Factors leading to dissemination of cutaneous anthrax: an international ID-IRI study

U. S. Elbahr1, R. Tekin2, M. Papic3, N. Pandak4, H. Erdem1, F. K. Can5, S. N. Alpat6, A. U. Pekok7, F. Pehlivanoglu8, M. Karamese9, P. C. Petru10, S. Kulzhanova11, S. Tosun12, M. Dogan13, R. Morosi14, E. Ramosaco15, H. Alay5, E. Puca15, J. Arapovic16, N. Pshenichnaya17, T. Fasciana18 and A. Giammanco18

1) Bahrain Oncology Center, King Hamad University Hospital, Department of Infectious Diseases, AlMuharraq, Bahrain, 2) Department of Infectious Diseases, School of Medicine, Dicle University, Diyarbakir, Turkey, 3) Libertas International University, Zagreb, Croatia, 4) The Royal Hospital, Muscat, Oman, 5) Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Ataturk University, Erzurum, 6) Department of Infectious Disease and Clinical Microbiology, Faculty of Medicine, Eskisehir Osmangazi University, Eskisehir, 7) Department of Infectious Diseases, VM Medical Park, Pendik Hospital, Istanbul, 8) Department of Clinical Microbiology and Infectious Diseases, University of Health Sciences, Haseki Training and Research Hospital, Istanbul, 9) Department of Medical Microbiology, Kafkas University, Kars, Turkey, 10) Dr Victor Babes Clinical Hospital of Infectious and Tropical Diseases, Bucharest, Romania, 11) Department of Infectious Diseases, Astana Medical University, Nur-Sultan, Kazakhstan, 12) Department of Infectious Diseases and Clinical Microbiology, Izmır Bzyaka Training and Research Hospital, Izmır, 13) Department of Infectious Diseases, Namık Kemal University School of Medicine, Tekirdag, Turkey, 14) National Institute for Infectious Diseases ‘Matei Balş’, and ‘Carol Davila’ University of Medicine and Pharmacy’, Bucharest, Romania, 15) Infectious Diseases Clinic, University Hospital Center “Mother Teresa”, Tirana, Albania, 16) Department of Infectious Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina, 17) Central Research Institute of Epidemiology, Moscow, Russia and 18) Department of Health Promotion, Maternal-Childhood, Internal Medicine of Excellence G. D’Alessandro, University of Palermo, 90127 Palermo, Italy

Abstract

Background: Although anthrax is a rare zoonotic infection, it still causes significant mortality and morbidity. In this multicenter study, which is the largest anthrax case series ever reported, we aimed to describe the factors leading to dissemination of cutaneous anthrax.

Methods: Adult patients with cutaneous anthrax from 16 referral centers were pooled. The study had a retrospective design, and included patients treated between January 1, 1990 and December 1, 2019. Probable, and confirmed cases based upon CDC anthrax 2018 case definition were included in the study. A descriptive statistical analysis was performed for all variables.

Results: A total of 141 cutaneous anthrax patients were included. Of these, 105 (74%) patients had probable and 36 (26%) had confirmed diagnosis. Anthrax meningitis and bacteremia occurred in three and six patients, respectively. Sequelae were observed in three patients: cicatricial ectropion followed by ocular anthrax (n = 2) and movement restriction on the left hand after surgical intervention (n = 1). One patient had gastrointestinal anthrax. The parameters related to poor outcome (p < 0.05) were fever, anorexia, hypoxia, malaise/fatigue, cellulitis, fasciitis, lymphadenopathy, leukocytosis, high CRP and creatinine levels, longer duration of antimicrobial therapy, and combined therapy. The last two were seemingly the consequences of dissemination rather than being the reasons. The fatality rate was 1.4%.

Conclusions: Rapid identification of anthrax is crucial for prompt and effective treatment. Systemic symptoms, disseminated local infection, and high inflammatory markers should alert the treating physicians for the dissemination of the disease.

