Postmortem Diagnosis of Dengue as an Epidemiological Surveillance Tool

Luciano Pamplona de Góes Cavalcanti,* Deborah Nunes de Melo Braga, Livia Maria Alexandre da Silva, Marina Gondim Aguiar, Mariana Castiglioni, José Udevanier Silva-Junior, Fernanda Montenegro de Carvalho Araújo, Renata Allana da Costa Pereira, Danielle Lima Malta, and Margarida Maria de Lima Pompeu

Department of Community Health, Federal University of Ceara, Fortaleza, Brazil; Departamento de Patologia, Universidade Federal do Ceará, Fortaleza, Brazil; Faculdade de Medicina, Centro Universitário Christus, Fortaleza, Brazil; Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, Brazil; Central Public Health Laboratory, Department of Health of Ceará State, Fortaleza, Brazil; Curso de Medicina, Universidade de Fortaleza, Fortaleza, Brazil; Programa de Pós-graduação em Patologia da Universidade Federal do Ceará, Departamento de Patologia, Universidade Federal do Ceará, Fortaleza, Brazil

Abstract. Dengue remains a problem in Brazil, and a substantial number of cases that progress to death are not diagnosed by health services. We evaluated the impact of a protocol adopted by the Coroner's Office Rocha Furtado (CO-RF) for the detection of unreported deaths from dengue in Brazil. We evaluated prospectively cases of deaths referred to the CO-RF with suspicion of dengue and those referred with other diagnosis in which the pathologists suspected dengue as the cause of death. Biological material was collected from all bodies autopsied, for which the suspected cause of death was dengue, between January 2011 and December 2012. Of the 214 bodies autopsied, 134 (62.6%) tested positive for dengue; of these cases, 121 were classified as dengue according to the World Health Organization's case definition (1997 or 2009, as appropriate). Thus, CO-RF detected 90 deaths from dengue, which were not suspected during disease progression. This CO-RF protocol, through a combined effort of the surveillance and laboratory teams, increased the detection of fatal dengue cases by 5-fold. This is the largest series of autopsies performed in cases of death related to dengue in the world to date.

INTRODUCTION

Dengue remains an important global public health problem.¹ It was reintroduced in Brazil in the early 1980s, and at present > 60% of confirmed cases occur in the southern hemisphere,² including large epidemics.^{3–5} Successive epidemics and the co-circulation of all serotypes of dengue viruses contributed to an increase in cases in younger people with unusual and severe manifestations.^{6–9} However, not all cases that progress to death are diagnosed by the health services. In fatal cases, a retrospective diagnosis of dengue can sometimes be made by the detection of viral antigen in tissue samples from autopsied cases, using techniques such as immunohistochemistry¹⁰ or in situ hybridization.¹¹

In Ceará, a state in northeastern Brazil, in the Coroner's Office Rocha Furtado (CO-RF) performs autopsies in cases of natural death without medical assistance and ill-defined causes that are referred by health services. Approximately 430 deaths are referred each month; 65% of these patients died at home without medical assistance. Autopsies are performed by pathologists after authorization by the family in 35% of the cases reported to the service. In many of the deaths reported to CO-RF without clinical suspicion of dengue, signs, symptoms, and morphological findings were present that led the pathologists to suspect dengue.

The objective of this study is to evaluate the impact of a protocol used by the CO-RF for detecting unreported deaths from dengue fever in a region of Brazil where the disease is endemic.

METHODS

Study design. This was a longitudinal study conducted in the CO-RF in Fortaleza, Ceará, Brazil, between January 2011 and December 2012.

* Address correspondence to Luciano Pamplona de Góes Cavalcanti, Departamento de Saúde Comunitária da Universidade Federal do Ceará. Rua Professor Costa Mendes, 1608 – Bloco Didático – 5º andar – Rodolfo Teófilo – Fortaleza, Ceará, Brasil. CEP 60.430-140. E-mail: pamplona.luciano@gmail.com

The work consisted of collecting samples for diagnosis of dengue fever according to the standards of the Ministry of Health, Brazil,¹² and achievement of complete clinical autopsies. The bodies were stored under refrigeration until the time of autopsy.

