Int J Legal Med (2008) 122:35–41
DOI 10.1007/s00414-007-0163-9

ORIGINAL ARTICLE

Pathology of fatal traumatic and nontraumatic clostridial gas gangrene: a histopathological, immunohistochemical, and ultrastructural study of six autopsy cases

Michael Tsokos · Sarah Schalinski · Friedrich Paulsen · Jan P. Sperhake · Klaus Püschel · Ingo Sobottka

Received: 24 August 2006 / Accepted: 20 February 2007 / Published online: 17 March 2007

Abstract We prospectively investigated six fatal cases of clostridial gas gangrene using autopsy, histology, immunohistochemistry, microbiology, and scanning electron microscopy. The causative pathogen was *Clostridium perfringens* in four cases, C. *sordellii* in one case, and a mixed infection with both *C. perfringens* and C. *sordellii* in one case. According to the previous medical history and autopsy findings, clostridial infection was related to trauma in three cases. Characterized by extensive tissue necrosis and total absence of an accompanying leukocyte infiltration and tissue inflammatory response, the histopathological picture of clostridial gas gangrene is distinctly different from other bacterial infections. In medicolegal casework, the proof of the source of infection and the portal of entry of the responsible pathogen is not always an easy task, especially in the absence of trauma.

M. Tsokos (✉) · S. Schalinski
Institute of Legal Medicine and Forensic Sciences,
Charité—Universitätsmedizin Berlin, Turmstr. 21 (Haus L),
10559 Berlin, Germany
e-mail: mtsokos@web.de

F. Paulsen
Department of Anatomy and Cell Biology, Martin Luther
University of Halle-Wittenberg,
Halle (Saale), Germany

J. P. Sperhake · K. Püschel
Institute of Legal Medicine,
University Hospital Hamburg-Eppendorf,
Hamburg, Germany

I. Sobottka
Institute of Infectious Medicine,
University Hospital Hamburg-Eppendorf,
Hamburg, Germany

Keywords Gas gangrene · Clostridial myonecrosis · *Clostridium perfringens* · Autopsy · Forensic pathology

Introduction

Clostridial gas gangrene (clostridial myonecrosis) is one of the most fulminant necrotizing infections affecting humans [3, 22]. Clostridiae are anaerobic, Gram-positive, rod-shaped organisms commonly found in the environment in niches such as soil and sewage and in the gastrointestinal tract of both humans and animals. *Clostridium perfringens* is the most common organism isolated from patients with gas gangrene [3, 6]. Other *Clostridium* species identified as causative organisms of the disease include *C. septicum*, *C. novyi*, and *C. sordellii* [1, 4, 18, 19, 21, 25, 27].

Classically, gas gangrene used to be a well-known complication of war wounds. Nowadays, in autopsy cases of fatal gas gangrene investigated by forensic pathologists, infiltration of clostridiae into tissue is usually related to major or minor trauma sustained in the civilian setting. Clostridial infection may also be associated with injection drug abuse or surgical procedures involving the gastrointestinal or genitourinary tract. In addition, gas gangrene can also develop in the absence of trauma (referred to as "nontraumatic" or "spontaneous" gas gangrene), a fact that makes establishing the true pathogenesis of the disease more difficult.

Although a number of studies have dealt with the clinical picture and course of near-fatal and fatal events of gas gangrene and different therapeutical management strategies have been proposed, autopsy studies are sparse and especially the histological and ultrastructural correlates of clostridial gas gangrene are not yet well defined. Therefore, it was the objective of the present prospective autopsy study

to better describe the pathological features of this relatively rare disease.

Materials and methods

Study group

Six autopsy cases of fatal clostridial gas gangrene from the Institute of Legal Medicine, University of Hamburg, Germany, between 1999–2003 were prospectively investigated. Autopsies were performed within 8–35 h postmortem. Individual cases were analyzed as to the gender, age, previous medical history, survival time after onset of the disease, risk factors for the development of gas gangrene, location of the infection, responsible pathogens as identified by microbiological culture, pathogenesis of development of gas gangrene (probable portal of entry), and autopsy findings.

Microbiology

Appropriate autopsy specimens, wound exsudates, or aspirates were cultured on solid Columbia agar base +5% sheep blood and incubated for 48 h at 36°C under anaerobic conditions. Clostridiae were subcultured and determined using a biochemical identification system for the rapid identification of anaerobic Gram-negative bacilli (rapid ID 32A, bioMérieux, Marcy-l'Etoile, France).

