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Anthrax Threat: A review of Clinical and Diagnostic Measures

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Abstract

Anthrax is the plague of the ancient world and its existence is confirmed by the Roman poet Virgil. Also it is a threat in the modern world as it can be used in biological wars and bioterrorism. Anthrax is caused by *Bacillus anthracis* an unmovable, aerobic, gram-positive rod. It forms spores, which can survive for years in the environment. Three clinical forms result after exposure to anthrax spores: cutaneous, respiratory, and gastro- intestinal. The cutaneous anthrax commonly prevails among humans. The respiratory form occurs most likely due to inhalation of the bacterial spores, whereas the gastrointestinal form happens after spores' ingestion. Prophylactic, early diagnosis and proper treatment will reduce mortalities of anthrax. Thus, the physicians, senior nurses and individuals at risk should be aware of the danger of this disease.

Key words: Anthrax, Bioterrorism, Pathogenesis, treatment, Vaccination

Introduction

Anthrax is caused by *Bacillus anthracis*, is an uncommon illness in the United States. From 1980 through 2000, only seven cases of anthrax were reported to CDC (Hopkins *et al*. 2005). Twenty two bioterrorism related anthrax cases were confirmed or suspected in the United States. *B. anthracis* spores were sent in powder-containing envelopes through the mail. Rarely, sporadic cases of anthrax have occurred in the US among individuals exposed to contaminated animal hides while making traditional drums (Bush *et al*, 2001).

Review and discussion

The life cycle of was unraveled by Koch, who recognized the importance of dormant anthrax spores in the perpetuation of the organism in soil. These studies eventually led to Koch's postulates that have been the cornerstone for establishing a specific pathogen as the causative agent of human and animal diseases. Pasteur created the first successful antibacterial vaccine by successfully attenuating strains of *B. anthracis* and then proving that these strains could protect sheep from infection with fully virulent strains. *B. anthracis* is a sporulating gram-positive rod. It is non-motile and grows rapidly at 37ºC on blood agar plates under aerobic conditions. The individual colonies are non-hemolytic and sticky in shaped. A gamma bacteriophage can be used to confirm the identity of the organism and polymerase chain reaction techniques can be used to identify as

few as three spores of *B. an-thracis* in a single specimen. All virulent strains are pathogenic to mice.

Virulent *B. anthracis* has poly-D-glutamic acid capsule and three proteins (edema factor [EF], lethal factor [LF], and protective antigen [PA]) that associate into two protein exotoxins as described below. Toxin and capsule production are dependent upon the presence of two plasmids: pX01 (184.5 kbp) required for the production of the three exotoxins pX02 (95.3 kbp) contains the genes for synthesis of the poly-D-glutamic acid capsule. The capsule is antiphagocytic and weakly antigenic. The strains cured of pX02 plasmid are none encapsulated and are avidly phagocytosed by polymorphonuclear leukocytes (Mikesell *et al*, 1983).

Pathogenesis

Infection with anthrax requires the presence of three components that combine to form two binary exotoxins: edema factor (EF), lethal factor (LF), and protective antigen (PA) (Dixon *et al*. 1999).

Edema factor and lethal factor:

EF is a calmodulin-dependent adenylcyclase that causes edema when injected subcutaneously into experimental animals. It also impairs host defenses, including inhibition of phagocytosis (Bradley *et al,* 2001). LF causes death through an unknown mechanism when injected into susceptible animals. It is a zinc-dependent protease that causes lysis of macrophages.

But, neither EF nor LF is toxic alone; each produces deleterious effects only when combined with PA, so named because it is antigenic and antibodies binding PA are protective. LF was 10 times more lethal than EF in a rat model, on the other hand, EF produced more hypotension than LF and the combination of EF and LF had an additive effect compared to LF alone (Beall *et al*, 1962).

Liu *et al*. (2012) stated that tumor endothelium marker-8 (TEM8) and capillary morphogenesis protein-2 (CMG2) are the two well-characterized anthrax toxin receptors, each containing a von Willebrand factor A (vWA) domain responsible for anthrax protective antigen (PA) binding. They added that a cell-based analysis was used to implicate another vWA domain-containing protein, integrin β1 as a third anthrax toxin receptor. ExperimentallyTEM8 strongly suggested that is the only minor anthrax toxin receptor mediating direct lethality in vivo and that other proteins implicated as receptors do not play this role.

Protective antigen and Vaccination:

PA binds to a cell surface receptor. After binding, a 20 kDa N-terminal fragment (PA20) is proteolytically cleaved. Larger remaining cell-bound fragment (PA63) has an exposed binding site for either EF or LF (Mogridge *et al.* 2002). Availability program (AVAAP) offered extended antimicrobial PEP (>60 days) for persons at risk of IA, and 1727 individuals received anthrax vaccine in addition to extended antimicrobial PEP. Three serious adverse events with a probable or possible relationship to AVAAP protocol were identified: one case of allergic in interstitial nephritis was classified as

likely causally related to ciprofloxacin PEP, and two serious adverse events were determined to be possibly related to the doxycycline PEP. No serious adverse events were associated with anthrax vaccine use (Tierney *et al*, 2003). In planar phospholipid bilayers, PA63 forms cation-selective channels, suggesting that cleavage of PA20 permits insertion of PA63 as a true membrane-bound protein with channel properties (Wei *et al*, 2006).