Keywords: Anthrax, Bacteremia, Gastrointestinal, Meningitis, Mortality, Outcome

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Introduction

Anthrax is a rare zoonotic infection [1]. The disease is caused by Bacillus anthracis, a Gram-positive, sporulating, nonmotile,
rod-shaped, aerobic bacterium, which naturally exists in soil and infects mainly herbivorous animals. Humans acquire the agent from infected animals or animal products. No human-to-human transmission has been documented [2]. The microorganism can invade the body by four main routes: Transcutaneous inoculation, inhalation, ingestion, and by direct parenteral injection [3].

Although cutaneous form of anthrax is a benign and the most common form of the disease, the outcome can be fatal if the infection disseminates. To the best of our knowledge, in the literature no data exist for the parameters facilitating dissemination of anthrax. There are certain case series focusing characteristics of the disease, but in small numbers. Therefore, we performed an international study and provided the largest anthrax case series ever reported in the literature to make robust inferences.

**Material and methods**

This multicenter study pooled patients with cutaneous anthrax from a total of 16 medical centers in Turkey, Bosnia and Herzegovina, Albania, Romania, Kazakhstan, and Russia. Only adult patients with anthrax and aged > 17 years were enrolled. The study had a retrospective design, and included patients treated between January 1, 1990 and December 2019. No control groups were included for this study. The institutional review board of Dicle University, Faculty of Medicine in Diyarbakır, Türkiye, approved the study.

**Laboratory diagnosis of anthrax**

a) **Presumptive laboratory criteria:** Gram stain demonstrating Gram-positive rods, square-ended, in pairs or short chains.

b) **Confirmatory laboratory criteria:**
   i. Culture and identification from clinical specimens; or
   ii. Evidence of a four-fold rise in antibodies to protective antigen between acute and convalescent sera or a fourfold change in antibodies to protective antigen in paired convalescent sera using ELISA testing in an unvaccinated person; or
   iii. Detection of *B. anthracis* or anthrax toxin genes by PCR and/or sequencing in clinical specimens collected from a normally sterile site (such as blood or CSF) or lesion of other affected tissue.

**Case stratification**

All cases were divided into three main groups based upon Centers for Diseases Control and Prevention (CDC) anthrax 2018 case definition criteria [4]:

a) **Suspected case:** A case that meets the clinical criteria, but with no epidemiologic evidence relating it to anthrax.

b) **Probable case:** A case that meets the clinical criteria and has presumptive laboratory test results, or a case that meets the clinical criteria and has epidemiologic evidence relating it to anthrax.

c) **Confirmed case:** A case that meets the clinical criteria and has confirmatory laboratory test results.

**Exclusion criteria:** Suspected anthrax cases [4] were excluded from the study.

**Poor outcome:** Meningitis, bacteremia, sequel formation, and death were considered as poor outcome as a single block.

**Statistical analysis**

A descriptive statistical analysis was performed for all variables. Depending on the type of variable, absolute and relative frequencies, minimum, maximum, arithmetic mean, standard deviation, and asymmetry coefficient were calculated. The degree of correlation of all variables with the variable “poor outcome” (Pearson’s linear correlation coefficient, Pearson’s point biserial correlation coefficient, contingency coefficient - depending on the type of variable) was determined. The computer programs MS Excel and SPSS (SPSS version 16.0, Chicago, USA) were used in the analysis.

**Results**

In this study, the data of 252 cases with anthrax was submitted. 111 cases were found to be ineligible for the survey and were excluded. The mean age of the patients was 44.8 ± 13.2 years, and 47 of 141 patients were females (33%). A total of 105 (74%) patients had probable and 36 (26%) patients had confirmed diagnosis. The main risk factor was contact with an infected animal (n = 116, 82%) and 17 (12%) patients were sheep breeders. However, no risk factors could be detected in 8 (5%) patients. Cutaneous anthrax was recorded in all patients. In addition, one patient had gastrointestinal involvement and three patients had meningal involvement.

