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Clinical Characteristics, Histopathology, and Tissue Immunolocalization of Chikungunya Virus Antigen in Fatal Cases

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Abstract

Background.—Death in patients with chikungunya is rare and has been associated with encephalitis, hemorrhage, and septic shock. We describe clinical, histologic, and immunohistochemical findings in individuals who died following chikungunya virus (CHIKV) infection.

Methods.—We identified individuals who died in Puerto Rico during 2014 following an acute illness and had CHIKV RNA detected by reverse transcriptase–polymerase chain reaction in a pre- or postmortem blood or tissue specimen. We performed histopathology and immunohistochemistry (IHC) for CHIKV antigen on tissue specimens and collected medical data via record review and family interviews.

Results.—Thirty CHIKV-infected fatal cases were identified (0.8/100 000 population). The median age was 61 years (range: 6 days–86 years), and 19 (63%) were male. Death occurred a

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median of 4 days (range: 1–29) after illness onset. Nearly all (93%) had at least 1 comorbidity, most frequently hypertension, diabetes, or obesity. Nine had severe comorbidities (eg, chronic heart or kidney disease, sickle cell anemia) or coinfection (eg, leptospirosis). Among 24 fatal cases with tissue specimens, 11 (46%) were positive by IHC. CHIKV antigen was most frequently detected in mesenchymal tissues and mononuclear cells including tissue macrophages, blood mononuclear cells, splenic follicular dendritic cells, and Kupffer cells. Common histopathologic findings were intra-alveolar hemorrhage and edema in the lung, chronic or acute tenosynovitis, and increased immunoblasts in the spleen. CHIKV infection likely caused fatal septic shock in 2 patients.

Conclusions.—Evaluation of tissue specimens provided insights into the pathogenesis of CHIKV, which may rarely result in septic shock and other severe manifestations.

Keywords

chikungunya; Puerto Rico; fatal; pathology

Chikungunya virus (CHIKV) is an emerging pathogen that is primarily transmitted by *Aedes* species mosquitos and causes an acute febrile illness (AFI) characterized by severe arthralgia [1]. In 2006, an explosive CHIKV outbreak occurred on Reunion Island during which approximately 38% of the population was infected [2], and additional outbreaks followed in South Asia [1]. In 2013, CHIKV transmission was first identified in the Caribbean and soon after throughout the Americas [1, 3]. In Puerto Rico, local CHIKV transmission was reported in May 2014, and more than 28 000 suspected cases were reported in the following year [4]. In late 2014, screening of blood donors for anti-CHIKV immunoglobulin G (IgG) antibody demonstrated that 24% had been infected with CHIKV [5].

Over 244 000 chikungunya cases were reported during the 2006 outbreak in Reunion Island [6]. Among 610 patients with atypical manifestations of CHIKV infection (ie, multiple organ failure, hepatitis, meningoencephalitis, hemorrhage, encephalopathy) the case-fatality rate was 10.7% [7]. Other severe manifestations associated with CHIKV infection include Guillain-Barré syndrome and vesiculobullous skin lesions [8-11], the latter being common among newborns exposed through perinatal transmission [12, 13]. Multiple jurisdictions in the Pacific and Americas also reported septic shock in association with CHIKV infection [14-18] and excess deaths during outbreaks [19-22]; however, the potential contribution of comorbidities and coinfection to these severe phenotypes is difficult to rule out, particularly in the absence of tissue specimens to detect CHIKV infection and evaluate histopathologic changes.

The purpose of this investigation was to describe the demographic characteristics, medical history, clinical course, and postmortem findings of individuals who died following CHIKV infection. We also describe the clinicopathologic and immunolocalization of viral antigen in tissue specimens to better understand the pathophysiology of CHIKV and evaluate evidence for CHIKV as a cause of septic shock.

METHODS

Ethics Statement

This project underwent ethical and regulatory review in accordance with institutional policies and was determined to be outside the scope of institutional review board review requirements. Because cases were reported in the context of public health surveillance, informed consent from the decedents' families was not sought. Patient identifiers were removed from the dataset prior to analysis.

Case Detection and Specimen Collection

Since 2010, the US Centers for Disease Control and Prevention (CDC), Puerto Rico Department of Health, and the Puerto Rico Institute of Forensic Sciences (PRIFS) have operated the Enhanced Fatal Acute Febrile Illness Surveillance System (EFASS) [23]. The EFASS identifies patients who died following an AFI either through reporting to the Passive Arboviral Diseases Surveillance System (PADSS) or during autopsy conducted at PRIFS. Pathologists at PRIFS autopsy individuals who died following AFI, collect specimens from major organs and other tissues where atypical clinical findings were observed, and send them to CDC for diagnostic testing. Cases identified by EFASS were queried in the PADSS database to determine if premortem specimens had been submitted during the same illness from which the patient died, and if so, cases were consolidated.

Diagnostic Testing

Starting in March 2014, nucleic acid for diagnostic testing by reverse transcriptase–polymerase chain reaction (RT-PCR) was extracted from serum, plasma, or a combined formalin-fixed paraffin-embedded (FFPE) tissue block containing spleen, liver, kidney, and lung [24, 25]. The FFPE specimens were sectioned and stained with hematoxylin and eosin for evaluation. Additional assays for other infectious agents (eg, special stains, immunohistochemistry [IHC], molecular assays) were performed according to histopathologic findings. Immunohistochemistry utilized a polymer-based colorimetric indirect immunoalkaline phosphatase method on FFPE specimens [26]. Antibodies included a mouse polyclonal antibody raised against CHIKV (provided by Michel Huerre, Pasteur Institute) diluted 1:1000, and a mouse hyperimmune ascetic fluid raised in-house against CHIKV IND023574 and diluted 1:200. Both required pretreatment with proteinase K. CHIKV-infected Vero E6 cells embedded into paraffin blocks and normal mouse sera in place of primary antibodies were used as positive and negative controls, respectively. In sections with immunoreactivity, the overall amount of antigen detected and the cell types with immunostaining were recorded.