The study included deaths in the following situations: 1) referred with clinical suspicion of dengue; 2) referred with suspected diseases that make differential diagnosis of dengue fever (leptospirosis, meningitis, influenza [hemagglutinin type 1 and neuraminidase type 1]) pdm 09, melioidosis, and/or rash; and 3) sent without a hypothesis of dengue, but pathologists suspected dengue.

Pathologists at the CO-RF suspected a diagnosis of dengue after reviewing clinical records, during an interview with relatives, or during autopsy. The criteria for suspicion of dengue were 1) a recent report of fever (maximum 7 days) with no apparent bacterial infection, 2) presence of a rash, 3) presence of cavity effusion and/or bleeding.

Procedure for collecting samples for diagnosis of dengue.

Blood (5–10 mL) was collected by aortic puncture, after the pericardium section. Cerebrospinal fluid (CSF, 0.2–2.0 mL) collected by puncture of the subdural space after removing the skull. When effusion was detected, pleural and pericardial fluids were collected (5–10 mL) using a syringe. Collection of specimens from the peritoneal cavity was avoided because of the possibility of contamination with enteric pathogens. All materials were collected using sterile technique and stored in sterile glass tubes without anticoagulants.

Samples (2.0 cm) were collected from the organs (brain, lungs, heart, spleen, and liver) before removing them from the body cavity, according to the standards of the Ministry of Health, Brazil.¹² The samples were kept fresh in jars and wide mouth screw cap for molecular techniques, and the liver fragments were fixed in 10% formalin for immunohistochemistry. After samples were collected, a complete clinical autopsy was performed as per the CO-RF routine. Tissue samples were fixed in 10% formalin and subjected to routine histological processing for further analysis and issuance of autopsy.

The samples were sent to the Central Public Health Laboratory within 2 hours of collection for diagnostic tests, as described

elsewhere.¹² Frozen samples of human tissue, stored at 80°C, were ground in a mortar and pestle procedure in 1.5 mL of Leibovitz-15 medium® (Sigma) at a pH of 7.0–7.4 with 3% sodium penicillin/streptomycin sulfate. The suspension was incubated at 4°C for 60 minutes and centrifuged (800 × g at 4°C, 15 minutes). The supernatant obtained was transferred to a sterile cryotube and stored at –80°C until use. Samples were analyzed by nested reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) for antigen non-structural 1 (NS1Ag) and immunoglobulin M antibody (IgM Ab). Virus isolation was performed with CSF and blood using C6/36 (*Aedes albopictus*) cell monolayer cultures with the detection of infection by indirect immunofluorescence assay, with serotype-specific monoclonal antibodies, as described previously.¹³ Viral RNA was extracted from 140 µL of CSF and blood samples using the QIAamp Viral RNA Mini Kit® (QIAGEN, Valencia, CA), following the manufacturer's protocol, and stored at –80°C until tested.¹⁴ In this work, samples were tested for qualitative detection by nested RT-PCR.¹⁴ The nested RT-PCR was performed in two steps; the first step was performed according to the following cycles: 42°C for 60 minutes, followed by 30 cycles of 94°C for 35 seconds and 72°C for 2 minutes. For the second step, 5 µL of the amplicon according to the following 18 cycles: 94°C for 30 seconds, 55°C for 1 minute, and 72°C for 2 minutes. The amplicons were detected on a 2% agarose gel electrophoresis, stained with ethidium bromide and observed under ultraviolet light. Dengue virus serotype 1, 2, 3, and 4 were used as positive control in this study.

The NS1Ag Pan-E Dengue Early ELISA kit (Panbio Diagnostics®, Brisbane, Australia) was used to detect the dengue NS1Ag in samples in accordance with the manufacturers' instructions.¹⁵ Dengue IgM Capture ELISA (Panbio Diagnostics) was performed on samples according to the manufacturer's instructions.¹⁶ Samples were determined to be positive or negative using an index value calculated according to the manufacturer's instructions.

A portion of the viscera in formalin was transported to the Instituto Evandro Chagas for immunohistochemistry. The immunochemistry technique to determine the presence of dengue antigen in formalin-fixed paraffin-embedded liver tissue samples, alkaline phosphatase was used as the enzyme labeling system, with a red chromogen that contrasted with the tissue pigments, and mouse polyclonal antibody from ascitic fluid against dengue, using negative and positive controls, as described previously.¹⁷

All cases were reported as suspected dengue at the Center for Epidemiological Surveillance of the State Department of Health for epidemiological investigation.