LF is a protease that cleaves mitogen-activated protein (MAP) Kinases 1 and 2, leading to their inactivation and inhibition of the MAP Kinase signal transduction pathway. This inhibition of the MAP Kinase pathway leads to the inhibition of upstream signaling components that mediate NADPH oxidase assembly and thus effectively suppress human neutrophil-mediated innate immunity by inhibiting the generation of the superoxide (Crawford *et al,* 2006). In studies in vitro and in vivo, the combination of LF and PA directly inhibited the function of human B cells. In addition, in vitro studies of T lymphocytes isolated from the blood of the healthy volunteers and cultured in the presence of LF, showed down regulation of T lymphocyte activation and cytokine expression.

Subsequent treatment with Chloroquine significantly reduced the harmful effects of LF and protected against the activation and cytokine production of T lymphocytes. The protection of the normal cell response by Chloroquine may provide a new modality for treatment of anthrax, the efficacy of which has been suggested in animal models of infection (Artenstein *et al*, 2004).

Schiffer *et al.* (2012) reported that dried blood spot (DBS) matrix offers an alternative to serum for rapid and efficient sample collection with fewer on-site equipment requirements and considerably lower storage and transport costs. They developed and validated assay methods for using DBS in the quantitative anti-protective antigen IgG ELISA, one of the good assays to assess immunogenicity of anthrax vaccine and for confirmatory diagnosis of *B. anthracis* infection in humans. Also, they developed and validated high-throughput data analysis software to facilitate data handling for large clinical trials and emergency response.

Immune response:

Immune response to high-level anthrax exposure was evaluated in persons exposed or possibly exposed to anthrax when a letter containing anthrax spores was sent to the Senate Office Building in the United States in 2001. All highly exposed persons were immediately treated with antibiotics. No exposed individual developed a clinical anthrax, but post-exposure antibiotic prophylaxis did not prevent stimulation of the immune system. Antibodies to PA and LF were present and evidence of cell-mediated immunity to PA and LF was present in about 80 and 60%, respectively. Although immune responses were generally of low magnitude, there was a dose-response gradient, with immune responses primarily occurring in individuals with higher levels of exposure (Doolan *et al*, 2007).

Dissemination:

When introduced subcutaneously, spores of virulent *B. anthracis* become vegetative organisms and begin to multiply. Subsequent production of an antiphagocytic capsule facilitates local spread and exotoxin production produces extensive brawny edema and tissue necrosis, which are the hallmarks of cutaneous anthrax. The rapid growth of *B. anthracis* during infection requires iron. The organism's mandatory iron acquisition in an iron-scarce environment is promoted by local production of iron chelators (called siderophores) by *B. anthracis*. Two siderophores are produced: the bacillibactin and the petrobactin. Thus, petrobactin plays a key role in the growth of *B. anthracis* (Abergel *et al,* 2006). Thus, petrobactin may be the only siderophore necessary to ensure full virulence. Airborne anthrax spores greater than 5 microns in size pose no threat to the lung, since they are either physically trapped in the nasopharynx or cleared by mucociliary escalator system (Fischbach *et al*, 2006). However, spores between 2 and 5 microns in size are deposited in alveolar ducts or alveoli. These spores are phagocytosed by alveolar macrophages and transported to mediastinal lymph nodes, where they multiply and cause a hemorrhagic mediastinitis. Bacteremia and meningitis are frequent complications after mediastinal infection has become established. Gastrointestinal anthrax follows ingestion of grossly contaminated and undercooked meat. Following ingestion, anthrax bacilli are transported to mesenteric lymph nodes. Subsequently, hemorrhagic mesenteric adenitis, ascites, and septicemia may occur (Brachman, 1980).

Overwhelming infection due to *B. anthracis* results in uncontrolled intravascular multiplication and a fatal toxemia characterized by hypotension and hemorrhage. As an example, during the 12-hour period preceding death of Guinea pigs infected with anthrax, the number of bacteria in the blood rises from 300,000 to one billion organisms/ml.

If antibiotics are given after intravascular bacterial counts reach one million organisms/ml., the animals still die despite a marked reduction in bacterial numbers. Sterile blood from the animal reproduces a fatal toxemic syndrome when given to normal ones (Keppie *et al,* 1955). The organism has two distinct niches in which it can survive and grow: the soil and mammals, including humans.

Natural infection:

B. anthracis can be part of normal soil flora, and when conditions are favorable, it can undergo a burst of local multiplication, which in turn increases the risk of infection in grazing animals.

Systemic anthrax is primarily a disease of herbivores. Humans become accidentally infected through contact with infected animals or their products. In the 1950s and 1960s, over 80% of cases in the United States were related to products that were manufactured from imported goat hair. Inhalational anthrax, or woolsorters' disease, follows the inhalation of anthrax spores generated during the early cleaning of

contaminated goat hair (Brachman, 1965).

The reasons why anthrax bacilli proliferate in soil are not well understood. Studies of agricultural outbreaks have suggested that conditions for multiplication become favorable when: The soil pH is above 6.0, the soil is rich in organic matter. There are major changes in the soil micro-environments as occurs after abundant rainfall or drought (Titball *et al,* 1991).

Abdou (1991) reported that brucellosis, rabies, salmonellosis, anthrax and hydatidosis are among the main zoonotic diseases which constitute a threat to human health and welfare. Surveillance, prevention and control of such zoonoses and related food-borne diseases are problems of considerable magnitude. Despite their obvious importance, relatively few systematic control efforts have been made by national authorities.