Histopathology

In each case, a thorough light microscopical examination of the skin and subcutaneous tissue, musculature, and the internal organs, including the brain, was carried out. For histological examination, paraffin-embedded tissue specimens were cut in 40- to 5-μm sections and stained with hematoxylin and eosin (H&E), periodic acid-Schiff, phosphotungstic acid hematoxylin, and Gram's stain.

Immunohistochemistry

Immunohistochemical staining was carried out with specific monoclonal antibodies against neutrophil granulocytes (anti-CD15, M 0733, Dako, Glostrup, Denmark, 1:50) and macrophages (anti-CD68, M 0876, Dako, 1:100) that were applied using a standard alkaline phosphatase anti-alkaline phosphatase technique. The proteinase-K pretreated sections were finally mounted on Aquatex slides (Boehringer, Mannheim, Germany). Negative control sections were performed using PBS buffer instead of the primary antibody. As hemolytic effects of clostridial toxins within the infected host have been postulated [22], a specific antibody against hemoglobin (Hb; Dako, 1:50) was applied for better visualization of hemolysis, if present. For this purpose, a standard peroxidase-labeled streptavidin–biotin technique was used, either with microwave heating pretreatment or trypsination where appropriate. After counterstaining with hematoxylin, the sections were finally mounted with Aquatex (Boehringer). Two negative control sections were used in each case, incubated only with the primary or the secondary antibody, respectively. Standardized sections with submucosal blood vessels (staining of erythrocytes) served as positive controls.

Scanning electron microscopy

For scanning electron microscopy, tissue from the internal organs as well as muscle tissue previously fixed in 5% buffered formalin was cut longitudinally and halved. All tissue blocks were then impregnated with 2.5% tannic acid for 2 days. Counterfixation in 2% osmium tetroxide for 4 h was followed by dehydration in ethanol and drying in a critical-point dryer (Balzers CPD 030, Wiesbaden, Germany). The specimens were coated with gold (Ion Technology, Teddington, UK) and analyzed with a scanning electron microscope (Philips XL20, Kassel, Germany).

Results

Case characteristics

The individual case characteristics are summarized in Table 1.

Of the six deceased, two were men and four were women with age ranging from 30 to 85 years and a mean age of 57 years. Of the individuals, four had died in a hospital and two had died at home, all deceased were of Caucasian origin.

There were no diseases or injuries present to account for or contribute to death other than clostridial gas gangrene. *C. perfringens* was cultured in four cases, *C. sordellii* in one case, and a mixed infection with both *C. perfringens* and C. *sordellii* was found in another case.

According to the previous medical history and autopsy findings, the infection was most probably related to trauma in three cases and the nontraumatic form of the disease was found in the remaining three cases. The time between onset of the disease and death ranged between 4–20 h in four cases and could not be clearly determined in the remaining two cases. The average survival time after onset of disease was 15 h in the traumatic gas gangrene group compared to 8 h in the nontraumatic gas gangrene group (Table 1).

Underlying risk factors predisposing to the development of gas gangrene could not be established in three

Table 1 Individual case characteristics

Case no.	Sex (m/f)	Age (years)	Survival after onset of disease (h)	Etiological agent: pathogen as identified by culture	Infection location	Main autopsy findings other than clostridial myonecrosis	Risk factors for development of gas gangrene	Pathogenesis of development of gas gangrene
1	f	42	20	*C. perfringens*	Anterior and posterior trunk, both shoulders, upper arms, and thighs	No preexisting diseases	Alcoholism	Iatrogenic trauma: Perforation of small intestine during abdominal surgery
2	f	85	Unknown	*C. perfringens, C. sordellii*	Right thigh	Arteriosclerosis, coronary arteriosclerosis	No/ unknown	Blunt force trauma: Fall leading to fracture of neck of left femur; complicated wound healing after implantation of a total endoprosthesis
3	m	47	10	*C. sordellii*	Anterior and posterior trunk	Lung emphysema, arteriosclerosis	No/unknown	Sharp force trauma: Superficial self-inflicted cut wounds to both wrists
4	f	66	4	*C. perfringens*	Anterior and posterior trunk	Chronic pancreatitis	Von Willebrand's disease	Pancreatitis
5	f	30	12	*C. perfringens*	Anterior and posterior trunk	Acute pancreatitis, disseminated intravascular coagulation, fatty liver	Diabetes mellitus	Pancreatitis
6	m	73	Unknown	*C. perfringens*	Right pelvic region	Severe arteriosclerosis, old myocardial infarction, pulmonary emphysema, pulmonary edema, pleural effusions, recent amputation of right thigh	No/ unknown	Arterial occlusive disease with total necrosis of the right leg

cases, but in one case each, the deceased had suffered from von Willebrand's disease, diabetes mellitus, and chronic alcoholism.

