

Comprehensive Clinical and Laboratory Follow-up of a Female Patient With Ebola Virus Disease: Sierra Leone Ebola Virus Persistence Study

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The clinical, virologic, and immunologic findings in a female Ebola virus disease patient are described. During the long-term follow-up, Ebola virus RNA was detectable in vaginal fluid before 36 days after symptom onset, with nearly an identical genome sequence as in acute phase blood. Ebola-specific T cells retained activation at 56 days after disease onset.

Keywords. clinical sequelae; Ebola virus; immune responses; virus genome; virus persistence.

CLINICAL SYMPTOMS IN ACUTE DISEASE

A 38-year-old female presented signs of Ebola virus disease (EVD) while under contact surveillance after caring for a close relative who died of Ebola virus (EBOV) infection [1]. EVD was confirmed at day 2 after the onset of symptoms by a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test performed on blood.

At day 3, she was admitted to the Military Hospital 34 (MH34) Ebola Treatment Unit (ETU) in Freetown, Sierra Leone. On arrival, her body temperature was 38°C, she was conscious, she

able to walk unaided, and her oxygen pulse saturation was normal. All clinical signs are included in Table 1. Initial treatment is detailed in Box 1. On the same day, she consented to enroll in the Convalescent Plasma for Early Ebola Virus Disease in Sierra Leone study [2] and was transfused successfully with 450 mL of convalescent plasma. No abnormal signs were noted throughout the process.

The patient's clinical symptoms gradually improved from day 10 (Table 1). Ebola-specific RNA targets were detected in the blood at day 3, and again at day 9 [3, 4] (Figure 1A, Table 2).

At day 14, the patient presented a single episode of fainting with spasmodic generalized body contractions of unknown origin, treated as a grand mal seizure with a single dose of diazepam. One hour later, she was agitated and restless, with a Glasgow Coma Score of 10. Her pupils were responsive to light. Abnormal vitals included tachycardia (152 beats per minute) and elevated systolic blood pressure (148/88 mmHg). Her blood sugar was normal (5 mmols/L). She regained consciousness 2 hours later and continued her improved clinical course.

At day 15, a peripheral blood specimen tested by RT-PCR for EBOV was undetectable (Figure 1A). A second specimen collected at day 17 was also EBOV RNA undetectable, and the participant was discharged from the ETU on day 18.

FOLLOW-UP AS A SURVIVOR

On day 18, she was enrolled in the Sierra Leone Ebola Virus Persistence Study at the MH34 study site [5, 6]. The study participant reported no history of malaria, diabetes, or tuberculosis in the previous 3 months, and a rapid HIV screening test was negative. A blood sample and a first set of body fluid specimens were collected, as per routine study procedures [6].

During her second visit to the study site at day 36, relatives reported disorientation to time, space, and with numbers. Additional complaints can be found in Table 1. She was referred to a psychiatrist for clinical evaluation, and findings included depersonalization (a feeling that her personality has changed) and increased recent nightmares. She was diagnosed with acute traumatic or stress reaction and insomnia, and she received diazepam. Ophthalmologic examination was normal, although she complained of eye pain and had hemorrhagic sclera. During this visit, a blood specimen and a second set of body fluid specimens were collected.

At day 49, the participant came back for a third visit, during which blood and a vaginal swab specimen were collected. She did not report any health problems.

When seen again at the MH34 survivor clinic at day 57, she had additional symptoms (Table 1). She was treated with antimalarials (empirical treatment with artemether and

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Table 1. Signs Reported by a Female Ebola Patient During Admission in the Ebola Treatment Unit and During Follow-up in the Ebola Virus Persistence Study, Sierra Leone

	Days After Onset of Symptoms																		Post ETU Discharge				
	In ETU Discharge ↓																		36	57	73	176	261
	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18							
Signs assessed																							
Fever		X	X	X																			
Anorexia		X	X	X	X		X					X											
Generalized body weakness					X	X	X					X	X	X									
Generalized body pain		X	X	X	X	X						X	X	X				X			X		
Headache																							
Abdominal pain		X	X															X					
Chest pain			X				X					X	X	X									
Chest tightness					X																		
Muscle pain			X	X		X	X					X	X	X									
Joint pain			X	X	X	X	X					X	X	X			X				X		
Cough		X			X	X	X	X	X														
Difficulty breathing		X	X																				
Sore throat				X	X	X	X				X												
Red eyes		X	X	X	X	X	X		X	X		X			X	X	X						
Diarrhea		X	X	X																			
Nausea		X	X	X																			
Confusion				X								X											
Agitation				X								X											
Insomnia		X	X					X	X	X	X				X		X	X	X				
Itching			X	X	X		X																
Difficulty swallowing				X		X																	
Grand mal seizure												X											
Additional signs after ETU discharge																							
Depersonalization																		X					
Nightmares																		X					
Eye pain																		X					
Loss of appetite																	X	X	X		X		
Weight loss																					X		
Cutaneous abscess																					X		

Other signs assessed systematically and never reported for/by this patient during admission in the ETU: vomiting, coma, bleeding, sneezing, hiccupping, signs of shock, peripheral edema. Abbreviation: ETU, Ebola Treatment Unit.

lumefantrine), antibiotics (azithromycin), antipyretic/pain killer (paracetamol), multivitamin and mineral supplement, and oral rehydration salt solution. This clinic visit corresponded to the fourth visit as part of enrollment in the Sierra Leone Ebola Virus Persistence Study, and venous blood and a menstrual blood swab specimen (her second menstruation after ETU discharge) were collected.