Dengue death case. In this study, dengue death was classified as a positive case by RT-PCR and/or ELISA AgNS1 and/or IgM Ab and/or virus isolation and/or immunohistochemistry and features clinically consistent with dengue.

Statistical analysis. Data were analyzed using Epi-Info Version 3.5.1. Parametric (Student's *t* test) and nonparametric tests (Kruskal–Wallis test) were used, according to the characteristics of the study variables. Prevalence ratios and their respective confidence intervals were also calculated.

This study was approved by the Research Ethics Committee of the University Center Christus, registration number: 078/2011. Autopsies and sample collection were performed after authorization was received from the family.

RESULTS

During 2011 and 2012, complete autopsies were performed on and biological specimens were collected from 214 suspected dengue deaths. In this study, 143 (66.8%) were forwarded to the CO-RF without clinical suspicion of dengue. Of these cases, reasons for the pathologist to suspect dengue included reported fever for up to 7 days without apparent bacterial infection (41.3%), observation of a cavity spill during the autopsy (41.3%), differential diagnosis (7.0%), presence of rash (5.6%), and bleeding (4.9%).

Twice as many dengue-related deaths were detected by pathologists by postmortem suspicion of dengue than by clinical suspicion. However, positivity for dengue was approximately 62% for both (Table 1).

Of the 214 autopsies performed, the majority (86%) was admitted to the CO-RF on the day of death and 63.6% of the autopsies were performed within 12 hours of death. Of the samples tested, 134 (62.1%) were positive for dengue according to at least one of the techniques used. The tests with the highest positivity were immunohistochemistry (103/208, 49.5%), NS1Ag (33/165, 20.0%), IgM Ab (27/197, 13.7%), viral isolation (20/170, 11.8%), and RT-PCR (11/130, 8.5%). A total of 35/134 (26.1%) of these samples tested positive using more than one technique (Table 2).

The serotype was identified as dengue virus 1 (DENV-1) in 69.6% (16/23) of samples, DENV-3 in 8.7% (2/23), and DENV-4 in 21.7% (5/23). DENV-4 was first isolated in Ceará in a sample collected from a suspected dengue death at the end of 2011.

Bodies referred from 58/184 (31.5%) municipalities in Ceará were autopsied. Of these, 91/214 (42.5%) were living in Fortaleza, the state capital. The majority of deaths (54.5%) occurred in 2011.

The occupations most frequently cited were domestic workers (22.5%), students (15.7%), and farmers (12.7%). The majority (54.1%) had a family income above US\$307.77. Age ranged from 0 to 93 (median = 34), with a reduction in the median 34.5 to 33 years between 2011 and 2012, respectively. Disease duration among positive cases ranged from 0 to 21 days (median = 4).

Among the bodies autopsied, 16 were children aged < 1 year; of these, five met the dengue death case definition, based on World Health Organization 1997 case definition. In contrast, 18.7% of the deaths occurred in people > 60 years of age. For deaths without clinical suspicion of dengue, the median age was 32 years, and 25% of these were under 15 years of age. The median age among fatal dengue cases with clinical suspicion of dengue was 43 years. Males represented 55.7% of the clinically suspected cases and 53.8% of the clinically unsuspected group (*P* = 0.986).

TABLE 1

Percentage of deaths referred for autopsy with laboratory confirmed dengue, 2011–2012, Ceará State, Brazil

Clinically suspect (71)				Nonclinically suspect (143)			
Positive		Negative		Positive		Negative	
<i>N</i>	%	<i>N</i>	%	<i>N</i>	%	<i>N</i>	%
44	62.0	27	38.0	90	62.9	53	37.1