Gross pathology

At external examination, although different body regions were predominantly affected (Table 1), in all cases, the most prominent finding was a violaceous to magenta-bronze discoloration of the skin with hemorrhagic bullae formation (Fig. 1). The skin was taut in the affected regions. An emphysema of the subcutaneous tissue layers overlying the affected muscles was palpable, and the adipose tissue had a hemorrhagic, edematous appearance on cut surfaces. Myonecrosis appeared as a brownish smutty discoloration and soft consistency of the affected musculature with palpable crepitation.

Pathological findings in internal organs included a soft, spongy consistency of the parenchyma of liver, kidneys, and spleen, as well as edema and acute congestion of the lung parenchyma that had mostly a foamy appearance. On cut sections through the liver, the parenchyma appeared brownish-violaceous with numerous cystic cavity formations up to 1.4 cm in diameter, giving the cut surface a "Swiss-cheese appearance." On cut sections through the kidneys, the usually clear boundary between medulla and cortex was not detectable in any of the cases. Gross examination of the vascular system was unremarkable in all cases, and no thromboses were found.

Except for the presence of gas gangrene, arteriosclerosis as well as acute and chronic pancreatitis were the most frequent preexisting pathological conditions.

Histopathology, immunohistochemistry

The affected skeletal musculature showed separation of myofibers by abundant Gram-positive, rod-shaped bacteria but no

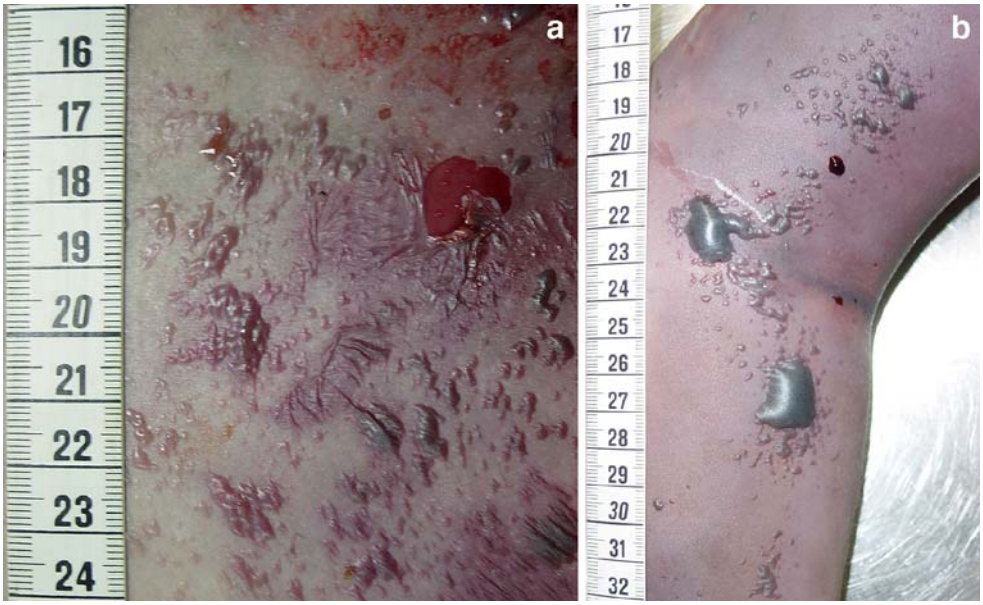

Fig. 1 Close-up view of skin lesions in clostridial gas gangrene. **a** Multiple, partly confluent hemorrhagic bullae and superficial desquamation of the skin, **b** tense bullae filled with serosanguinous fluid on one arm

significant adjacent inflammatory reaction (Fig. 2). In the brain, liver, and kidneys, numerous empty cystic spaces that were seen in at least a few visual fields were the predominant histopathological finding in all cases. These cystic spaces were frequently lined by abundant Gram-positive, rod-shaped bacteria corresponding to clostridiae (Fig. 3).