**Clinical presentation:** The complaints and the findings of the patients are shown in Table-1. The most common symptom was local edema 138 (98%). Three patients were diagnosed with meningitis and Glasgow coma scores were 3, 3, and 7. The
comorbidities were as follow; diabetes mellitus 8 (6%), coronary artery disease 7 (5%), chronic obstructive lung disease 4 (3%), cerebrovascular disease 2 (1%), chronic liver disease 2 (1%), collagen tissue disorder 1 (0.7%). One patient was pregnant, and three patients had trauma history.

**Microbiological diagnosis:** The diagnosis was established by culture in 20 (14%) cases, by Gram stain in 105 (74%) cases, by PCR test in 20 cases (14%), and by ELISA in 2 (1%) patients. B. anthracis was isolated from the skin lesions in 15 (11%) patients. Of these patients, B. anthracis was also isolated from blood culture in one case and from CSF culture in one case, in addition to skin lesions. One of the other two meningitis cases was diagnosed by Gram stain findings in the CSF, and the other one was diagnosed by PCR positivity in the CSF. The case with gastrointestinal involvement was diagnosed by clinical findings (abdominal tenderness, diarrhea, pharyngitis, ophthalmoplegic lesions) and Gram stain. Microbiological diagnosis of the patients is summarized in Table-2.

**Inflammatory markers:** The mean leukocyte, C-reactive protein (CRP) and creatinine levels were 9794 ± 3688/mm³, 3.8 ± 4.8 mg/dL, and 0.8 ± 0.2 (mg/dL), respectively. The leukocyte count was within the normal range (4000–10,000/mm³) in 88 (62%) patients. Serum CRP levels increased (> 0.5 mg/dL) in 115 (82%) patients.

**Therapeutic concerns:** In this study, 107 (76%) patients had no history of antibiotic use before admission to the hospital. Elapsing time between earliest possible exposure to start of treatment was 9.1 ± 3.9 days and elapsing time between the beginning of symptoms to start of treatment was 4.2 ± 3.1 days. Monotherapy was given to 95 (67%) patients and combination therapy was given to 46 (33%) patients. The antimicrobial therapies used for anthrax treatment are shown in Table-3.

**Antimicrobial Selection:** Penicillin and penicillin derivates (penicillin (n = 62, 44%), amoxicillin (n = 17, 12%), ampicillin (n = 20, 14%)) were the most commonly used antimicrobial therapy in both monotherapy and combined therapy, followed by ciprofloxacin (n = 59, 42%). The mean duration of antibiotic treatment was 11.4 ± 6.9 days. Eighty-two (58%) patients were hospitalized, and 58 (42%) patients were treated as outpatients. The mean hospitalization duration of inpatients was 10.3 ± 6 days.

**Outcomes:** Anthrax meningitis occurred in three patients and two of them died. The fatality rate was 1.4% in our study. Sequelae were observed in three patients; two of them had cicatricial ectropion followed by ocular anthrax and one patient had movement restriction on the left hand after surgical intervention. Bacteremia occurred in six patients. One patient with bacteremia had coexistent gastrointestinal anthrax. The parameters with statistical significance (p < 0.05) related to poor outcome were (Table-4):

a) **Symptoms:** Fever, anorexia, hypoxia, malaise/fatigue,
b) **Findings:** Cellulitis, fasciitis, lymphadenopathy,
c) **Laboratory findings:** Leukocytosis, high CRP and creatinine levels,
d) **Therapeutic factors:** Longer duration of antimicrobial therapy, and combined therapy

**Discussion**

Anthrax causes mortality and morbidity in developing countries in particular [5]. The fatality rate of cutaneous anthrax was reported to be less than 1% with appropriate antimicrobial treatment [6]. We had the similar result. Although, the fatality rate has been reported up to 18% in a cutaneous anthrax cases, that high fatality rate has been attributed to delayed presentation of the patients [7]. Complications of anthrax are diverse in the literature and include secondary