TABLE 2
Dengue laboratory confirmation according to tests in 121 autopsies cases

Case	IgM	IH	NSI	VI	RT-PCR	Case	IgM	IH	NSI	IgM	VI	RT-PCR	Case	IgM	IH	NSI	IgM	VI	RT-PCR
1	+	+	+	+	+	35	+	+	-	-	-	-	77	+	+	ND	-	-	ND
2	+	+	+	-	+	36	+	+	-	-	-	-	78	+	+	ND	-	-	ND
5	+	+	+	-	-	37	+	+	-	-	-	-	79	+	+	ND	-	-	ND
6	+	+	+	-	-	38	+	+	-	-	-	-	80	+	+	ND	-	-	ND
7	+	+	+	-	-	39	+	+	-	-	-	-	81	+	+	ND	-	-	ND
8	+	+	+	-	-	40	+	+	-	-	-	-	82	+	+	ND	-	-	ND
9	+	+	+	-	ND	41	+	+	-	-	-	-	83	+	+	ND	-	-	ND
14	+	-	+	+	-	42	+	+	-	-	-	-	84	+	+	ND	-	-	ND
16	+	-	-	+	+	43	+	+	-	-	-	-	85	+	+	ND	-	-	ND
17	+	-	ND	+	+	44	+	+	-	-	-	-	86	+	+	ND	-	ND	ND
18	+	ND	+	+	-	45	+	+	-	-	-	-	87	+	+	ND	-	ND	ND
19	+	ND	+	+	ND	46	+	+	-	-	-	-	88	+	+	ND	-	ND	ND
22	+	+	-	-	-	47	+	+	-	-	-	-	89	+	+	ND	-	ND	ND
23	+	+	-	-	-	48	+	+	-	-	-	-	90	+	+	ND	-	ND	ND
24	+	+	-	-	-	49	+	+	-	-	-	-	98	+	-	+	-	-	-
26	+	+	ND	ND	ND	50	+	+	-	-	-	-	99	+	-	+	-	-	-
27	+	-	+	-	-	51	+	+	-	-	-	-	100	+	-	+	-	-	ND
28	+	-	+	-	-	52	+	+	-	-	-	-	101	+	-	+	-	-	ND
29	+	-	+	ND	ND	53	+	+	-	-	-	-	102	+	-	+	-	ND	ND
105	+	-	-	-	-	54	+	+	-	-	-	-	103	+	-	+	-	ND	ND
106	+	-	-	-	-	55	+	+	-	-	-	-	104	+	-	+	-	ND	ND
107	+	-	-	ND	-	56	+	+	-	-	-	-	108	+	-	-	-	+	-
112	+	-	ND	-	-	57	+	+	-	-	-	-	109	+	-	-	-	+	ND
113	+	-	ND	ND	ND	58	+	+	-	-	-	-	110	+	-	-	-	+	ND
116	+	-	-	-	-	59	+	+	-	-	-	-	111	+	-	-	-	+	ND
117	+	-	+	-	-	60	+	+	-	-	-	-	114	+	+	-	-	-	-
119	+	-	-	-	ND	61	+	+	-	-	-	-	115	+	+	-	-	-	-
3	-	+	+	+	+	62	-	+	-	-	-	-	118	-	-	+	-	-	ND
4	-	+	+	+	+	63	-	+	-	-	-	-	15	-	-	+	ND	+	+
10	-	+	+	+	-	64	-	+	-	-	-	-	91	-	+	ND	ND	ND	ND
11	-	+	+	+	ND	65	-	+	-	-	-	ND	92	-	+	ND	ND	ND	ND
12	-	+	+	+	ND	66	-	+	-	-	-	ND	93	-	+	ND	ND	ND	ND
13	-	+	-	+	+	67	-	+	-	-	-	ND	94	-	+	ND	ND	ND	ND
20	-	+	+	-	-	68	-	+	-	-	-	ND	95	-	+	ND	ND	ND	ND
21	-	+	+	-	ND	69	-	+	-	-	-	ND	96	-	+	ND	ND	ND	ND
25	-	+	-	+	-	70	-	+	-	-	-	ND	97	-	+	ND	ND	ND	ND
30	-	-	+	-	+	71	-	+	-	-	-	ND	120	-	+	ND	ND	-	ND
31	-	-	-	+	+	72	-	+	-	-	ND	-	121	-	+	ND	ND	ND	ND
32	-	+	-	-	-	73	-	+	-	-	ND	-							
33	-	+	-	-	-	74	-	+	-	-	ND	ND							
34	-	+	-	-	-	75	-	+	ND	-	-	-							

IgM = immunoglobulin M; IH = immunohistochemistry; ND = not done; NSI = nonstructural 1; RT-PCR = reverse transcription-polymerase chain reaction; VI = viral isolation.