Microscopical features also included round to oval empty spaces corresponding to air bubbles that were predominantly seen in branches of the portal vein of all sizes (Fig. 4). In addition, in the liver, phagocytosed rod-shaped bacteria were found in Kupffer cells, but only a few single cell necroses of hepatocytes and a minimal inflammatory cell reaction in the form of an occasional sparse infiltration with CD15 immunopositive neutrophil granulocytes in the portal tracts could be detected.

In the lungs, accumulation of Gram-positive, rod-shaped bacteria within the alveolar capillaries (Fig. 5) was a frequent finding. Occasionally, accumulation of neutrophils as evidenced by conventional histology as well as anti-CD15 labeling was seen in medium-sized and small pulmonary vessels in a limited number of visual fields. Immunohistochemistry was unremarkable for the amount of macrophages in the immediate vicinity of the empty cystic spaces and aggregations of clostridiae within the different internal organs.

The myocardium was free of bacteria in all cases and was not affected by an inflammatory reaction in any of the cases.

In the brain, Gram-positive, rod-shaped bacteria were seen occasionally in the leptomeninges in four cases, but no adjacent inflammatory reaction could be observed here with immunohistochemistry being unremarkable for the amount

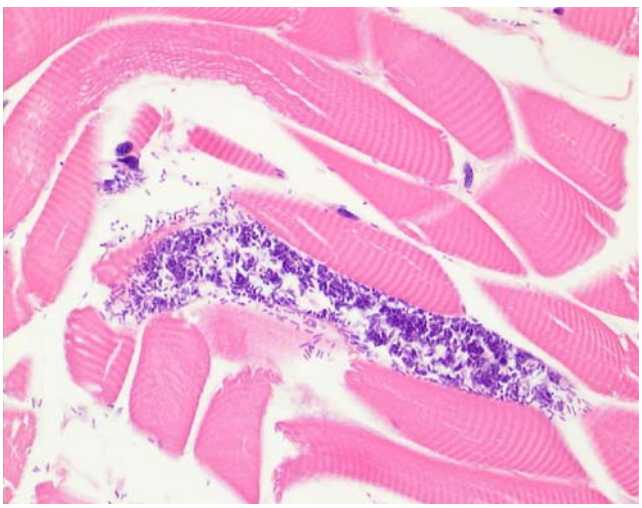

Fig. 2 Clostridial myonecrosis. Infiltration of affected skeletal musculature by rod-shaped bacteria (H&E ×400)

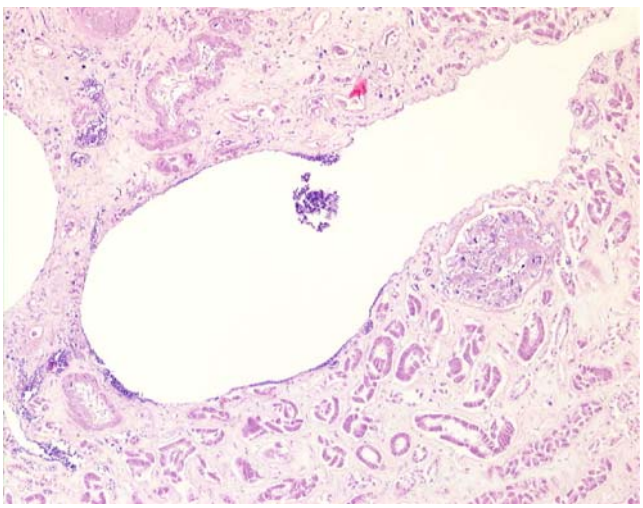

Fig. 3 Section of kidney in clostridial gas gangrene showing Gram-positive, rod-shaped bacteria lining a cystic space (Gram's stain ×50)