At day 73, she reported fever, persisting insomnia, and loss of appetite (Table 1). She was treated with an antibiotic (ciprofloxacin), antidepressant (amitriptyline), antipyretic/pain killer (paracetamol), and multivitamin supplement. During this clinic visit, which corresponded to her fifth visit in the study, venous blood, tears, and vaginal swab specimens were collected.

During her next 2 rounds of follow-up at days 176 and 210 and at days 261 and 268, respectively, additional specimens were collected (Table 2). At her follow-up visit at day 176, she

reported weight loss, abscess in the right axilla of 1 month's duration, and a 1-week history of fever and joint pain. Blood smear was positive for malaria. She was treated with antimalarials (artemether and lumefantrine), antibiotics (amoxicillin), antipyretic/pain killer (paracetamol), nonsteroidal anti-inflammatory drugs (ibuprofen), and mineral supplements. She did not complain of any new health problems at day 210. At day 261, the participant complained of loss of appetite and generalized body pain. She did not complain of any new health problems at her visit at day 268.

VIROLOGIC AND IMMUNOLOGIC TESTING

Six vaginal swabs were collected and tested by RT-PCR [3] in the SLE-CHN Bio-safety Lab [7, 8]. EBOV RNA was detected in the first 2 specimens at days 18 and 36 after symptom onset,

Box 1. Treatment on Admission to Ebola Treatment Unit (From Day 2 After Onset of Disease)

1. Rehydration: Dextrose 500 mL + normal saline 500 mL, every 8 hours
2. Antiemetic: Metoclopramide 10 mg IV, PRN
3. Antibiotics: Ceftriaxone 1 g IV, once per day, 3 days
Metronidazole 500 mg/100-mL IV, 3 times per day, 2 days
Ciprofloxacin 200 mg/100-mL IV, twice per day, 2 days
4. Antimalaria: Artesunate 120-mg IV, once per day, 3 days
5. Analgesic: Paracetamol tablets 500 mg, twice per day, 2 days
6. Vitamin supplement: Immunoboost capsules, once per day, 3 days
7. Immunotherapy: 450 mL of convalescent plasma, 1 time

Abbreviations: IV, intravenous; PRN, as needed.

respectively (Figure 1A, Table 2). The threshold cycle (C_t) values increased over time, indicating a decreasing amount of EBOV RNA detected. Subsequent vaginal swab specimens were RT-PCR negative. Virus culture of the specimens collected at days 18 and 36 after symptom onset did not yield viable virus. A nearly complete viral sequence was generated using unbiased next-generation sequencing (Illumina MiSeq) from the vaginal swab acquired on day 18 (MF326641). A comparison of viral sequences acquired from the blood on day 2 (KX121194; 99.7% coverage) and vaginal swab on day 18 (99.9% coverage with an average depth of 255 reads) indicated nearly identical sequences. Sequence differences included (1) a single nucleotide insertion at position 3040 within the VP35 5' noncoding region in the blood reference sequence (KX121194) that was only observed in 2/1537 sequences from the outbreak and was not observed in the sequence from the index case (KX121193), which may represent a sequencing or assembly artifact; and (2) a silent mutation in the polymerase (T11755A) of the vaginal swab sequence that represents a unique mutation to this outbreak. The sequences from both specimens were very closely related to the index patient's sequence from an acute blood specimen (KX121193).

As per study protocols, the participant received counseling regarding sexual risk reduction recommendations, and condom demonstrations were performed when she received the RT-PCR test results from her body fluids. As it was the first time, the study counselor had to deliver a positive test result for vaginal fluid, and additional briefings and preparations ensured that the results were provided to the female participant with measured caution and appropriate counseling.