Spores can persist in the soil for long periods of time. Surface decontamination is not practical except in unusual circumstances; thus, epizootic anthrax will continue to occur in highly endemic areas, such as Iran, Iraq, Turkey, Pakistan, and sub-Saharan Africa, where the use of animal anthrax vaccine is not comprehensive. In addition, an epidemic occurred in Sverdlovsk in the former Soviet Union due to accidental release from a military microbiologic facility (Meselson *et al*, 1994).

B. cereus can produce disease that simulates inhalational anthrax. Three cases of severe pneumonia have been described. All were due to *B. cereus* strains that were genetically closely related to *B. anthracis* and carried *B. anthracis* virulence plasmids and/or genes. Two of these cases were fatal, and both occurred in non-immunocompromised metal workers (Hoffmaster *et al,* 2006).

Clinical manifestations:

There are three major anthrax syndromes: cutaneous, respiratory, and alimentary tract anthrax. Cutaneous one is the most common form of the disease. Naturally occurring cases of cutaneous anthrax develop after spores of *B. anthracis* are introduced subcutaneously, often as a result of contact with infected animals or animal products. Cuts or abrasions increase susceptibility to cutaneous infection. Spores vegetate and multiply, and the antiphagocytic capsule facilitates local spread (Pile *et al,* 1998).

The incubation period is usually five to seven days with a range of one to 12 days. However, during an anthrax outbreak in Sverdlovsk, Union of Soviet Socialist Republics, cutaneous cases developed up to 13 days following the aerosol release of spores. An outbreak in Algeria was reported with a median incubation period of 19 days (Abdenour *et al,* 1987). The case-fatality rate of cutaneous anthrax is <1% with antibiotic therapy; however, without appropriate therapy, mortality can be as high as 20% (Freedman *et al*, 2002).

Over 90% of cutaneous anthrax lesions occur in exposed areas such as the face, neck, arms, and hands. The disease begins as a small, painless, but often pruritic papule and quickly en-

larges and develops a central vesicle or bulla, followed by erosion leaving a painless necrotic ulcer with a black, depressed eschar. Extensive edema of the surrounding tissues, due to toxin release, is often present along with regional lymphadenopathy and lymphangitis. Systemic symptoms, including fever, malaise, and headache can accompany the cutaneous lesion. In one case during the bioterrorism (BT) event of 2001, a micro-angiopathic hemolytic anemia, thrombocytopenia, coagulopathy, and renal dysfunction developed in a seven-month old child; these manifestations resolved following treatment with antibiotics (Wenner and Kenner, 2004).

Baykam *et al*. (2009) in Turkey reviewed charts of patients hospitalized between 1992 and 2008 found that of 58 cases with cutaneous anthrax with mean age of 49.8, and 36.2% were female. They were farmers (62%), butchers (19%), and housewives (15%) of whom 62% acquired infection when butchering sick animals. Affected sites were hands (39%), fingers (29%), forearms (12%), eyelids (7%) and necks (3%). All cases initially had painless ulcers with vesicles; dissemination of the lesion was in 27.5% of patients.

Doganay and Metan (2009) reviewed anthrax from 1900 to 2007 recorded in the central and eastern parts of Turkey, 426 out of 926 cases, of which 413 cases were cutaneous, 8 gastrointestinal and 5 anthrax meningitis. They stated that anthrax is an endemic disease in Turkey, and acquisition of infection is generally through contact with ill or dying animals or animal products and that controlling human infection depends on controlling infection in animals. Doganay *et al*. (2010) reviewed clinical experience with twenty-two cases of cutaneous anthrax in the last 7 years. Ten were severe form, 10 cases mild form and 2 cases were toxemic shock due to cutaneous anthrax. The incubation period was between 1 and 17 days. The main clinical characteristics of the cases with severe cutaneous anthrax were fever, hemorrhagic bullous lesions surrounded by an extensive erythema and edema, and leukocytosis. Two cases with toxemic shock had low systolic blood pressure, apathy and toxemic appearance, leukocytosis, hypoalbuminemia & hyponatremia.

Inhalation anthrax (Respiratory anthrax = Woolsorters Disease) results from the inhalation of *B. anthracis* spore-containing particles. This may occur when anthrax spores are aerosolized while working with contaminated animal products such as wool, hair, or hides. It has also been resulted from the inhalation of weaponized and intentionally released spore preparations. Inhaled airborne particles >5 microns in size are either physically trapped in the nasopharynx or cleared by the mucociliary escalator system. In comparison, inhaled particles <5 microns in size can be deposited on alveolar ducts or alveoli. The *B. anthracis* spores are phagocytosed by alveolar macrophages and transported to mediastinal lymph nodes there they germinate, multiply, and release toxins, causing hemorrhagic necrosis of the thoracic lymph nodes draining the lungs, which results in a hemorrhagic mediastinitis, and, in oc-

casional cases, a necrotizing pneumonia. The organisms then become bloodborne, causing bacteremia and, in some cases, meningitis (Stern *et al*, 2008).

The incubation period for inhalation anthrax is estimated to be one to seven days, but was reported to be as long as 43 days for fatal cases in the 1979 outbreak in Sverdlovsk. Information from a single case report suggests that the incubation period can be as short as one day. During the bioterrorism (BT) event in USA, the time between known exposure and symptom onset ranged from four to six days, with a mean of 4.5 days. In primate studies, spores have been found in the lungs up to 100 days following exposure, and inhalation anthrax has developed up to 58 days following experimental aerosol exposure in primates receiving 30 days of post-exposure antibiotics (Jernigan *et al*, 2001).