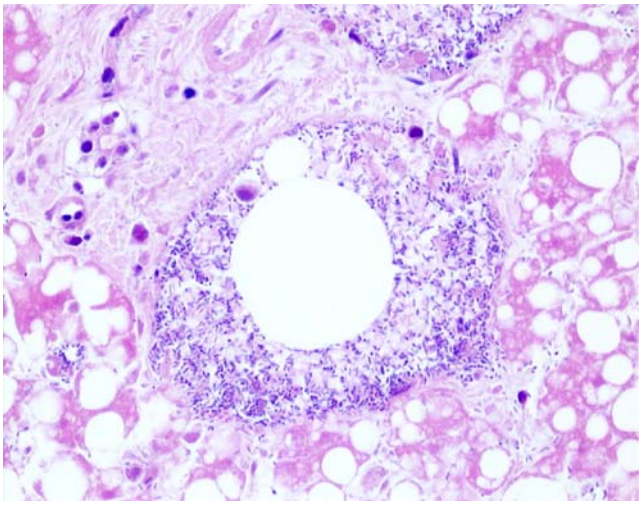

Fig. 4 *Round empty spaces* corresponding to air bubbles surrounded by abundant rod-shaped bacteria and blood cells within a portal venule in the portal tract of the liver. Accompanying fatty change of hepatocytes was unrelated to gas gangrene (Gram's stain ×100)

of neutrophil granulocytes and macrophages in the leptomeninges. In these, four cases where bacteria were seen within the leptomeninges, in addition to empty cystic spaces, aggregations of Gram-positive, rod-shaped bacteria within the white matter of the brain were another frequent finding. These aggregates of bacteria were located within and adjacent to small-sized vessels and capillaries in the white matter of the brain (Fig. 6). Intravascular accumulation of neutrophils as evidenced by histology and immunohistochemistry was a frequent finding in smaller cerebral vessels and capillaries, but no perivascular or extravascular leukostasis could be detected.

As evidenced by immunohistochemistry, Hb immunopositivity was almost exclusively restricted to intravascular erythrocytes (Fig. 7), and the observed degree of hemolysis

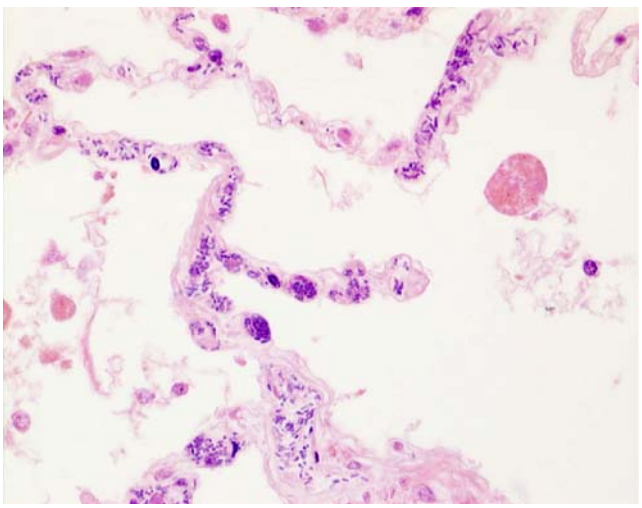

Fig. 5 Accumulation of rod-shaped bacteria within the alveolar capillaries. Note total absence of bacteria within the alveolar spaces (H&E ×400)

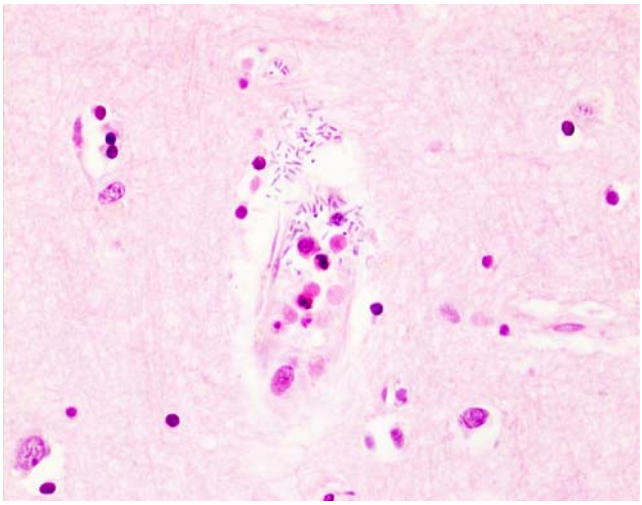

Fig. 6 Accumulation of bacteria located within and adjacent to a capillary in the white matter of the brain (H&E ×600)

did not exceed the average extent as usually seen postmortem as a result of autolysis in any of the cases.

Microthromboses were not detectable in any of the cases.