Data Collection and Analysis

A standardized chart abstraction form was used to collect demographic data, laboratory test results, clinical and discharge diagnoses (from medical records and death certificates), and other relevant clinical information from medical records and autopsy reports. Variables not documented in the medical record were considered to be absent. For cases in which no or incomplete clinical details were available (eg, individuals who died outside of the healthcare

system), the decedent's next of kin was contacted and queried regarding the decedent's recent medical history, underlying illnesses, medications, and behaviors including illicit drug use.

Modified surveillance case definitions for sepsis and septic shock were used [27]. Among patients in whom CHIKV antigen was detected by IHC in at least 1 tissue specimen and who had no evidence of acute infection with another pathogen, sepsis was defined by the following findings made within 1 week of death: (1) temperature of 38°C or higher; (2) leukocytosis (white blood cell [WBC] count >12 000 cells/mL^3) or leukopenia (WBC count <4000 cells/mL^3); and (3) acute organ dysfunction as evidenced by (a) mechanical ventilation, (b) serum bilirubin ≥2.0 mg/dL, (c) serum creatinine ≥2.0 mg/dL, or (d) thrombocytopenia (<100 000 cells/mL^3). Septic shock was defined by the above criteria for sepsis in a patient who received vasopressor therapy.

RESULTS

Identification of Fatal Cases With CHIKV Infection

Among 58 fatal cases of AFI identified by EFASS in 2014, CHIKV nucleic acid was detected by RT-PCR in a pre- or postmortem blood or tissue specimen from 30 cases (52%; 0.8 cases per 100 000 population) (Figure 1). At least 1 blood specimen was available for all 30 cases, of which 20 (67%) were positive by RT-PCR, including 5 that only tested positive in a premortem specimen. Autopsy was performed and tissue was available for 24 cases, of which 18 (75%) were positive by RT-PCR and 11 (46%) were also positive by IHC. Most (63%) fatal cases with CHIKV infection were male, and the median age was 61 years (range, 6 days–86 years).

Among 27 fatal cases with CHIKV infection for whom medical information was available, nearly all (93%) had an identified comorbid condition, most frequently hypertension, diabetes, and obesity (Table 1). Three (10%) cases had only 1 comorbid condition and 7 (23%) cases had a severe comorbid condition (eg, coronary artery disease, chronic kidney disease).

Clinical Characteristics

Among 27 fatal cases with CHIKV infection for whom medical information was available, the median time from illness onset to death was 4 days (range, 1–29 days) (Table 2) and was similar among cases who only tested positive by RT-PCR in a premortem blood specimen (n = 5; median, 4 days; range, 2–29 days) and those who tested positive in a postmortem specimen (n = 22; median, 5 days; range, 1–8 days). Ten (37%) fatal cases with CHIKV infection died at home, as 4 (15%) had not sought medical care and 9 (33%) were evaluated as outpatients only. Among 14 (52%) fatal cases who were admitted to the hospital, the median time from hospitalization to death was 2 days (range, 0–23 days); 9 were admitted to the intensive care unit.

The most common signs and symptoms were fever, arthralgia, lethargy, myalgia, and rash (Table 2). The most common clinical complications were dyspnea, cyanosis, edema, hematemesis, and clinically diagnosed disseminated intravascular coagulopathy (DIC),

encephalitis, and myocardial infarct. The most common discharge diagnoses were diabetes/diabetic ketoacidosis, viral syndrome, respiratory failure, sepsis or septic shock, DIC, encephalitis, and myocardial infarct. Among 25 cases for whom at least 1 blood count was available, 11 (44%) had thrombocytopenia and 16 (64%) had leukocytosis. Cerebrospinal fluid from 2 cases had pleocytosis with predominance of lymphocytes. In a separate case, blood culture was positive for growth of *Staphylococcus* species bacteria.

Evidence of Septic Shock and Clinical Cases

Among 11 fatal cases for whom CHIKV antigen was detected in at least 1 tissue specimen, 3 case-patients met the case definitions for sepsis. Two cases (#2 and #6) also met the case definition for septic shock; abundant CHIKV antigen was detected in multiple tissues from both.

Noteworthy cases include a 9-year-old female with no identified comorbid conditions who died at home 2 days after illness onset due to apparent septic shock with DIC; CHIKV RNA was detected in serum by RT-PCR, and tissue specimens were negative by RT-PCR and IHC. Second, CHIKV RNA and antigen were detected in a rib specimen from a 21-year-old, 38-week pregnant female with sickle cell disease and asthma (Case #10); neither CHIKV RNA nor antigen were detected in fetal serum or tissue. Last, nucleic acid of CHIKV and *Leptospira* spp. bacteria were detected in a plasma specimen from a 54-year-old male who died from head trauma suffered after falling at home during an AFI with rash and arthralgia.