The disease course is usually biphasic. Prodromal symptoms of inhalation anthrax are nonspecific and variable, complicating assessment and diagnosis (La Force, 1994). The early symptoms, such as myalgia, fever, and malaise, may mimic those of influenza. However, a variety of symptoms less suggestive of influenza may also be present such as nausea, hemoptysis, dyspnea, odynophagia or chest pain. Prodromal symptoms last an average of 4 to 5 days and followed by a rapidly fulminant bacteremic phase with development of progressive respiratory symptoms, including severe dyspnea, hypoxemia & shock (Brachman, 1980). The fulminant phase is a catastrophic illness that almost uniformly leads to death within days. It does not appear that modern intensive care has changed the outcome once the fulminant phase is reached. However, antibiotic therapy can be successful if initiated during the prodromal phase of the disease. For instance, 6 of 11 cases (55%) associated with the 2001 BT event in the United States responded to treatment, but none of the five patients who required mechanical ventilation or tracheostomy survived. The challenge for the clinician is to appropriately treat patients during the prodromal stage, even though anthrax is a rare disease with a nonspecific and variable presentation. Imaging studies can aid in establishing the diagnosis. Widening of the mediastinum, secondary to mediastinitis, is considered a classic finding in inhalation anthrax (and 7 of the first 10 cases associated with the 2001 BT event had this finding (Borio *et al*, 2001).

Other chest radiographic findings seen with inhalation anthrax include hilar abnormalities, pulmonary infiltrates or consolidation, and pleural effusion. One or more of these abnormalities were documented in all 11 cases associated with the 2001 BT event. Abnormalities, however, were often subtle, and chest radiographs obtained early in the course of illness were interpreted as normal in 3 of 11 cases (Barakat *et al*, 2002).

The hematogenous spread can result in lesions in other organ systems, including hemorrhagic meningitis and the sub-mucosal gastrointestinal lesions. Inhalation anthrax is usually fatal; among 71 cases in the world's literature from 1900 to 2005, excluding the

six survivors during the 2001 BT event, mortality rate was 92% (La Force, 1994).

Alimentary tract anthrax presents as one of two clinical forms, or pharyngeal or gastrointestinal anthrax. *B. anthracis* infects all alimentary tract regions from the mouth to the ascending colon Infection develops after consumption of undercooked infected meat from animals infected with anthrax, and tends to occur in family clusters or point source outbreaks.

Gastrointestinal involvement is more common than orpharyngeal disease, but its incidence is probably underestimated because it occurs mostly in medically underserved areas. The incubation period is one to six days (Holty *et al,* 2005). The spores infect the alimentary tract epithelium. Necrotic ulcers, often similar to eschars on the skin, are surrounded by extensive edema of the infected intestinal segment and its adjacent the mesentery; mesenteric lymph nodes may be enlarged and hemorrhagic. Ulcerations can occur in the stomach, esophagus, and duodenum and may result in gastrointestinal hemorrhage. The case-fatality rate of gastrointestinal anthrax is estimated to range from 4 to 60% (Brachman and Kaufmann, 1998). The lower estimate is derived from point source outbreaks studied by public health officials in Uganda and Thailand, where large numbers of people ate uncooked meat from animals that died of anthrax. Most of the people who ate the uncooked meat became sick with gastroenteritis, which cleared with oral antibiotics. In more than 100 Lebanese patients with gastrointestinal anthrax, illness started with asthenia, headache, low grade fevers, facial flushing, and conjunctively injection. This was followed by abdominal pain of variable intensity, nausea, vomiting, and to a lesser extent, diarrhea. Typically, patients at this point had ascites and intravascular depletion. Later, the abdominal pain tended to become more severe and patients had progression of ascites and hypotension. At surgery, segmental disease was found in the distal small bowel and/or proximal colon. Although they do not cite the survival rate, most patients (even those who required surgery) survived.

Oropharyngeal santhrax is less frequent, develops after consumption of undercooked infective meat. Edematous lesions develop, progress to necrotic ulcers covered with a pseudomembrane. Edema and painful swelling may develop in the oropharynx and neck, accompanied by the cervical lymphadenopathy, pharyngitis, and fever (Sirisanthana and Brown, 2002). The mortality can be substantial even with parenteral antibiotic treatment.

Meningitis is association with cutaneous, inhalation and gastrointestinal anthrax cases. One-half of patients with inhalation anthrax developed hemorrhagic meningitis (Abramova *et al*, 1903). The cerebrospinal fluid analysis reveals an elevated protein (70%), low glucose (37%), and a positive Gram's stain (77%) and culture (81%). Parenchymal brain hemorrhage may be so severe that a grossly bloody lumbar puncture may be confused with a traumatic tap. Delirium or coma follows

quickly and refractory seizures, cranial nerve palsies, and myoclonus have been reported (Dixon *et al,* 1999).In 44 well-documented cases 75% of patients died within 24 hours with an overall survival of only 6% (Lanska, 2002).

