin (g/dL)		2.7	2.3-3		2.8	2.4-3.4	0.045
AST (U/L)		444	167-1,000		382	166-946	0.732
ALT (U/L)		178	95-363		174	94-336	0.694
Lactate dehydrogenase (U/L)		781	503-1,372		788	489-1,368	0.706
Calcium (mg/dL)	7.71±0.71			8.06±0.8			**0.008**
Phosphorus (mg/dL)		3.5	2.3-5		3.2	2.2-5.2	0.482
Sodium (mEq/L)		136	131-140		136	132-139	0.722
Potassium (mEq/L)		4.4	3.8-5		4.3	3.8-5	0.919
Lactate (mmol/L		1.95	1.54-2.58		1.58	1.28-2.01	**0.002**
White blood cell count (10^9/L		14.05	10.8-19.05		14.02	10.71-18.30	0.991
Hemoglobin (g/dL)		10	9.1-12.2		11.4	10.2-13.9	**0.003**
Platelet count (10^9/L)		226	166-293		248	169-360	0.202
C-reactive protein (mg/L)		161	116-219		110.5	65.3-152	**<0.001**
Procalcitonin (µg/L)		0.72	0.28-3.79		0.48	0.16-1.72	0.072

SD: Standard deviation; Q1: First quartile; Q3: Third quartile; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

between the two groups (p=0.903, p=0.313, and p=0.159, respectively; Table III). In the amputation group, 43 (72.9%) patients had a single-site amputation, 14 (23.7%) had two-site amputations, and two (3.4%) had three-site amputations. Thirty-five patients who underwent amputation had previously received fasciotomy. The most frequently performed amputation was transtibial amputation (n=30). The most commonly performed fasciotomies were in the thigh and upper arm regions (n=30 and n=22, respectively). The distribution of other amputation and fasciotomy sites is shown in Table IV.

Univariate and multivariate regression analyses were performed to investigate factors predicting sepsis in the overall study population. Multivariate analysis revealed that the number of traumatized sites (odds ratio [OR]=3.68, 95% confidence interval [CI]: 2.13-6.33, p<0.001), amputation at more than two sites (OR=4.27, 95% CI: 1.15-15.9, p=0.022), and fasciotomy at more than two sites (OR=2.71, 95% CI: 1.10-6.69, p=0.021) were significant predictors of sepsis. An increase in the number of traumatized sites, amputation at more than two sites, and fasciotomy at more than two sites were found to increase the risk of sepsis by 3.7, 4.2, and 2.7 times, respectively (Table V).

TABLE III
Comparison of trauma characteristics between the two groups

	Amputation (n=59)				Fasciotomy (n=79)				
Parameters	n	%	Median	Q1-Q3	n	%	Median	Q1-Q3	*p*
Number of trauma sites*			2	2-3			2	2-3	0.089
Bone fracture	20	33.9			26	32.9			0.903
Duration of entrapment under rubble (h)			30	6-48			12	8-32	0.030
Burn injury	3	5.1			1	1.3			0.313
Trauma characteristics									
Head trauma	3	5.1			7	8.9			0.516
Thoracic trauma	19	32.2			18	22.8			0.217
Abdomino-spinal trauma	25	42.4			23	29.1			0.106
Extremity trauma									0.159
Single extremity	14	23.7			28	35.4			
Two extremities	37	62.7			47	59.5			
Three extremities	6	10.2			3	3.8			
Four extremities	2	3.2			1	1.3			

Q1: First quartile; Q3: Third quartile; * Refers to the number of traumatized sites in seven regions: the four extremities, head, abdomen, and thorax.

TABLE IV
Distribution of amputation and fasciotomy sites between the two groups

Amputation (n=59)			Fasciotomy (n=79)		
	n	%		n	%
Number of amputated sites			Number of fasciotomy sites		
Single site	43	72.9	Single site	50	63.3
Two sites	14	23.7	Two sites	28	35.4
Three sites	2	3.4	Three sites	1	1.3
Amputation sites			Fasciotomy sites		
Hand	3	5.1	Hand	9	11.4
Transradial	7	11.9	Forearm	10	12.7
Transhumeral	6	10.2	Upper arm	22	27.8
Foot	6	10.2	Foot	6	7.6
Transtibial	30	50.8	Cruris	14	17.7
Transfemoral	10	16.9	Thigh	30	38
Hip disarticulation	2	3.4			