Autopsy and Histopathologic Findings

Among 11 cases for which CHIKV antigen was detected by IHC, common gross autopsy findings were pleural effusions and petechia/ecchymosis on extremities (Table 3). Although no pathognomonic histologic findings were observed, changes were observed consistent with viral syndrome, including both systemic and localized inflammation and immune stimulation (Table 4). Common microscopic findings included pulmonary congestion, pulmonary edema, intra-alveolar hemorrhage, increased number of immunoblasts in the spleen, and changes consistent with chronic comorbidities.

In hand-tendon specimens, predominantly perivascular, minimal-to-mild mononuclear cell inflammatory infiltrates were observed in the connective tissue surrounding the tendon and within the synovial sheath; 2 patients with this finding had reported hand pain. In addition, tendon from 1 case with edema of the extremities (including the hand) had acute inflammation characterized by neutrophilic inflammation and expansion of surrounding connective tissue by fibrin, edema, neutrophilic and macrophage infiltrates, and vascular congestion.

Among 7 cases with perivascular to interstitial mononuclear cell infiltrates observed in the skin (dermis), 4 (57%) had congestion/extravasation, of whom 3 (75%) had a clinical history of rash and 2 (50%) had dermal petechia or ecchymosis noted during autopsy. Increased numbers of leukocytes within vascular channels were observed in the lung (Figure 2A and 2B), liver, and heart. Common findings in the bone marrow were left shift and hypercellularity; hemophagocytosis was observed in 2 cases.

Immunolocalization of CHIKV Antigen

CHIKV antigen was observed in a wide range of tissues including the spleen, skin, tendon, bone, and skeletal muscle (Tables 4, 5, and 6). Antigen was frequently detected in vessels and the cytoplasm of monocytes, macrophages, and cells of mesenchymal origin including fibroblasts, vascular endothelium, muscle, and adipose tissue.

In the lung, CHIKV antigen was detected surrounding or within vessels (Figure 2C) and vascular endothelium in the alveolar septa (Figure 2D) and in alveolar and septal monocytes (Figure 2E). In the liver, CHIKV antigen was detected in vessels, Kupffer cells, vascular lumens, capsule, sinusoidal lining cells, and portal connective tissue (Figure 2F). In the heart, CHIKV antigen was detected in fibroblasts and interstitial connective tissue (Figure 2G), vessels or vascular endothelium, epicardial adipose tissue, surrounding nerves, and within vascular lumens (Figure 2F).

CHIKV antigen was also observed within vessels of the spleen, liver, heart (Figure 2G), brain, thyroid gland, adrenal gland, and gastrointestinal tract, within aggregates of discrete eosinophilic material consistent with platelet aggregates (Figure 2C and 2G) or within circulating mononuclear cells.

In the spleen, CHIKV antigen was observed in white pulp mononuclear cells consistent with follicular dendritic cells (Figure 3A) and in red pulp macrophages (Figure 3B). In the skin, CHIKV antigen was observed in interstitial connective tissue, including the subcutis/hypodermis and surrounding or within adnexal structures and nerve (Figure 3C), within the cytoplasm of cells consistent with fibroblasts and cells of monocyte/macrophage lineage, and within vessels and vascular endothelium. In hand tendon, CHIKV antigen was frequently observed in fibroblasts and surrounding connective tissue, surrounding and within nerves, and in vessels and vascular endothelium. In the aforementioned case-patient with acute inflammation in the tendon, CHIKV antigen was observed within the cytoplasm of infiltrating macrophages. In bone and bone marrow from the rib, CHIKV antigen was observed in the periosteum (Figure 3D), osteonal endosteum (Figure 3E), and within the bone marrow in mononuclear cells and megakaryocytes (Figure 3D and 3F).

DISCUSSION

By conducting enhanced surveillance for fatal AFI in Puerto Rico, we identified 30 individuals who died following CHIKV infection. Systematic review of available medical records and interview with the decedents' families enabled description of the clinical course, while autopsy enabled elucidation of postmortem findings. CHIKV antigen was detected in multiple tissues of 11 individuals, most frequently in mesenchymal tissues and cells of the mononuclear phagocytic system. Two patients had a clinical course consistent with septic shock with CHIKV antigen detected in multiple tissues. For the remaining cases, although death was temporally associated with CHIKV infection, the role of CHIKV in the patients' fatal outcome could not be confidently assessed.

In this investigation, CHIKV antigen was identified in multiple tissues, including skin, bone, muscle, spleen, liver, kidney, lung, and heart, with major tropism for mesenchymal

cells such as fibroblasts, adipocytes, and endothelial cells, and mononuclear cells including tissue macrophages, blood monocytes, splenic follicular dendritic cells, and Kupffer cells. Although overt cytopathic effects were not observed, CHIKV-induced changes to endothelial cell function may be involved in the pathogenesis of pulmonary edema, pleural effusions, and peripheral edema.

Detection of CHIKV antigen in circulating blood monocytes supports their role in systemic dissemination of CHIKV [28]. Study of CHIKV-infected nonhuman primates suggested that CHIKV replicates in skin fibroblasts; disseminates hematogenously to organs including the liver, kidneys, spleen, lymph nodes, muscle, and joints; and infects epithelial and mesenchymal cells [29, 30]. Detection of CHIKV in multiple, diverse tissues in this investigation suggests a similar tropism and pathology of CHIKV in humans and nonhuman primates.