Diagnosis:

Because of the public health implications of any form of anthrax and the rapid course of inhalation anthrax, clinicians and the laboratories should coordinate the diagnostic evaluation as rapidly as possible with an appropriate laboratory response network reference (Morse *et al*, 2003). Several diagnostic tests are available. The standard culture and susceptibility testing can be done as for other pathogens, although most clinical laboratories can offer only presumptive identification of *B. anthracis* with confirmation at a reference laboratory. Standard or real-time PCR can be done on a variety of isolates, including blood cultures, tissue, and blood samples. Susceptibility to lysis by gamma phage differentiates the organism from *B. thuringensis*. In late acute case or in convalescence, antibodies can be detected qualitatively and quantitatively (Quinn *et al*, 2002). The organism can be identified by direct observation through immuno-histochemical (IHC) staining (Shieh *et al*, 2003). Baykam *et al*. (2009) stated that cutaneous anthrax should be considered in cases with a painless ulcer with vesicles, edema, and a history of exposure to animals or animal products.

Criteria for diagnosis:

CDC (2001) developed interim case definitions for anthrax. A confirmed anthrax was defined as a clinically compatible case that was laboratory confirmed by *B. anthracis* isolation from the patient, or by laboratory evidence based on at least two other supportive tests using non culture methods for *B. anthracis*. Supportive laboratory tests include the Laboratory Response Network (LRN) PCR, immuno-histochemical staining (IHC) of tissues and an anti-protective antigen (PA) IgG detected by an enzyme-linked immunosorbent assay (ELISA).

A suspect case was a clinically compatible illness without isolation of *B. anthracis* and with only a single supportive test, or a clinically compatible case epidemiologically linked to a confirmed exposure to *B. anthracis* but without corroborative laboratory evidence (Hupert *et al,* 2003).

Laboratory response network:

The Laboratory Response Network (LRN) was established in 1999 by the CDC, the Association of Public Health Laboratories (APHL), the Federal Bureau of Investigation (FBI) and the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) for the rapid identification of select agents including *B. anthracis*. The LRN is part of a linked hierarchy of sentinel, reference, and national level laboratories. There are LRN reference laboratories (generally state public health laboratories) in all 50 states (Swartz, 2001). Kracalik *et al*. (2012) compared a local clustering and a cluster morphology statistic using anthrax outbreaks in large (cattle) and small (sheep and goats) domestic ruminants

across Kazakhstan. The results showed important differences in spatial statistical methods for defining local clusters and highlight the importance of selecting appropriate levels of data aggregation

Specimen collection and transport:

Generally, the guidelines below should be applied: specimens of stool, sputum, pleural fluid, CSF, and blood stored at 2 to 8ºC, swabs at room temperature, frozen fresh tissue samples, formalin fixed specimens at room temperature. Blood specimens for PCR testing should optimally be collected in tubes containing EDTA or citrate as anticoagulant and not heparin. Isolates of *Bacillus* can be transported on most nonselective laboratory media at room temperature.

Clinical Diagnostic Syndrome:

Inhalation Anthrax Distinction from Common Respiratory Infections: It is important to distinguish potential inhalation anthrax cases from more common disorders such as community-acquired pneumonia (CAP), influenza, and influenza-like illnesses (ILI). As mentioned above, this may be difficult. If the patient has influenza, a positive test for this disease can allay concerns about anthrax. The epidemiologic setting is important; especially with regards to occupational history and hobbies e.g. drum makers if there is an association with other cases as in the occurrence of a suspected bioterrorism (BT) event (CDC, 2004). The BT event of 2001 illustrated the importance of screening for inhalation anthrax because the window of opportunity for successful treatment is narrow once symptoms appear. Clinical signs more frequently associated with inhalation anthrax compared to CAP or ILI included shortness of breath, nausea, vomiting, altered mental status, pallor or cyanosis, and hematocrit >45%.

In contrast, symptoms more suggestive of an ILI included rhinorrhea and sore throat (Hupert *et al,* 2003). Unexplained mediastinal widening on chest radiography in a compatible clinical setting should raise the possibility of inhalation anthrax. Other radiographic findings are probably not well specific to be helpful in an unsuspected sporadic case, but such findings can be helpful in an outbreak situation or if there was a known risk of exposure. In the 2001 outbreak, pleural effusion was more common in patients with inhalation anthrax than in those with CAP. Although chest radiographs are almost always abnormal in patients with inhalation anthrax, these findings are sometimes subtle and they may be initially overlooked. Thus, the diagnosis of inhalation anthrax cannot be ruled out even if a chest radiograph is interpreted as normal early in the course of illness.

Diagnostic testing should be done on specimens from patients being evaluated for inhalation anthrax, including patients with a known exposure or high risk of exposure, patients with a clear epidemiologic link presenting with the symptoms of inhalation anthrax, and patients with a clinical presentation suggestive of anthrax in the absence of an alternate diagnosis (CDC, 2001) developed recommendations for clinical evaluation of persons with possible

inhalation anthrax during bioterrorism event, available online at http: //www.cdc.gov/mmwr/preview/mmwrhtml/mm5044a5.htm.

Diagnostic tests of patients with suspected inhalation is recommended. Specimens of blood obtained prior to antimicrobial therapy for routine culture and for PCR at the Laboratory Response Network (LRN) laboratory/ pleural fluid, if present, for Gram stain, culture, and PCR Cerebrospinal fluid, in patients with meningeal signs, for Gram stain, culture, and PCR Acute and convalescent serum samples for the serologic testing pleural and/or bronchial biopsies for the immunohistochemistry, if other tests are negative CDC developed recommendations for clinical evaluation of persons with cutaneous anthrax, available online at http://www.cdc.gov/mmwr/preview/mwrhtml/mm5044a5.htm.