Scanning electron microscopy

Scanning electron microscopy confirmed the results obtained by routine histopathology by revealing numerous empty cystic spaces within the parenchyma of the inner organs (Fig. 8). No significant inflammatory cell reaction was observed adjacent to these cystic lesions, but numerous clostridiae, appearing as rod-shaped bacilli with blunt ends, were frequently detected in the marginal zones of the cystic lesions within the parenchymatous organs and the brain as well as adjacent to myofibers in the skeletal musculature.

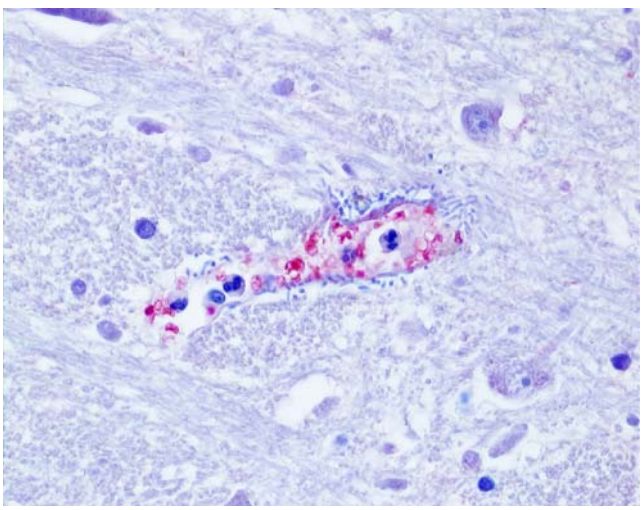

Fig. 7 Representative view of Hb immunopositivity restricted to intravascular erythrocytes in clostridial gas gangrene as seen in a capillary within the white matter of the brain (Hb immunohistochemistry ×600)

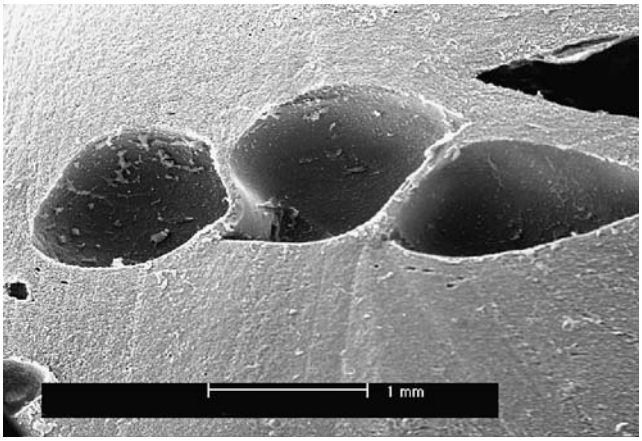

Fig. 8 Scanning electron microscopic appearance of empty cystic spaces within the white matter of the brain

Discussion

The most comprehensive information on the pathological features of gas gangrene is derived from a paucity of autopsy cases and a few animal studies, thus leading to a primarily anecdotal rather than systematic approach towards the pathology of the disease. To better describe the pathological features of this rare disease, we conducted the present autopsy-based study using microbiology, histology, immunohistochemistry, and scanning electron microscopy.

The clinical course of gas gangrene is characterized by a rapid onset, aggressive progression, multiple organ failure, and high mortality rate [1, 10, 18, 24, 27]. Left untreated, the disease is almost always fatal, with death often occurring within hours after the onset of the first symptoms [1, 5, 8, 14, 22]. In the present series, the time span between onset of the disease and death ranged between 4 and 20 h in four cases and was unknown in the remaining two cases. Therefore, these cases have to be handled as sudden and unexpected deaths with all medicolegal implications associated with such cases [9, 12, 17, 26]. Interestingly, in our cases with *C. perfringens* infection, the progression of nontraumatic gas gangrene seems to have been more fulminant with an average survival time of 8 h when compared to the traumatic gas gangrene cases with an average survival time of 15 h. Nonetheless, we are well aware that the case numbers of the present study are far too small to draw any general conclusions. However, until now, this more severe course of infection has only been described for *C. septicum* in association with nontraumatic gas gangrene [23].