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**TABLE 1. Signs and symptoms of anthrax patients.**

| Symptoms                        | Total n (%) |
|--------------------------------|-------------|
| Edema                          | 138 (98%)   |
| Erythema                       | 134 (95%)   |
| Eschar                         | 97 (69%)    |
| Vescicles                      | 75 (53%)    |
| Malaise/fatigue                | 54 (38%)    |
| Pruritis                       | 31 (22%)    |
| Anoxia                         | 9 (6%)      |
| Headache                       | 3 (2%)      |
| Abdominal pain/tenderness      | 1 (0.7%)    |
| Abdominal distension           | 1 (0.7%)    |
| Diarrhea/vomiting              | 1 (0.7%)    |
| Dyspea                         | 1 (0.7%)    |
| Oropharyngeal lesions          | 1 (0.7%)    |

| Findings                        | Total n (%) |
|--------------------------------|-------------|
| Cellulites                      | 108 (77%)   |
| Fever                          | 47 (33%)    |
| Lymphadenopathy                | 36 (26%)    |
| Lymphangitis                   | 18 (13%)    |
| Fascitis                       | 9 (6%)      |
| Cyanosis                       | 8 (6%)      |
| Hypoxia                        | 3 (2%)      |
| Altered mental status          | 3 (2%)      |
| Coma                           | 3 (2%)      |
| Convulsions                    | 2 (1%)      |
| Neck pain/suffness             | 2 (1%)      |
| Pharyngitis                    | 1 (0.7%)    |
| Acute respiratory distress     | 1 (0.7%)    |

**TABLE 2. Microbiological diagnosis of anthrax patients.**

| Gram stain, n (%) | Culture n (%) | PCR n (%) | Total n (%) |
|-------------------|---------------|-----------|-------------|
| SST Lesion        | 105 (74%)     | 15 (11%)  | 9 (6%)      | 129 (91%)   |
| Blood             | —             | —         | 6 (4%)      | 7 (5%)      | 13 (8%)     |
| CSF               | 1 (0.7%)      | 1 (0.7%)  | 1 (0.7%)    | 3 (2%)      |
| Total             | 106 (75%)     | 22 (16%)  | 22 (16%)    |             |
TABLE 3. Antimicrobial therapies used for anthrax treatment.

| Antimicrobial therapy                        | Monotherapy, n (%) | Combined therapy, n (%) |
|---------------------------------------------|--------------------|------------------------|
| Penicillin and penicillin derivates (amoxicillin, ampicillin) | 62 (44%)           | 34 (24%)               |
| Ceftriaxone                                  | 2 (1%)             |                        |
| Ciprofloxacin                                | 28 (20%)           |                        |
| Clindamycin                                  | 2 (1%)             |                        |
| Doxycycline                                  | 3 (2%)             |                        |
| Gentamicin                                   | 4 (3%)             |                        |
| Levofloxacin                                 | 7 (5%)             |                        |
| Meropenem                                    | 3 (2%)             |                        |
| Rifampicin                                   | 1 (0.7%)           |                        |
| Vancomycin                                   | 3 (2%)             |                        |

Clinical presentations are mostly manifested by local symptoms such as edema, erythema and eschar formation. However, the systemic symptoms such as fever, malaise are less commonly seen and often present along with regional lymphadenopathy [12,13]. In our study, systemic symptoms were suggestive of poor outcome and sepsis may occur as a result of spreading of the bacterium via lymphohematogenous route from the primary lesion. Severe toxemia and shock may cause death in a short time [14]. In addition, massive tissue damage may result in prerenal azotemia during anthrax [15], which is reflected by the significant association between high creatinine levels and poor outcome. Thus, it is of particular importance that early and effective treatment should be maintained before the dissemination of the disease.