The presence of an eschar especially with extensive edema out of proportion to the size of the lesion and the presence of gram-positive rods and few polymorpho-nuclear leukocytes on the Gram stain are strongly suggestive of cutaneous anthrax (Meselson *et al,* 1994).

Diagnostic tests of patients with suspected cutaneous anthrax is recommended: for vesicular lesions, two swabs of vesicular fluid from an unopened vesicle, one for Gram stain and culture, the second for PCR for eschars, the edge should be lifted and two swabs rotated underneath and submitted, one for Gram stain and culture, the second for PCR for ulcers, the base of the lesion should be sampled with two saline moistened swabs and submitted, one for Gram stain and culture, the second for PCR.

Also, a full thickness punch biopsy of a papule or vesicle including adjacent skin from all patients should be submitted in 10 percent formalin for histopathology and immunohistochemistry. In patients not on therapy for <24 hrs, a second biopsy specimen should be submitted for Gram stain, culture, and PCR (Carucci *et al*, 2002).Information regarding the reliability of diagnostic testing in alimentary tract anthrax is limited. Culture from stool frequently does not yield *B. anthracis,* but Gram stain or culture of oropharyngeal lesions or ascitic fluid may be positive. Blood cultures may be positive when collected prior to initiating antimicrobial therapy. Serologic tests were positive in seven of 10 oropharyngeal cases (Sirisanthana *et al*, 1988).

Gastro-intestinal Anthrax diagnostic tests of patients with suspected alimentary tract anthrax is recommended. Blood cultures and blood for PCR obtained prior to antimicrobial therapy ascites fluid for Gram stain, culture, and PCR testing stool or rectal swab for Gram stain, culture, and PCR testing oropharyngeal lesion, if present, for Gram stain, culture, and PCR testing; Acute and convalescent serum samples for serologic testing If the patient undergoes surgery, affected tissue can be obtained for Gram stain, culture and PCR testing. The immunohistochemistry can be performed on formalinized tissue.

Treatment

Artenstein *et al*. (2004) found that Chloroquine enhances survival in *B. anthracis* intoxication. Doganay and, Metan (2009) stated the endemicity of Anthrax in Turkey, among other countries of the world as an important global issue. On reviewing human cases recorded from 1990 to 200, most cases were recorded from the central and eastern parts of Turkey. They reviewed 426 out of 926 cases, of which 413 (96.9%) cases were cutaneous, 8 (1.9%) gastrointestinal and 5 (1.2%) anthrax meningitis. Of all the affected patients, 95.2% had contact with contaminated materials. Most of the patients (88.7%) had received Penicillin G., total mortality was 2.8%. Anthrax is an endemic disease in Turkey, and acquisition of infection is generally through contact with ill or dying animals or animal products. Sheep and cattle are generally involved. Most clinical disease in humans is cutaneous anthrax, although other clinical forms are seen and have a greater mortality. Penicillin remains the drug of choice in treating the disease. Controlling anthrax in humans depends on controlling the animals' infections.

Baykam *et al*. (2009) successfully treated cutaneous anthrax with Penicillin G and/or Ciprofloxacin or Imipenem. One patient with a disseminated lesion on the neck died

Bioterrorism

In USA, 22 cases of anthrax, 18 confirmed and four suspected, resulted from the attempts to deliberately expose selected individuals or organizations to weaponized anthrax spores. Eleven of them were inhalational and 11 cutaneous; all but two inhalational cases resulted from exposure to *B. anthracis* in a powder sent through the mail and most of the inhalational cases occurred in postal employees (Bush *et al*, 2001).

Madle-Samardzija *et al*. (2002) mentioned that anthrax has been developed as a weapon of mass destruction since World War I. During accidental release from a biological warfare factory in the former Soviet Union, 68 people died. The ease of laboratory production and its dissemination via aerosol led to its adoption by terrorists. They added that grass-eating animals are usually infected by the bacilli from the grass and ground. The disease is transmitted to people by contact with the sick animals or their products, such as wool, skin, meat etc.

Two unexpected findings resulted from the investigations of these bioterrorism cases. First, airborne dissemination of anthrax spores occurred from sealed envelopes during their travel through high-speed mail sorting machines. Second, re-aerosolization of infective spores occurred long after airborne spores had settled onto surfaces. The Ames strain had been used widely by the United States military in Bio-defense research. Investigations by the US CDC and the Federal Bureau of Investigation (FBI) implicated a biodefense researcher working at the US Army Medical Research Institute in Frederick, Maryland, although the case was never submitted to the scrutiny of a court of law since the researcher

committed suicide before the case could be tried (Jernigan *et al,* 2001). Twenty-five days after the Senate Office Building was closed, a study was conducted in the office of a United States senator who had received an envelope that was opened by his staff. Individuals wearing sterile protective suits initially placed sampling devices around the office suite and then left the area. Later they returned to the contaminated areas and simulated office activity such as walking, sorting mail, and moving trash cans. The airborne spore concentrations increased 65-fold during simulated active period, proving that reaerosolization of anthrax spores is possible (Bhattacharjee, 2009).

Friedman *et al*. (2010) addresses how Israel might best (1) prevent hostile elements from obtaining, from Israel's biological research system, materials, information and technologies that facilitate their carrying out a biological attack, while (2) continuing to promote academic openness, excellence and other hallmarks of that system. This important and sensitive issue was assessed by a special national committee, and their recommendations are presented and discussed. One particularly innovative element is the restructuring and use of Israel's extensive biosafety system to also address biosecurity goals, with minimal disruption or delay.