Clinical suspicion of dengue was 3-fold more likely among patients who died in the hospital ($P = 0.013$) than those who did not, 3-fold more likely for those who were older than 16 years ($P = 0.015$) than those who were younger; nearly three times the deaths occurring within the first semester ($P = 0.012$). No significant association of suspected dengue was observed with sex, place of residence, family income, education, comorbidity, or year of death (Table 3).

Among the 134 positive tests, 121 (90.3%) met the clinical case definition and the other 13 (9.7%) were discarded after clinical investigation.

The three major immediate causes of death (terminal event) declared by the CO-RF that met the clinical case definition of WHO 1997¹⁸ were respiratory failure (47.1%), septic shock (13.2%), and hypovolemic shock (9.9%).

With the use of the CO-RF protocol, mortality from dengue increased from 0.154 to 0.780/100,000 inhabitants in 2011 and 0.142 to 0.651/100,000 inhabitants in 2012.

Among the fatal dengue cases, 77.7% (94/121) occurred in health facilities, 10.7% (13/121) occurred at home without medical assistance, and 11.5% (14/121) occurred on the way to the hospital. Males represented 66/121 (54.5%) of the deaths, but

the difference in fatality rate between the sexes was not significant ($P = 0.092$). Seizure and fever were reported in 34.9% and 87% of deaths, respectively.

In 72.2% of the fatal dengue cases, a comorbidity was reported, including hypertension (36.4%), obesity (31.0%), heart disease (28.8%), diabetes (22.9%), and hematologic disease (17.5%). Alcoholism (32.9%) and smoking (30.6%) were also associated.

Among the fatal dengue cases that were not clinically suspected, the predominant symptoms were respiratory distress in 47/56 (83.9%), dyspnea in 47/58 (81.0%), cough in 42/54 (77.8%), vomiting in 43/57 (75.4%), headache in 34/47 (72.3%), and prostration in 20/31 (64.5%) cases. Petechiae (54.8%) and headache (76.5%) were the only signs and symptoms significantly more prevalent among deaths with clinical suspicion of dengue ($P < 0.05$). No significant differences were observed among other signs and symptoms of patients with clinical suspicion of dengue and those in which dengue was first suspected by the CO-RF (Table 4).

Of the confirmed cases, 11/121 (9.1%) were diagnosed as dengue hemorrhagic fever (DHF) and four (3.3%) as dengue shock syndrome (DSS). Among the criteria used to classify

TABLE 3

Demographic characteristics and risk factors of the groups clinically suspected and nonclinically suspected in positive cases in Ceará, Brazil, 2011 and 2012

Key features	Clinically suspected <i>N</i> (%)	Nonclinically suspected <i>N</i> (%)	RR	CI	<i>P</i> value
Sex					
Female	20 (35.7)	36 (64.3)	1.25	0.60–2.59	0.277
Male	24 (30.8)	54 (69.2)			
Age(year)					
> 16	39 (37.5)	65 (62.5)	3.00	1.06–8.48	0.015
≤ 15	5 (16.7)	25 (83.3)			
City of residence					
Other municipalities	19 (26.8)	52 (73.2)	0.56	0.27–1.15	0.059
Fortaleza	25 (39.7)	38 (60.3)			
Family income					
> US\$307	25 (37.9)	41 (62.1)	1.63	0.75–3.53	0.112
≤ US\$307	15 (27.3)	40 (72.7)			
Years of schooling (year)					
No	4 (18.2)	18 (81.8)	–	–	–
≤ 3	9 (33.3)	18 (66.7)	0.44	0.12–1.71	0.193
4–7	13 (43.3)	17 (56.7)	0.29	0.07–1.07	0.051
8–11	13 (36.1)	23 (63.9)	0.39	0.11–1.41	0.122
≥ 12	3 (27.3)	8 (72.7)	0.59	0.11–3.29	0.429
Comorbidities or associated factors					
Alcoholism	10 (29.4)	24 (70.6)	0.75	0.30–1.87	0.278
Smoking	8 (27.6)	21 (72.4)	0.66	0.25–1.73	0.207
Diabetes	4 (20.0)	16 (80.0)	0.35	0.10–1.78	0.044
Hypertension	13 (40.6)	19 (59.4)	1.22	0.49–3.03	0.340
Obesity	7 (33.3)	14 (66.7)	10.6	0.36–3.10	0.455
Place of death					
Hospital	40 (37.7)	66 (62.3)	3.64	1.18–11.25	0.013
Other	4 (14.3)	24 (85.7)			
Year					
2011	26 (36.1)	46 (68.9)	1.38	0.66–2.87	0.196
2012	18 (29.0)	44 (71.0)			
Semester					
First	37 (38.5)	59 (61.5)	2.78	1.11–6.95	0.012
Second	7 (18.4)	31 (81.6)			

CI = confidence interval; RR = relative risk.