Definitive diagnosis of anthrax requires the isolation of *B. anthracis* from the infected tissue or blood. *Bacillus anthracis* grows on sheep blood agar easily [14]. The low culture positivity rate is likely to be related to prior antibiotic use since cutaneous anthrax may be treated as common soft tissue infections, with which anthrax could be easily confused [16]. The infected tissue may be culture-negative within several hours of initiating antibiotic therapy so that isolation of the pathogen may not be feasible [9]. On the other hand, the pathogen can be misidentified as other *Bacillus* strains and might be misinterpreted as contaminant by the microbiologist. In minimizing the diagnostic dilemma, clinicians should notify the microbiologist on the suspicion of anthrax so that microbiological evaluation can be done properly. In overcoming these obstacles, Gram-stain, serological and molecular methods like ELISA and PCR can provide evidence of the disease [17]. Microbiological diagnosis was established by culture and Gram stain in in this study, and other confirmatory microbiological tests like PCR, immunohistochemical staining, detection of lethal factor in serum specimens by mass spectrometry, or ELISA [4] could not be done systematically in the hospitals due to infrastructure related issues. In addition, since anthrax is a skin and soft tissue infection (SSTI) and cannot be differentiated from other common SSTIs, the patients commonly apply to hospitals after using antibiotics, which decreases the efficacy of wound cultures. Consequently, 74% of the patients are probable cases in this study and microbiological diagnosis is limited with Gram-stain. On the other hand, probable cases with compatible clinical presentation with a positive Gram-stain are of utmost importance for such a rare disease with the potential to end up with mortality. Therefore, they were included in our study. Similar to other reports [18], *B. anthracis* was isolated only in one-tenth of the patients in our study. Hence, physicians were forced to diagnose anthrax cases with the combination of clinical assessment and the non-culture diagnosis methods, PCR in particular [14] and our data supports this point of view.
B. anthracis is susceptible to a variety of antimicrobial agents including penicillin, fluoroquinolones, tetracycline, macrolides, carbapenems, linezolid and clindamycin. The recommended first regimen treatment for naturally occurring cutaneous anthrax is penicillin and penicillin derivates. Ciprofloxacin or doxycycline can be given as an alternative treatment [19]. Although combination therapy is recommended for systemic anthrax and anthrax meningocerebralitis [5,20], there was no correlation between monotherapy and poor outcome in our case series. Hence, our data disclosed that preventing dissemination of infection seems to be a priority rather than the antimicrobial selection.

The duration of antimicrobial therapy is controversial in anthrax. The recommended duration is 3–7 days for uncomplicated cutaneous anthrax and 10–14 days for systemic and injection anthrax [21]. The mean duration of antimicrobial therapy was over 10 days in our study. Seemingly, anthrax panic originating from bioterrorism fears [22] were reflected in clinical practices by longer and conservative treatment. On the other hand, although longer therapeutic duration and combined therapy were associated to poor outcome in this study, it is most likely to be related to severity of the cases.

This study is limited by its observational and retrospective design; thus, it cannot account for potential unmeasured confounding effects. On the other hand, it is very difficult to perform prospective analysis for such a rare disease. In conclusion, rapid identification of anthrax is crucial for prompt and effective treatment. Systemic symptoms, disseminated local infection, and high inflammatory markers should alert the treating physicians for poor outcomes.

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Transparency declaration

The authors have no relevant financial or non-financial interests to disclose.

Data availability statement

The datasets generated during and/or analysed during the current study are not publicly available. But it can be provided by the corresponding author on reasonable request.

Ethical approval and informed consent

The present research was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. The Institutional Review Board of Dicle University, Faculty of Medicine in Diyarbakır, Türkiye, approved the study without the need for individual informed consent.

Author contributions

All authors contributed to the study conception and design. Conceptualization, Recep Tekin, Nenad Pandak and Hakan Erdem; Data curation, Fatma Kesmez Can, Saygin Nayman Alpat, Abdullah Pekok, Filiz Pehlivanoglu, Murat Karamése, Popescu Corneliu Petru, Sholpan Kulzhanova, Selma Tosun, Mustafa Doğan, Ruxandra Moroti, Ergys Ramosasco, Handan Alay, Edmond Puca, Jurica Arapovic, Natalia Pshenichnyaya, Teresa Fasciana, Anna Giannanco; Data analysis, Umran Elbahr, Milan Papić and Hakan Erdem; Methodology and Statistics; Milan Papić; Supervision, Hakan Erdem; Writing – original draft, Umran Elbahr and Hakan Erdem; Writing – review & editing, Umran Elbahr, Hakan Erdem and Ruxandra Moroti. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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