Steelfisher *et al.* (2012) emphasized the need for outreach that would more effectively support racial/ethnic minority populations during a bioterrorism incident. They used a nationally representative poll of 1,852 adults, including 1,240 whites, 261 African Americans, and 282 Hispanics. The poll examined public reactions to a "worst-case scenario" in which cases of inhalation anthrax are discovered without an identified source and the entire population of a city or town is asked to receive antibiotic prophylaxis within 48 hours. They suggested the need for tailored outreach to racial/ethnic minorities through, for example, emphasis on key messages and enhanced understandability in communications, increased staff for answering questions in relevant dispensing sites, and long-term trust building with racial/ethnic minority

Other modes of acquisition:

During the 20th Century, improvements in industrial hygiene, a decrease in the use of imported, contaminated animal materials, and immunization of at-risk workers resulted in a reduction in the incidence of inhalational anthrax (only 18 cases in the United States). Before the 2001 bioterrorism attack, the last prior fatal case of anthrax in the United States occurred in 1976 when a weaver by hobby died of inhalational anthrax after working with yarn imported from Pakistan (Brachman, 1980). Although the risk of anthrax associated with the handling of animal hides is low, such cases still sporadically occur. As an example, a man in Connecticut developed cutaneous anthrax in 2007 after processing a contaminated African goat hide to make a traditional drum. His eight year old child also developed cutaneous anthrax despite having had no direct contact with the hide. An investigation re-

vealed widespread contamination of multiple areas of the home with *B. anthracis*, although all drum-making activities were confined to a backyard shed (Bhattacharjee, 2009).

Despite the rarity of human cases, anthrax remains a potential threat in USA for two reasons: Anthrax epizootics still occur in the United States. In 2000, 32 farms in North Dakota were quarantined because of anthrax: a total of 157 animals died during this epizootic and a single ranch worker who helped move dead animals developed cutaneous anthrax. Anthrax remains an important potential agent of bioterrorism and biological warfare.

Grunow *et al*. (2012) mentioned that injected anthrax rarely affects heroin users. But, there were one fatal out of four cases in Germany, as well as a small number of cases in other European countries, including Denmark, France, and England. Three cases among drug users occurred in Germany in 2009/2010, in the setting of a larger outbreak centered on Scotland, where there were 119 cases.

Anthrax Vaccine Adsorbed

U.S. Brand Names: BioThrax® Vaccine, Inactivated (Bacterial):

Adults Dosing: Primary immunization: I.M.: Five injections of 0.5 ml each given at 0- and 4 weeks, then 6-, 12-, and 18 months. Subsequent i.m. booster injections of 0.5 ml, at 1-year interval, are recommended for maintenance of immunity in persons who remain at risk. Pediatric safe dosing and efficacy have not been established. Also elderly safe dosing, and efficacy have not been established in persons >65 years of age.

Forms of anthrax vaccines:

Two forms of anthrax vaccines are available for prophylaxis against the disease, a) BioThrax® suspension for injection (5ml)**,** it contains *B. anthracis* proteins (contains aluminum, natural rubber/ natural latex in packaging) b) BioThrax®: suspension for injection (5ml)**,** it contains *B. anthracis* proteins

Administration: shake well before use. Do not use if discolored or contains particulate matter. Do not use same site for more than one injection. Do not mix with other injections. For i.m.; do not inject i.v. or intradermally. For patients at risk of hemorrhage following intramuscular injection, vaccine can be administered subcutaneous.

Anthrax vaccine with other inactivated vaccines: may be given simultaneously or at any interval between doses. Anthrax vaccine with live vaccines: may be given simultaneously or at any interval between doses.

Vaccine administration with antibody-containing products: Anthrax vaccine and antibody-containing products may be given simultaneously at different sites or at any interval between doses. Examples of antibody-containing products include i.m. and i.v. immune globulin, hepatitis B immune globulin, tetanus immune globulin, varicella zoster immune globulin, and rabies immune globulin, whole blood, packed red cells, plasma, and platelet products.

USE: Immunization against *B.* anthracis in persons at high risk for infection.

The Advisory Committee on Immunization Practices (ACIP) recommends routine vaccination for the following: Persons who work directly with the organism in the laboratory Persons who may come in contact with animal products which come from anthrax endemic areas and may be contaminated with *B. anthracis* spores, such as veterinarians who travel to other countries or persons who work with imported animal hides/ furs from areas where standards are insufficient to prevent anthrax spores. Military personnel deployed to areas with high risk of exposure

Routine immunization for general population is not recommended. Use - Unlabeled/Investigational: Post-exposure prophylaxis in combination with antibiotics.