TABLE 4

Signs and symptoms in 121 dengue death cases, Ceará, Brazil, 2011 and 2012

Signs	Clinically suspected <i>N</i> (%)	Nonclinically suspected <i>N</i> (%)	RR	CI	<i>P</i> value
Fever	39 (90.7)	61 (84.7)	1.76	0.52–5.91	0.267
Respiratory discomfort	32 (88.9)	47 (83.9)	1.53	0.43–5.40	0.366
Vomiting	33 (86.8)	43 (75.4)	2.15	0.70–6.57	0.090
Cough	25 (73.5)	42 (77.8)	0.79	0.29–2.15	0.327
Prostration	21 (80.8)	20 (64.5)	1.40	0.53–3.68	0.254
Irritability/agitation	20 (62.5)	27 (62.8)	0.99	0.38–2.54	0.489
Diarrhea	18 (66.7)	21 (51.2)	1.90	0.69–5.22	0.110
Sensorial loss	8 (36.4)	24 (55.8)	0.45	0.16–1.30	0.075
Hematemesis	14 (48.3)	11 (30.6)	2.12	0.77–5.86	0.078
Petechiae	17 (54.8)	7 (21.2)	4.51	1.51–13.47	0.003
Convulsion	6 (23.1)	16 (43.2)	0.39	0.13–1.20	0.054
Melena	8 (30.8)	5 (16.1)	2.31	0.65–8.22	0.160
Bruise	8 (32.0)	4 (12.9)	3.18	0.83–12.19	0.080
Exanthema	6 (25.0)	5 (16.1)	1.73	0.46–6.55	0.222
Hematoma	6 (26.1)	3 (10.7)	1.54	0.49–4.86	0.238
Symptoms					
Dyspnea	33 (86.8)	47 (81.0)	1.93	0.57–6.59	0.221
Abdominal pain	30 (81.1)	38 (71.7)	1.69	0.61–4.68	0.162
Headache	26 (76.5)	13 (27.7)	8.50	3.07–23.52	0.000
Myalgia	25 (75.8)	24 (60.0)	2.08	0.75–5.76	0.082
Somnolence	15 (60.0)	30 (66.7)	0.75	0.27–2.06	0.293
Arthralgia	10 (50.0)	14 (41.2)	1.07	0.34–3.35	0.455
Retro-orbital pain	7 (30.4)	7 (25.0)	1.31	0.38–4.50	0.339

CI = confidence interval; RR = relative risk.

DHF and DSS, two were missed: plasma leakage in 58.5% and thrombocytopenia (count < 100,000 mm³) in 47.2%.

DISCUSSION

The enhanced CO-RF protocol increased the detection of fatal dengue cases around by approximately 5-fold in Ceará in 2011 and 2012, compared with deaths reported through the health system. The use of this assessment protocol increased the rate of deaths attributed to dengue from 0.154 to 0.780/100,000 inhabitants in 2011 and from 0.142 to 0.651/100,000 in 2012. This increase in detection of the deaths can be attributed to factors such as the adoption of the Protocol of Suspected Dengue at the CO-RF; the role of the Central Public Health Laboratory, which uses five diagnostic techniques for blood, CSF, and viscera; as well as improved surveillance. These factors combined in an integrated and effective way to improve dengue detection.

This perhaps justifies, in part, because the northeast region has a higher mortality from dengue, compared with other regions of Brazil.^{19,20} Moreover, these results suggest that deaths related to dengue may be underreported in regions where there is no verification service or integrated epidemiological surveillance. The rate of clinical suspicion of dengue was low in the cases referred to the CO-RF, although it was during an epidemic. This led the CO-RF to adopt a protocol based on the presence of either acute febrile syndrome and plasma leakage, or hemostasis disorders. As a result of the adoption of the protocol, the pathologists identified twice as many deaths as were referred by health services.