Although gas gangrene is a necrotizing infection that is considered to predominantly affect the skeletal musculature (clostridial *myonecrosis*) and soft tissue, in the present series, we were able to document pathological findings attributable to the disease process in almost every internal organ. In contrast to infections caused by bacteria such as *Staphylococcus aureus*, *Haemophilus influenza*, or *Streptococcus pneumoniae* that show minimal tissue destruction and an intense leukocyte response at the site of infection, it is now widely accepted that gas gangrene is characterized by a total absence of leukocyte infiltration and tissue inflammatory response [2, 4, 22] as verified by the present findings on both the micromorphological and ultrastructural level. Despite the application of Hb immunohistochemistry, we were unable to demonstrate any hemolytic effects related to human clostridial infection as reported earlier from experimental animal studies [22].

Several aspects provide evidence that infection with clostridiae was acquired *intra vitam* in the cases studied here. The fact that postmortem microbiological cultures yielded a single infectious pathogen in five cases (*C. perfringens* in four cases, *C. sordellii* in one case) and a mixed infection with two different clostridial species (*C. perfringens* and C. *sordellii*) in a sixth case proves, in combination with the deceased's individual previous history, symptoms, and clinical course before death, the existence of antemortem clostridial infection [16]. In addition, phagocytosed clostridiae were a frequent finding in Kupffer cells in the liver, and this finding can be considered as a vital reaction likewise verifying infection before death.

The nontraumatic form of the disease, as observed here in three cases, is very rare accounting for approximately 10% of all reported cases in both adults and children [20]. Clostridiae are bowel commensals and also known to colonize the biliary tree [11, 15]. The large bowel is probably the site of entry for nontraumatic ("spontaneous") clostridial infections where the clostridiae gain access to the circulation via mucosal ulcerations related to or in association with these aforementioned pathological conditions. This type of gas gangrene has a strong association with severe underlying diseases and colorectal or hematological malignancies [1, 7, 10, 13, 18]. In addition, nontraumatic gas gangrene has also been described to occur in association with a variety of more or less debilitating disorders such as pancreatitis, cholezystitis, perforated duodenal ulcer, liver cirrhosis, diabetes mellitus, and alcohol abuse [1, 8, 11, 14, 18, 24]. Here, nontraumatic gas gangrene originated from acute pancreatitis in two cases, and the disease was associated with von Willebrand's disease, diabetes mellitus, and chronic alcoholism in one case each.

Conclusions

Although rare, gas gangrene is not a disease of the past. The fact that clostridial gas gangrene may develop in the absence of trauma is less well recognized. In medicolegal casework, the proof of the source of infection and the portal of entry of

the responsible pathogen is not always an easy task, especially in the absence of trauma, and even sophisticated diagnostic methods such as immunohistochemistry and scanning electron microscopy seem to be of little or no value in the elucidation of the route of infection. However, performing the autopsy as early as possible and a close cooperation with the field of clinical microbiology is, in addition to the careful analysis of individual case characteristics, a prerequisite for a conclusive medicolegal opinion. Because of the rapid, fulminant course of the disease with death often occurring within hours after the onset of first symptoms, most fatalities because of clostridial gas gangrene will have to be regarded as unavoidable from the ex post viewpoint. Also termed "myonecrosis," clostridial gas gangrene is also capable of causing a number of typical pathological changes in a variety of other organ systems such as a characteristic violaceous to magenta-bronze discoloration of the skin with hemorrhagic bullae formation and a soft and spongy consistency of the parenchyma of liver, kidneys, and spleen at autopsy. Moreover, empty cystic spaces lined by numerous clostridiae in the parenchymatous organs and the brain in addition to accumulation of clostridiae within the alveolar capillaries as well as phagocytosed clostridiae in Kupffer cells in the liver are typical histopathological features. Characterized by an extensive tissue necrosis and an absence of accompanying leukocyte infiltration and tissue inflammatory response, the histopathological picture of clostridial gas gangrene is distinctly different from other bacterial infections.