Arthralgia, myalgia, and bone pain are common symptoms of chikungunya [1]. In this report, abundant CHIKV antigen was observed in fibroblasts and connective tissue, mononuclear cells, vascular endothelium, and within the periosteum and osteonal endosteum in the bone. While histologic abnormalities, including inflammation and myocyte necrosis, were observed in hand tendon and skeletal muscle, destructive arthritis or active bone remodeling was not. The detection of CHIKV antigen in megakaryocytes of the bone marrow was unexpected. Infection of megakaryocytes by other viruses has been proposed to result in apoptosis and consequent decreased expression of receptors to trigger thrombopoiesis [31], which could explain why some individuals experience thrombocytopenia following CHIKV infection [1, 32]. CHIKV antigen was also abundant in the hand tendon, muscle, and bone, suggesting that, in addition to cell-mediated damage, inflammatory mediators and cellular apoptosis may be the origin of joint, muscle, and bone pain.

CHIKV antigen was detected in 8 of 9 skin specimens collected from cases with and without rash. In cases with rash, no significant histologic changes were identified; however, tissue sampling was not standardized and did not specifically target tissue with lesions. CHIKV antigen was detected primarily in fibroblasts, mononuclear cells, and vascular endothelium. CHIKV antigen was frequently noted in stromal cells within and surrounding nerves and may be involved in the pathogenesis of paresthesia or pruritus. Extensive apoptosis of fibroblasts has been reported in patients with persistent chronic arthralgia and is proposed to contribute to connective tissue damage and clinical disease [33]. Contrary to a previous report in which CHIKV antigen was limited to muscle satellite cells and not mature myofibers [34], we identified antigen within myofibers in 1 case-patient. This patient had extensive antigen throughout multiple tissues and no concurrent histologic skeletal muscle changes.

Whereas chikungunya was reported at approximately equivalent rates among all age groups in Puerto Rico in 2014 [4], nearly all (93%) individuals in the present series of fatal cases were adult and two-thirds were aged more than 50 years. It is therefore not unexpected that nearly all fatal cases had at least 1 comorbid condition, most frequently obesity, hypertension, and diabetes, consistent with previous associations of comorbid conditions

with increased morbidity in patients infected with CHIKV [14, 35]. Moreover, as diabetes and obesity both increase the risk of sepsis mortality due to impaired immune response [36], this observation is also consistent with reduced immune response to CHIKV infection in aged nonhuman primates, in which infectious CHIKV persists in the spleen, liver, and muscle up to 44 days postinfection [37]. These characteristics and others that were prevalent among fatal cases with CHIKV infection should be further evaluated as potential risk factors associated with poor outcome.

Previous reports suggest that CHIKV may cause septic shock [14-18]. Although bacteria are the archetypal mediators of sepsis, multiple viruses including influenza virus, hantavirus, and dengue virus also cause septic shock [38, 39]. In the present case series, 2 case-patients had a clinical course consistent with septic shock, both of whom had prominent intravascular leukocytosis in multiple organs, with leukocytosis and neutrophil left shift consistent with a leukemoid reaction. Similar observations have been made for patients infected with hantavirus or Middle East respiratory syndrome coronavirus [39, 40]. These observations in combination with the detection of CHIKV antigen in multiple tissues and absence of another identified pathogen are suggestive of CHIKV as an etiologic agent of septic shock.

Although we exhausted all avenues to describe infected individuals' medical history and clinical course, the clinical findings herein may be incomplete, particularly for individuals who died outside of the healthcare system or did not have an autopsy. An additional limitation of this investigation is that testing was not available to detect replicating viral RNA in human tissue specimens, which is necessary to conclusively identify the cell types in which CHIKV replicates. Last, due to underreporting and potential lack of clinical suspicion for CHIKV infection in patients presenting with septic shock or other severe manifestations, some fatal cases associated with CHIKV infection may have been missed. Consequently, the true incidence of fatal outcome following CHIKV infection was likely underestimated [22].

In summary, inflammation of the hand tendon, pulmonary edema, and splenic immunoblasts was present in many fatal cases with CHIKV infection. Due to widespread detection of viral antigen, histopathologic findings, and lack of other etiologic agents identified, CHIKV infection was assessed to be the cause of death in 2 individuals who died from septic shock. In 9 other individuals, CHIKV infection may have contributed to death as evidenced by the detection of CHIKV antigen in multiple tissue specimens. In several individuals, CHIKV antigen was abundant in multiple tissues, including cells of mesenchymal origin, circulating monocytes, and tissue macrophages. CHIKV antigen was often present in tissues without associated histologic abnormalities but with correlating clinical manifestations. Clinicians should be aware that CHIKV can rarely result in severe disease and death, including a clinical course consistent with septic shock, and that such outcomes appear to be more common among the elderly and those with comorbid conditions.