The immunohistochemical and NS1Ag ELISA tests made the largest contribution to diagnosis, as reported by Araújo and others¹⁵ and Tomashek and others.²¹ Therefore, in this study, the NS1Ag test was critical despite the predominance of DENV-4 in 2012, which is difficult to detect with the NS1Ag test.²² Virus isolation and RT-PCR had low detection sensitivities of 11.5% and 8.5%, respectively. Several factors may have contributed to this low sensitivity: viral isolation and RT-PCR would be less sensitive if death occurred after defervescence; the possibility of bacterial contamination; the lability of RNA, considering that it was isolated from post-mortem material. This study showed the importance of using all available techniques for dengue diagnosis in postmortem cases, as demonstrated in other studies.²²⁻²⁴

The most common signs and symptoms were fever, respiratory distress, dyspnea, cough, vomiting, abdominal pain, headache, and prostration. Dengue is traditionally defined as an acute febrile illness. However, in our study, fever was not reported in 13% of dengue deaths, as reported previously.^{4,25}

Cough not has been reported to be a symptom of dengue, but it was observed in 76.1% of fatal cases in this study. Convulsion was observed in 34.9% of the dengue deaths, confirming the findings of Araújo and others²⁶ and warning of the importance of this clinical manifestation in severe cases.

Comorbidity was reported in 72% of fatal dengue cases, hypertension, heart disease, and diabetes showed the highest prevalence, as reported in Singapore and in an epidemic in southeastern Brazil.²⁷⁻³⁰ The presence of some of these comorbidities, such as heart and kidney problems, were identified as factors associated with death.^{29,32} New studies should be encouraged to improve the understanding of the role of comorbidities in severe disease progression.

The three major immediate causes of deaths reported by CO-RF were respiratory failure, septic shock, and hypovolemic shock; similar causes were reported in 2010 in Thailand.³¹ It is also necessary to better understand the role of coinfections in more serious cases, as it often remains unclear whether dengue complicated a preexisting disease or a preexisting disease was exacerbated by infection with dengue virus.

In endemic areas, it is necessary to sensitize health professionals to consider dengue in the differential diagnosis with other diseases. If this can be accomplished, it is possible to provide a more adequate assistance and prevent deaths. Similar findings have been reported in both Brazil³³ and other countries.³⁴

The refusal of the family to allow the completion of the autopsy in 65% of deaths might interfere with the representativeness of the sample. Gaps in the epidemiological knowledge of dengue in Brazil are partly due to the limitations of passive surveillance, as in other countries. Thus, we believe that surveillance of dengue in Brazil underestimate the true mortality from dengue. The implementation of the partnership between surveillance and laboratory allowed to capture a greater number of deaths due to dengue and better characterize the profile of deaths from dengue, with the inclusion of unusual forms of the disease.³⁵ Most deaths had no clinical record of adequate hydration volume. This study reinforces the need to improve primary health care to identify cases of dengue with increased risk of becoming severe allowing for proper treatment to prevent deaths.

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Authors' addresses: Luciano Pamplona de Góes Cavalcanti, Department of Community Health, Federal University of Ceará, Fortaleza, Brazil, E-mail: pamplona.luciano@gmail.com. Deborah Braga, Departamento de Patologia, Universidade Federal do Ceará, Fortaleza, Brazil, E-mail: deborahmb@gmail.com. Livia Alexandre, Marina Aguiar, and Mariana Castiglioni, Faculdade de Medicina, Centro Universitário Christus, Fortaleza, Brazil, E-mails: livinhalexandre@hotmail.com, ina_aguiar@hotmail.com, and marianacastiglioni@gmail.com. José Udevanier Silva-Junior and Renata Allana, Faculdade de Medicina, Universidade Federal do Ceará, Fortaleza, Brazil, E-mails: udevanier_reboucas@hotmail.com and renata.allana1@gmail.com. Fernanda Araújo, Central Public Health Laboratory, Department of Health of Ceará State, Fortaleza, Ceará, Brazil, E-mail: fernanda.montenegro@lacen.ce.gov.br. Daniele Lima Malta, Curso de Medicina, Universidade de Fortaleza, Fortaleza, Brazil, E-mail: danimalta37@gmail.com. Margarida Lima Pompeu, Programa de Pós-Graduação em Patologia da Universidade Federal do Ceará, Departamento de Patologia, Fortaleza, Brazil, E-mail: margarida.pompeu@gmail.com.

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