Adverse reactions significant: All serious adverse reactions must be reported to the U.S. Department of Health and Human Services (DHHS) Vaccine Adverse Event Reporting System (VAERS) 1-800-822-7967 or online at https://secure.vaer

Note: Percentages reported with I.M. administration: >10%:

CNS: Headache (4% to 64%), fatigue (5% to 62%)

Local: Tenderness (10% to 61%), erythema (8% to 31%), pain (4% to 23%), edema (1% to 16%), limitation of arm motion (1% to 16%), induration (3% to 14%), warmth (1% to 11%)

Neuromuscular and skeletal: Myalgia (2% to 72%)

Respiratory: Nasopharyngitis (12% to 15%), pharyngolaryngeal pain (12%)

1% to 10%:

Dermatologic: Pruritus (≤ 2%), rash (≤ 2%)

Endocrine & metabolic: Dysmenorrhea (7%)

Gastrointestinal: Diarrhea (6% to 8%), nausea (6%)

Local: Itching (≤ 9%), bruising (3% to 6%), nodule (1% to 6%)

Neuromuscular & skeletal: Back pain (7% to 9%), neck pain (3%), joint sprain (≤ 2%), and rigors (1% to 2%)

Respiratory: Sinusitis (5% to 7%), upper respiratory tract infection (2% to 3%), sinus headache (1% to 3%)

Miscellaneous: Hypersensitivity (2% to 4%), lymphadenopathy (2% to 3%), flu-like illness (2%), tender/painful axillary adenopathy (≤ 1%)

Post-marketing and/or case reports: Allergic reactions, alopecia, anaphylactoid reaction, arthralgia, arthropathy, erythema multiforme, injection site reactions (cellulitis), paresthesia, pyrexia, rhabdomyolysis, Stevens-Johnson syndrome, syncope, tremor, ulnar nerve neuropathy

Contraindications: Hypersensitivity to anthrax vaccine or any component of the formulation; history of anthrax

Warnings/Precautions: Concerns related to adverse effects: Anaphylactoid/ hyper-sensitivity reactions: Immediate treatment (epinephrine 1:1000) for anaphylactoid and/or hypersensitivity reactions should be available during vaccine use.

Disease-related concerns: acute illness: May consider deferring admin-

istration in patients with moderate or severe acute illness (with or without fever); may administer to patients with mild acute illness (with or without fever). Anthrax disease: Persons with a history of anthrax disease may have an increased risk for adverse reactions from the vaccine.

Bleeding disorders: use with caution in patients with a history of bleeding disorders; and those on anticoagulant therapies, bleeding/hematoma may occur from i.m administration. For patients at risk of hemorrhage following intramuscular injection, vaccine can be administered Subcutaneous.

Special populations: altered immunocompetence: Use with caution in severely immunocompromised patient; chemo/radiation therapy or other immunosuppressive therapy (high dose corticosteroids)); may reduce response to vaccination. Elderly: safety and efficacy not established in adults >65 years. Pediatrics: Safety and efficacy not established in children.

Concurrent drug therapy issues, vaccines: in order to maximize vaccination rates, the ACIP recommends simultaneous administration of vaccines appropriate for all ages (live or inactivated) for which a person is eligible at a single clinic visit, unless contraindications exist.

Dosage form specific issues: Latex: Packaging may contain natural latex rubber.

Restrictions: Not commercially a available in the U.S.; presently, all anthrax vaccine lots are owned by the U.S. Department of Defense. The CDC does not currently recommend routine vaccination of the general public.

Drug Interactions:

Immuno-suppressants may diminish the therapeutic effect of vaccines (Inactivated). Risk C: Monitor therapy

The pregnancy implications adverse events were not observed in animal develop-mental toxicity studies. Use during pregnancy only if clearly needed. Data from the Department of Defense suggest the vaccine may be linked with a slightly increased number of birth defects when given during the first trimester of pregnancy. Male fertility is not affected by vaccine administration.

Lactation: excretion in breast milk is unknown, however use with caution. For breast-feeding no adequate and well-controlled studies using this vaccine in breast-feeding women; however, the administration of non-live vaccines during breast-feeding is generally not medically contraindicated.

Monitoring Parameters: for local reactions, chills, fever, anaphylaxis; syncope for ≥ 15 minutes after vaccination

Mechanism of active immunization: the vaccine is prepared from a cell-free filtrate of *B. anthracis*, but no dead or alive bacteria. Completion of the entire vaccination series is required for full protection.

Patient Information: immunization using the vaccine consists of a series of 5 injections. The vaccine should be used by people who may be exposed to the anthrax bacteria, such as laboratory workers, veterinarians, and military personnel. Most people receiving the

vaccine will experience soreness, redness, or itching at the injection site, which should clear up within 48 hours.

Conclusion

Anthrax is still an endemic disease in some countries in the world and has become a re-emerging disease in western countries with recent intentional outbreak. A good knowledge of anthrax, its transmission and potentials as a biological weapon for timely prevention and protection is a must. Two clinical forms exist: outer--cutaneous and inner, including inhalation and gastrointestinal anthrax. While cutaneous anthrax is easily cured, the inner forms cause high mortality rates. The diagnosis is easily established in cutaneous cases, characterized by black eschar. Severe intoxication and collapse during the course of bronchopneumonia or hemorrhagic enteritis indicated anthrax suspicion. Hospitalization of patients is a must. *B. anthracis* is susceptible to a number of antibiotics, including Penicillin, Erythromycin, Tetracyclines, Cephalosporins etc. Timely treatment can be life-saving. General vaccination of livestock and control of products is very important. The vaccine consists of anthrax bacillus that is attenuated. The endangered population, such as animal workers and military personnel should be vaccinated. Annual schedule of booster immunization must be maintained.

Capsular polypeptide and anthrax toxin are the principal virulence factors of *B. anthracis*. Toxin consists of three proteins called protective antigen, edema factor, and lethal factor. The inflammatory mediator--lethal factor is stored within the macrophage during the early stage of infection and rapidly released in huge numbers in blood stream and once the threshold for lysis is reached, it may cause sudden death.

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