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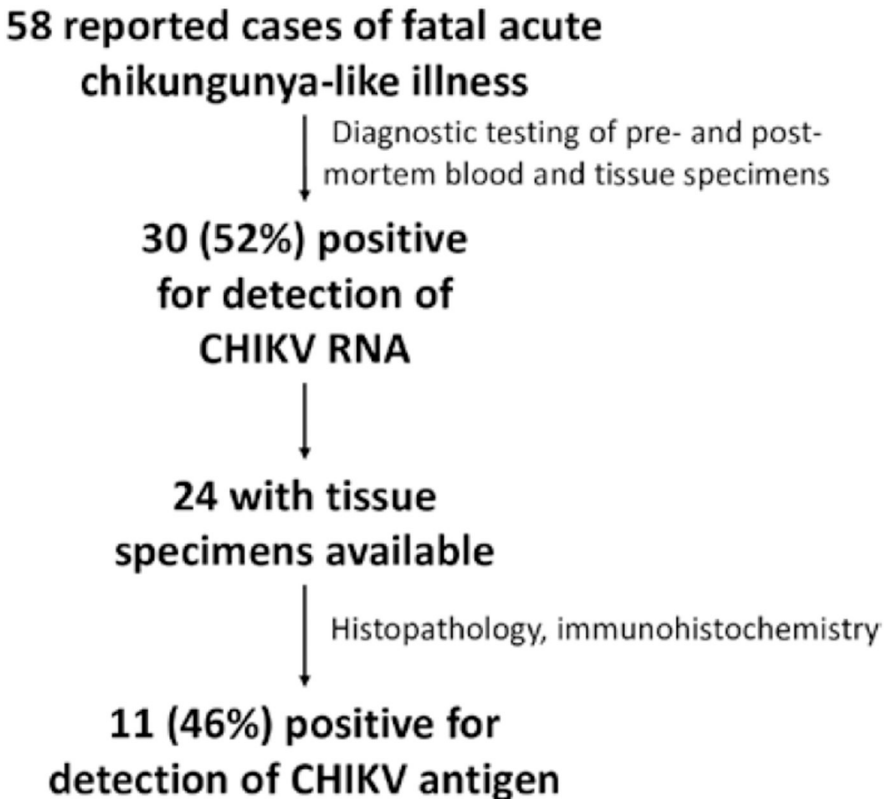

Figure 1.
Flow diagram of identification of individuals who died associated with CHIKV infection: Puerto Rico, 2014. Abbreviation: CHIKV, chikungunya virus.

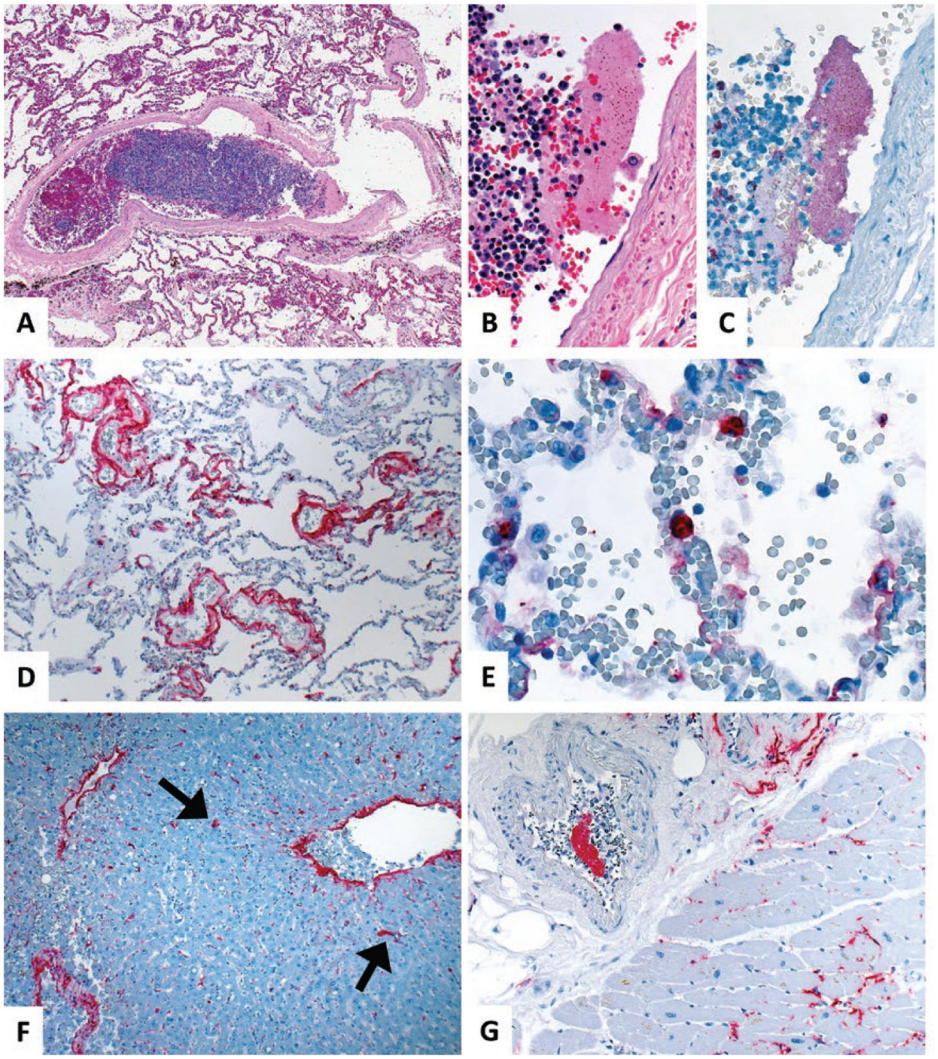

Figure 2.

Histopathologic and immunohistochemical evaluation of tissue specimens collected postmortem from fatal cases with CHIKV infection: Puerto Rico, 2014. Numerous leukocytes within vascular channels of the lung (*A*, *B*; hematoxylin and eosin). Immunochemical detection of CHIKV antigen (red staining) in the lung (*C*–*E*), liver (*F*), and heart (*G*). CHIKV antigen in vascular lumens (*C*), connective tissue of alveolar septa (*D*), and within alveolar and septal monocytes of the lung (*E*). Prominent immunoreactivity within connective tissue of portal and centrilobular regions and within sinusoidal Kupffer cells (arrows) of the liver (*F*). CHIKV antigen within vessels in the heart as well as perivascular and interstitial connective tissue (*G*). Original magnifications: *A*, *D*–*G*, ×10; *B*, *C*, ×63. Abbreviation: CHIKV, chikungunya virus.