TABLE V
Parameters predicting sepsis

	Univariate analysis			Multivariate analysis		
	OR	95% CI	*p*	OR	95% CI	*p*
Age	0.99	0.97-1.03	0.930			
Sex						
Female	1.40	0.69-2.84	0.351			
Major comorbidity	0.71	0.26-1.98	0.518			
Number of trauma sites*	3.81	2.28-6.38	<0.001	3.68	2.13-6.33	<0.001
Duration of entrapment under rubble	0.99	0.98-1.01	0.713			
Amputation at two or more sites	7.17	2.17-23.71	0.001	4.27	1.15-15.9	0.022
Fasciotomy at two or more sites	2.56	1.21-5.41	0.014	2.71	1.10-6.69	0.021
Bone fracture	2.02	0.971-4.21	0.060			

OR: Odds ratio; CI: Confidence interval; * Refers to the number of traumatized sites in seven regions: the four extremities, head, abdomen, and thorax.

Additionally, a correlation analysis was performed between the time under rubble and the parameters included in the univariate analysis, such as age, sex, major comorbidities, number of traumatized sides, having amputation or fasciotomy in more than two sides, and bone fractures. Significant correlations were found only with the presence of bone fractures (correlation coefficient=0.208, p=0.014) and having amputations in more than two sides (correlation coefficient=0.210, p=0.013), while no significant correlation was observed with the other parameters.

DISCUSSION

This single-center, retrospective earthquake study presented a comprehensive comparison of patients who underwent either amputation or fasciotomy following crush injuries and identified sepsis predictors within these groups. Our findings revealed no significant difference in sepsis rates between the two groups. Although the duration of time under debris did not increase the risk of sepsis, patients who underwent amputations or fasciotomies in more than two sides had a 4.2- and 2.7-fold increased risk of developing sepsis, respectively.

In the initial days following the earthquake, early deaths are reported to be predominantly due to hypovolemia and hyperkalemia associated with crush injuries, whereas delayed mortality is largely attributed to sepsis-induced multiorgan failure.[18] While fasciotomy is an early intervention necessary for patients developing compartment syndrome, subsequent surgical site infections (SSIs) can initiate a process leading to sepsis, amputation, and, ultimately, multiorgan failure and death. Following fasciotomy, SSI rates can reach up to 30%.[19] There are various studies in the literature investigating predictors of SSI. Most studies indicate no association between age and the development of SSI.[20,21] Similarly, our study found no correlation between age and the development of sepsis. Regarding patient sex, while some publications suggest that SSI is more common in males, there is also data indicating the opposite.[19,21,22] In our study, no relationship was found between sex and the development of sepsis. Similarly, there was no association between the presence of major comorbidities and sepsis in our study. The relatively young mean age and low number of comorbidities in the earthquake survivor population may have contributed to these results. Most studies in the literature define SSI in various ways, such as positive culture growth or the need for repeat surgery. Due to the heterogeneity in SSI definitions, our study investigated sepsis predictors using the SOFA score, which more clearly represents organ failure development and infection status. Given that sepsis is the most crucial factor in mortality following infection, predicting sepsis development may be more clinically valuable.

The duration of entrapment under rubble plays a significant role in many decisions regarding earthquake survivors, such as the choice of antibiotic therapy, or the selection of the patient's follow-up setting. Studies have shown that the duration of entrapment under rubble increases the frequency of amputations.[23,24] Consistent with this, in our study, the median duration of entrapment under

rubble was higher in the amputation group. Patients who remain under rubble for extended periods are exposed to trauma for longer, leading to more pronounced tissue necrosis and ischemia following rescue. Although there is data suggesting that the duration spent under rubble increases the frequency of infections,[25] our study found no association between the duration of entrapment under rubble and the development of sepsis in regression analysis. These findings suggest that timely surgical intervention and appropriate antibiotic therapy in patients rescued from the rubble at a late stage can reduce the incidence of sepsis.

As observed in our study, post-earthquake injuries most frequently occur in the extremities, particularly the lower extremities.[6,26] The larger muscle mass in the lower extremities leads to a higher incidence of rhabdomyolysis and crush syndrome, leading to worse clinical outcomes. Proper fluid replacement, close monitoring of fluid and electrolyte balance, and timely fasciotomy or amputation when necessary after rescue can prevent the development of crush syndrome and adverse clinical outcomes. In our study, there were no significant differences between the amputation and fasciotomy groups regarding the incidence of crush syndrome or the need for renal replacement therapy. However, a study by Koyuncu et al.[23] reported that the fasciotomy group had higher incidences of acute kidney injury and a greater need for renal replacement therapy. There are also studies indicating a higher incidence of crush syndrome in the amputation group.[27] The variability in the results observed in the literature suggests that local factors, such as the severity of trauma and intervention time, play a crucial role in the development of crush syndrome.