References

1. Abella BS, Kuchinic P, Hiraoka T, Howes DS (2003) Atraumatic clostridial myonecrosis: case report and literature review. J Emerg Med 24:401–405
2. Awad MM, Ellemor DM, Boyd RL, Emmins JJ, Rood JI (2001) Synergistic effects of alpha-toxin and perfringolysin O in Clostridium perfringens-mediated gas gangrene. Infect Immun 69:7904–7910
3. Bryant AE (2003) Biology and pathogenesis of thrombosis and procoagulant activity in invasive infections caused by group A streptococci and *Clostridium perfringens*. Clin Microbiol Rev 16:451–462
4. Bryant AE, Bayer CR, Aldape MJ, Wallace RJ, Titball RW, Stevens DL (2006) *Clostridium perfringens* phospholipase C-induced platelet/leukocyte interactions impede neutrophil diapedesis. J Med Microbiol 55:495–504
5. Burke MP, Opeskin K (1999) Nontraumatic clostridial myonecrosis. Am J Forensic Med Pathol 20:158–162
6. Caplan ES, Kluge RM (1976) Gas gangrene: review of 34 cases. Arch Intern Med 136:788–791
7. Chetta SG, Weber MJ, Nelson CL (1982) Non-traumatic clostridial myonecrosis: report of two cases. J Bone Joint Surg Am 64:456–457
8. Delbridge MS, Turton EPL, Kester RC (2005) Spontaneous fulminant gas gangrene. Emerg Med J 22:520–521
9. Fineschi V, Riezzo I, Centini F et al (2007) Sudden cardiac death during anabolic steroid abuse: morphologic and toxicologic findings in two fatal cases of bodybuilders. Int J Legal Med 121:48–53
10. Hausmann R, Albert F, Geißdörfer W, Betz P (2004) Clostridium fallax associated with sudden death in a 16-year-old boy. J Med Microbiol 53:581–583
11. Ikegami T, Kido A, Shimokawa H, Ishida T (2004) Primary gas gangrene of the pancreas: report of a case. Surg Today 34:80–81
12. Karger B, Fechner G (2006) Sudden death due to giant cell coronary arteritis. Int J Legal Med 120:377–379
13. Keogh G, Unsworth I, Vowels M, Kern IB (1994) Spontaneous *Clostridium septicum* myonecrosis in congenital neutropenia. Aust N Z J Surg 64:574–575
14. Leung FW, Serota AI, Mulligan ME, George WL, Finegold SM (1981) Nontraumatic clostridial myonecrosis: an infectious disease emergency. Ann Emerg Med 10:312–314
15. Levy P, Boudet MJ, Perniceni T et al (1999) Spontaneous gas gangrene of the pancreas caused by *Clostridium perfringrens*. Gastroenterol Clin Biol 23:1248–1250
16. Roberts FJ (1998) Procurement, interpretation, and value of postmortem cultures. Eur J Clin Microbiol Infect Dis 17:821–827
17. Sakai K, Takatsu A, Shigeta A, Abe S, Ikegami M, Takagi K (2007) Sudden death due to undiagnosed acute promyelocytic leukemia: a case report. Int J Legal Med (in press) DOI:10.007/s00414-006-0106-x
18. Sasaki T, Nanjo H, Takahashi M, Sugiyama T, Ono I, Masuda H (2000) Non-traumatic gas gangrene in the abdomen: report of six autopsy cases. Gastroenterology 35:382–390
19. Siemann M, Koch-Dörfler M, Rabenhorst G (2000) *Clostridium difficile*-associated diseases. The clinical courses of 18 fatal cases. Intensive Care Med 26:416–421
20. Smith-Slatas CL, Bourque M, Salazar JC (2006) *Clostridium septicum* infections in children : a case report and review of the literature. Pediatrics 117:e796–e805
21. Soper DE (1986) Clostridial myonecrosis arising from an episiotomy. Obstet Gynecol 68(3 Suppl):26S–28S
22. Stevens DL, Bryant AE (2002) The role of clostridial toxins in the pathogenesis of gas gangrene. Clin Infect Dis 35(Suppl 1):S93–S100
23. Stevens DL, Musher DM, Watson DA et al (1990) Spontaneous, nontraumatic gangrene due to *Clostridium septicum*. Rev Infect Dis 12:286–296
24. Takahira N, Shindo M, Tanaka K, Soma K, Ohwada T, Itoman M (2002) Treatment outcome of nonclostridial gas gangrene at a level 1 trauma center. J Orthop Trauma 16:12–17
25. Thys JP, Ectors P, Noel P (1980) Non-traumatic clostridial myositis: an unusual feature of brain death. Postgrad Med J 56:501–503
26. Tsokos M, Herbst H (2005) Black oesophagus: a rare disorder with potentially fatal outcome. A forensic pathological approach based on five autopsy cases. Int J Legal Med 119:146–152
27. Valentine EG (1997) Nontraumatic gas gangrene. Ann Emerg Med 30:109–111