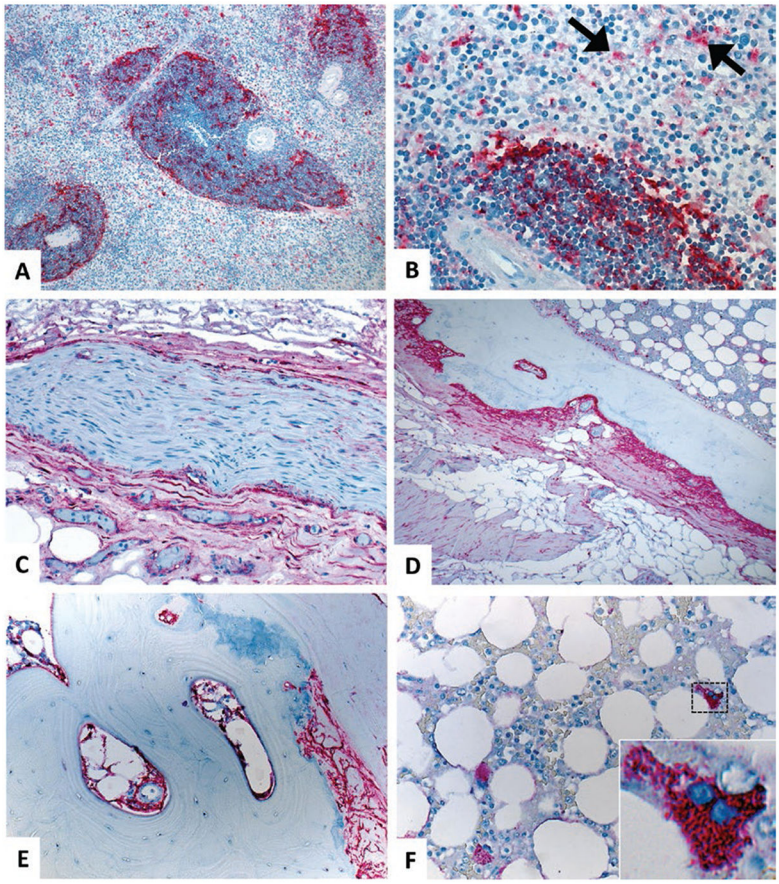

Figure 3.

Immunohistochemical evaluation of tissue specimens collected postmortem from fatal cases with CHIKV infection: Puerto Rico, 2014. Immunohistochemical detection of CHIKV antigen (red staining) in the spleen (*A*, *B*), skin (*C*), and bone and bone marrow (*D*–*F*). CHIKV antigen within mononuclear cells of the white pulp and within red pulp macrophages (arrows) of the spleen (*A*, *B*). CHIKV antigen within connective tissues surrounding a nerve in the skin (*C*) and within the periosteum and surrounding connective tissue (*D*) and osteonal endosteum (*E*) of the bone. CHIKV antigen within megakaryocytes (inset in *F*) of the bone marrow (*D*, *F*). Original magnifications: *A*, *C*, *D*–*F*, ×10; *B*, ×63. Abbreviation: CHIKV, chikungunya virus.

Table 1.

Comorbid Medical Conditions of Fatal Cases With Chikungunya Virus Infection: Puerto Rico, 2014

Condition	CHIKV-Positive Fatal Cases (N = 27), n (%)
Smoking	5 (19)
Alcohol abuse	1 (4)
Intravenous drug use	2 (7)
Pregnancy	1 (4)
Hypertension	16 (59)
Diabetes	14 (52)
Obesity	11 (41)
Asthma	4 (15)
Arthritis	4 (15)
Lupus	2 (7)
Coronary artery disease	5 (19)
Chronic kidney disease	4 (15)
Chronic obstructive pulmonary disease	1 (4)
Hyperlipidemia	3 (11)
Sickle cell disease	1 (4)
Neurologic illness	2 (7)
Mental illness	2 (7)
Dementia	2 (7)
Coinfection	5 (19)
Hepatitis C virus	2 (7)
Leptospira	1 (4)
Human T-cell lymphotrophic virus	1 (4)

Abbreviation: CHIKV, chikungunya virus.

Table 2.

Clinical Course, Signs and Symptoms, and Diagnoses Among Fatal Cases Infected With Chikungunya Virus: Puerto Rico, 2014

	CHIKV-Positive Fatal Cases (N = 27)
Clinical course	
Number of medical visits, median (range)	1 (0–4)
Hospitalized, n (%)	14 (52)
Admitted to the intensive care unit, n (%)	9 (33)
Day of death post–illness onset, median (range)	4 (1–29)
Clinical signs and symptoms, n (%)	
Fever	25 (93)
Lethargy	18 (67)
Rash	15 (56)
Vesiculobullous skin lesions	4 (15)
Headache	11 (41)
Myalgia	16 (59)
Bone pain	11 (41)
Eye pain	4 (15)
Conjunctivitis	5 (19)
Arthralgia	22 (81)
Arthritis	5 (19)
Cough	7 (26)
Dyspnea	8 (30)
Anorexia	12 (44)
Nausea	7 (26)
Vomiting (≥3 times)	9 (33)
Diarrhea	11 (41)
Abdominal pain	8 (30)
Splenomegaly	1 (4)
Petechia	12 (44)
Jaundice	3 (11)
Epistaxis	2 (7)
Bleeding gums	2 (7)
Hematemesis	4 (15)
Hematuria	4 (15)
Melena	2 (7)
Seizures	4 (15)
Clinically diagnosed syndrome	
Cardiac arrhythmias	11 (41)
Cyanosis	8 (30)
Edema	8 (30)
Encephalitis	5 (19)