Disaster situations often lead to a lack of standardized surgical interventions due to the unique challenges posed by mass casualty events. This issue has been highlighted in various studies, attributing it to logistical constraints, resource variability, and the chaotic nature of disaster environments.[28,29] Similarly, in our study, which focuses on an earthquake disaster, surgical interventions such as amputations and fasciotomies were not standardized. The 59 patients who underwent amputation may have had their procedures performed in different settings and for various indications, further contributing to variability. Additionally, some patients who underwent amputation had previous fasciotomies. While mortality was higher in the amputation group, confounding factors such as the lack of standardization in surgical procedures complicate the evaluation of these outcomes. For a clearer understanding of mortality and other clinical outcomes, future studies should aim to control for these confounding factors and standardize surgical interventions whenever possible.

The incidence of bacteremia was higher in our amputation group. In the literature, the most frequently isolated pathogens in tissue cultures have been reported as *A. baumannii* and *Pseudomonas aeruginosa* among individuals who have survived an earthquake.[30,31] Similarly, in our study, these two pathogens were the most frequently observed. The higher incidence of bacteremia in the amputation group necessitated the administration of broad-spectrum antibiotics for an extended period, resulting in prolonged hospitalization in this group. A study by Merchan et al.[21] also reported longer periods of wound healing and hospitalization in patients who underwent amputation due to SSI. Although bacteremia was more prevalent in the amputation group, there was no significant difference between the two groups in terms of sepsis rates. This suggests that the timely and appropriate execution of surgical procedures and antibiotic therapies may have limited the infection, resulting in no difference in sepsis rates between the groups.

Although some data in the literature indicates that fasciotomies result in poorer clinical outcomes with respect to sepsis, other studies contradict this, indicating that fasciotomies do not increase the risk of sepsis.[32-34] However, these studies compared all patients who underwent a fasciotomy with those who did not, which may not accurately represent the data. There is a paucity of literature specifically comparing amputation and fasciotomy patient groups, and no studies have directly compared these two groups in terms of sepsis risk. Our study further demonstrated that having more than two amputations or fasciotomies was significantly associated with an increased risk of sepsis in the overall population. These findings suggest that patients undergoing multiple amputations or fasciotomies should be more closely monitored for signs of sepsis and that more aggressive antibiotic therapy regimens may be a reasonable approach in these cases.

This study had several limitations. The main limitation was its single-center and retrospective design. The unpredictable nature of natural disasters, such as earthquakes, limits the feasibility of conducting prospective studies. The lack of

standardization in surgical procedures is another limitation. As our study is based on an earthquake disaster, surgical interventions such as fasciotomy and amputation were performed under varying field conditions by different surgical teams and for diverse indications. Additionally, there was a lack of data on the timing of fasciotomy and closure techniques, as late fasciotomies or different closure methods may affect patient outcomes. This situation introduces potential confounding factors that may have influenced clinical results. Moreover, some patients in the amputation group had previously undergone fasciotomy, complicating the interpretation of results between the groups. While this was recognized as a potential limitation, these patients were not excluded from the amputation group to better reflect real-world data and avoid focusing solely on a restricted cohort. Finally, due to the retrospective nature of our study, we had limited access to detailed trauma assessments and severity ratings that were initially documented by the first surgical teams. The absence of comprehensive data on trauma severity may have restricted some of our analyses.

In conclusion, a comprehensive evaluation of the potential benefits and risks is essential for patients undergoing fasciotomies or amputations. Patients undergoing amputations or fasciotomies at more than two sites should be closely monitored for the development of sepsis. Future multicenter studies with larger patient populations are necessary to achieve a more comprehensive understanding of the clinical outcomes associated with amputation and fasciotomy.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Conception, design, analysis and interpretation of data, and drafting the article: M.C.; Collection of data, analysis and interpretation of data: F.A., B.F.D., Y.Y., M.O., E.K., BB.; Contributed to the critical revision of the manuscript for important intellectual content and provided administrative and technical support: B.S., G.O., O.B., E.S.S.All authors discussed and commented on the results, contributed and approved the final manuscript.

Conflict of Interest: The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding: The authors received no financial support for the research and/or authorship of this article.

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