	CHIKV-Positive Fatal Cases (N = 27)
Disseminated intravascular coagulopathy	4 (15)
Meningoencephalitis	2 (7)
Myocarditis	1 (4)
Discharge diagnoses, n (%)	
Diabetes/diabetic ketoacidosis	16 (59)
Viral syndrome/viral infection	10 (37)
Respiratory failure	8 (30)
Sepsis/septic shock	8 (30)
Disseminated intravascular coagulopathy	4 (15)
Myocardial infarct	4 (15)
Encephalitis	2 (7)

Abbreviation: CHIKV, chikungunya virus.

Table 3.

Gross Autopsy Findings Among Fatal Cases in Whom Chikungunya Virus Antigen Was Detected by Immunohistochemistry: Puerto Rico, 2014

Autopsy Finding	IHC-Positive Fatal Cases (N = 11), n (%)
Pleural effusions	8 (73)
Unilateral	2
Bilateral	6
Petechia/ecchymosis	5 (45)
Abdominal effusions[a]	2 (18)
Splenomegaly	2 (18)
Gastrointestinal hemorrhage or ulceration	2 (18)
Cardiomegaly and atherosclerosis	1 (9)
Pulmonary congestion and fibrous adhesion	1 (9)
Splenitis	1 (9)

Abbreviation: IHC, immunohistochemistry.

[a] Ascites associated with cirrhosis and hemoabdomen.

Table 4.

Histologic Findings in Fatal Cases in Whom Chikungunya Virus Antigen Was Detected in Any Tissue: Puerto Rico, 2014

Tissue	Cases With Tissue Specimen Available, n	Histopathological Feature	Frequency, n (%)	CHIKV Antigen Detected by IHC, n (%)	Localization of CHIKV Antigen	Frequency, n (%)
Spleen	11	Increased immunoblasts[a]	8 (73)	10 (91)	Round cells in white pulp	9 (90)
		White pulp depletion	3 (27)		Capsule/trabeculae	6 (60)
					Red pulp macrophages	4 (40)
					Vascular	3 (30)
					Intravascular	1 (10)
Lung	11	Congestion	9 (82)	7 (64)	Alveolar septa/connective tissue	6 (86)
		Intra-alveolar hemorrhage	8 (73)		Vascular	4 (57)
		Circulating leukocytosis	6 (55)		Mononuclear cells	3 (43)
		Perivascular mononuclear infiltrates	5 (45)		Intravascular	3 (43)
		Interstitial mononuclear infiltrates	2 (18)		Pleura	2 (29)
		Edema	8 (73)			
		Fibrin	2 (18)			
		Emphysema	4 (36)			
Kidney	11	Glomerulosclerosis (extensive)	3 (27)	6 (55)	Vascular	6 (100)
		Glomerulosclerosis (mild/moderate)	5 (45)		Glomeruli	4 (67)
		Atherosclerosis	7 (64)		Interstitial connective tissue/capsule	2 (33)
		Interstitial mononuclear infiltrates	10 (91)		Inflammatory infiltrates	2 (33)
		Interstitial fibrosis	7 (64)		Tubular epithelium	1 (17)
Liver	11	Minimal/mild chronic portal hepatitis	11 (100)	6 (55)	Vascular	6 (100)
		Minimal/mild steatosis	7 (64)		Endothelium and/or Kupffer cells	4 (67)
		Steatohepatitis	2 (18)		Connective tissue (portal/capsular)	2 (33)
		Rare single-cell necrosis/apoptosis	5 (45)		Intravascular	3 (50)
		Sinusoidal leukocytosis[a]	3 (27)			
		Congestion	2 (18)			
Heart	11	Myocyte hypertrophy	9 (82)	5 (45)	Connective tissue	5 (100)
		Edema	6 (55)		Vascular	5 (100)
		Interstitial fibrosis	4 (36)		Intravascular	3 (60)

Tissue	Cases With Tissue Specimen Available, n	Histopathological Feature	Frequency, n (%)	CHIKV Antigen Detected by IHC, n (%)	Localization of CHIKV Antigen	Frequency, n (%)
		Atherosclerosis	2 (18)		Epicardial adipose	4 (80)
		Mononuclear interstitial cells	2 (18)		Nerves	3 (60)
		Circulating leukocytosis[a]	2 (18)		Endocardium	3 (60)
					Myocardium	2 (40)
Bone	11			7 (64)	Periosteum	7 (100)
					Osteonal endosteum	4 (57)
Bone marrow	10	Left shift	7 (70)	4 (40)	Mononuclear cells and/or megakaryocytes	4 (100)
		Hypercellularity	6 (60)			
		Hemophagocytosis	2 (20)			
Skeletal muscle (rib)	10	Chronic inflammation[a]	2 (20)	7 (70)	Tendons/aponeurosis	5 (71)
		Acute inflammation[a]	1 (10)		Connective tissue	4 (57)
		Myocyte necrosis/degeneration/regeneration[a]	2 (20)		Vascular	4 (57)
					Nerves	3 (43)
					Adipose	3 (43)
					Myofibers	1 (14)
Skin	9	Perivascular inflammation	7 (78)	8 (89)	Connective tissue	7 (88)
		Congestion/extravasation[a]	4 (44)		Mononuclear cells	7 (88)
					Vascular	5 (63)
Gastrointestinal tract	6	Inflammatory infiltrates	3 (50)	3 (38)	Vascular endothelium	3 (100)
					Smooth muscle	2 (67)
					Tunica muscularis and serosa within fibroblasts and interstitial connective tissue	2 (67)
Tendon (hand)	5	Chronic inflammation[a]	3 (60)	4 (80)	Tendon	4 (100)
		Acute inflammation[a]	1 (20)		Nerves	2 (50)
Adrenal gland	4	Inflammatory infiltrates[a]	1 (25)	2 (50)	Periadrenal adipose	2 (100)
					Vascular	2 (100)
					Capsule	2 (100)
					Intravascular	2 (100)
Synovium (hand)	3	Synovitis[a]	3 (100)	2 (67)	Vascular	2 (100)
Thyroid gland	2			1 (50)	Vascular	1 (100)

Clin Infect Dis. Author manuscript; available in PMC 2024 August 08.

Tissue	Cases With Tissue Specimen Available, n	Histopathological Feature	Frequency, n (%)	CHIKV Antigen Detected by IHC, n (%)	Localization of CHIKV Antigen	Frequency, n (%)
					Connective tissue	1 (100)
					Intravascular	1 (100)
Brain	2	Congestion[a]	1 (50)	1 (50)	Vascular	1 (100)
					Intravascular	1 (100)

N = 11.

Abbreviations: CHIKV, chikungunya virus; IHC, immunohistochemistry.

[a]Finding was only observed in tissue specimens in which CHIKV antigen was also detected.

Table 5.

Demographic and Clinical Characteristics and Evidence of Septic Shock Among Fatal Cases With Chikungunya Virus Infection Detected by Immunohistochemistry: Puerto Rico, 2014

Case ID	Age/ Sex	Comorbid Condition(s)	Fever	Highest WBC Count, Cells/mL^3	Lowest Platelet Count, Cells/mL^3	Mechanical Ventilation	Vasopressors Given?	Cause of Death[a]
2	45/M	Diabetes	Yes	37 000	174 000	Yes	Yes	Septic arthritis, acute renal failure
6	85/M	Hypertension, prostate cancer, cholecystectomy, Parkinson's disease	Yes	34 900	53 000	Yes	Yes	Septic shock, viral infection
7	80/F	Hypertension, diabetes, COPD, heart disease, obesity, hysterectomy	Yes	10 470	269 000	No	No	Viral syndrome, septic ulcers
8	38/F	Lupus, sarcoidosis, arthritis	Yes	NA	NA	Yes	Yes	Septic shock
10	21/F	Sickle cell disease, asthma, 38 weeks pregnant	Yes	16 660	261 000	No	No	Sickle cell crisis, viral infection
12	84/M	Hypertension, obesity	Yes	6200	134 000	Yes	Yes	Acute cardiorespiratory failure
13	54/M	Prediabetes, throat cancer, endocrine disorders, osteoarthritis, obesity, high cholesterol, anemia	Yes	NA	NA	No	NA	NA
20	29/M	Diabetes, lupus, arthritis, asthma, IV drug user, smoker	Yes	NA	NA	No	NA	NA
21	71/F	Hypertension, diabetes, thyroid disease, coronary artery disease	Yes	5800	196 600	No	No	Viral syndrome
22	61/M	Diabetes, hypertension, alcoholism, liver cirrhosis	Yes	4080	20 200	Yes	Yes	Viral syndrome, encephalitis
29	68/M	Hypertension	Yes	NA	NA	No	NA	NA

N = 11.

Abbreviations: COPD, chronic obstructive pulmonary disease; F, female; IV, intravenous; M, male; NA, data not available; WBC, white blood cell.

[a]As listed on the death certificate or hospital discharge diagnosis.

Table 6.

Scoring of Antigen Staining by Tissue Type Among Fatal Cases With Chikungunya Virus Infection Detected by Immunohistochemistry: Puerto Rico, 2014

Case ID	Spleen	Skin	Hand Tendon	Bone	Skeletal Muscle	Lung	Kidney	Liver	Brain	Thyroid Gland	Adrenal Gland	Gastrointestinal Tract	Heart	Bone Marrow
2	+++	NE	NE	+++	+++	+	++	++	NE	NE	NE	NE	+++	−
6	+++	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++
7	++	+	NE	+	+	−	−	+	NE	NE	NE	−	−	−
8	++	NE	NE	−	−	+	−	−	NE	NE	NE	NE	−	−
10	−	−	−	+	−	−	−	−	NE	NE	NE	−	−	−
12	+++	+++	+++	+++	+++	+++	+++	+++	NE	NE	+++	+++	+++	+++
13	++	+	++	−	−	+	−	−	NE	NE	NE	NE	−	+
20	++	++	NE	+++	+++	−	+	+	−	−	−	NE	+++	−
21	+	+	+	−	+	+	−	−	NE	NE	−	−	−	−
22	++	+	NE	NE	NE	−	+	−	NE	NE	NE	NE	−	NE
29	+++	+++	NE	++	+	++	++	+++	NE	NE	NE	++	++	+++

N = 11.

Abbreviations: NE, not evaluated; +++, moderate to abundant immunostaining; ++, mild immunostaining; +, rare to mild immunostaining; −, no immunostaining.