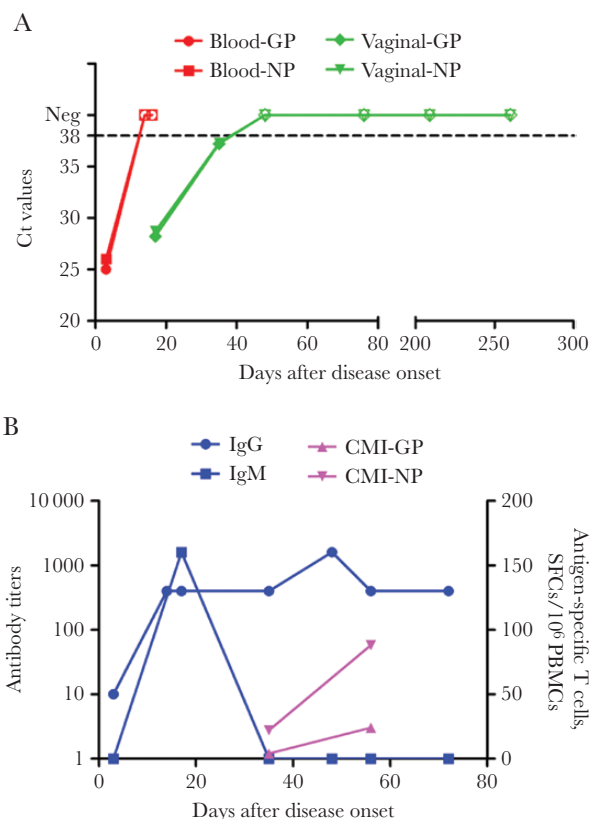


Figure 1. Virologic and immunologic laboratory findings in the blood and vaginal fluid of a female Ebola case during 9 months of follow-up; Sierra Leone. A, The virus shedding and persistence in body fluids. The Ebola virus (EBOV) RNA detected by reverse transcriptase polymerase chain reaction (RT-PCR)—targeting the glycoprotein (GP) and nucleoprotein (NP) genes in the SLE-CHN Bio-safety Lab are included; the EBOV RNA detection results from other labs are shown in Table 2. The threshold cycle (C_t) values of blood and vaginal fluids were represented with red and green points linked with lines, respectively. The negative results were denoted as empty points at $C_t = \text{Neg}$. The cutoff C_t value 38 was shown as a dashed black line. Six vaginal fluid swabs were collected and tested by RT-PCR. EBOV RNA was detected in the first 2 vaginal specimens collected on day 18 and day 36. During all her follow-up, all the viral RNA tests for vaginal fluid swabs were negative, including days 49 and 73, a follow-up visit at day 210, and a follow-up at day 261. B, The EBOV-specific immune responses. The EBOV NP-specific IgG and IgM were detected through commercial enzyme-linked immunosorbent assay kits (Wantai, Beijing, China). The antibody titer measurement of the blood on days 4 and 15 was performed in the P3 core area of the SLE-CHN Bio-safety Lab [19], and the 1 on day 16 and thereafter was performed in the P2 lab area. Peripheral blood mononuclear cells were isolated from the blood collected on days 36 and 57 for the test of EBOV GP- and NP-specific T-cell responses (CMI-GP and CMI-NP) by ELISPOT assays. The IgG and IgM values refer to the left y-axis, and the CMI-GP and CMI-NP values refer to the right y-axis. Abbreviations: PBMCs, peripheral blood mononuclear cells; SFCs, spot-forming cells.

All other body fluids (menstrual blood, oral swab, rectal fluid, sweat, tears, and urine) collected from the participant at time points ranging from day 18 to day 268 after symptom onset tested RT-PCR negative (Table 2).

Immune responses were evaluated on 7 convalescent venous blood samples collected from days 4 to 73 covering both the acute and convalescent phases [9]. The serological responses

Table 2. Laboratory Test Results for Various Specimens From a Female Case, Ebola Virus Persistence Study, Sierra Leone

Day	Specimen	Test	Result
2	Blood	RT-PCR	Positive (C _t values: NP = 25.00) ^a
3	Blood	RT-PCR	Positive (C _t values: GP = 24.57; NP = 24.64) ^b
4	Blood	RT-PCR	Positive (C _t values: GP = 25.06; NP = 25.51) ^c
		IgG	10 ^d
9	Blood	RT-PCR	Positive (C _t values: GP = 24.14; NP = 36.09) ^b
15	Blood	RT-PCR	Negative ^c
		IgG	400 ^d
17	Blood	RT-PCR	Negative ^c
18	Vaginal secretions	RT-PCR	Positive (C _t values: GP = 28.21; NP = 28.72) ^c
		Virus isolation	Negative ^e
	Urine, oral, sweat, rectal fluid	RT-PCR	Negative ^c
	Blood	IgG IgM	400 ^d 1600 ^d
36	Vaginal secretions	RT-PCR	Positive (C _t values: GP = 37.22; NP = 37.31) ^c
		Virus isolation	Negative ^e
	Urine, oral, tears, sweat, rectal fluid	RT-PCR	Negative ^c
	Blood	IgG IgM	400 ^d <100 ^d
		Cellular immune responses	GP = 4; NP = 22 ^f
49	Vaginal secretions	RT-PCR	Negative ^c
	Blood	IgG IgM	1600 ^e <100 ^e
57	Menstrual blood	RT-PCR	Negative ^c
	Blood	IgG IgM	400 ^d <100 ^d
		Cellular immune responses	GP = 23; NP = 85 ^f
73	Vaginal secretions, tears	RT-PCR	Negative ^c
	Blood	IgG IgM	400 ^d <100 ^d
79	Menstrual blood	RT-PCR	Negative ^c
176	Menstrual blood, urine, oral, tears, sweat, rectal fluid	RT-PCR	Negative ^c
210	Vaginal secretions	RT-PCR	Negative ^c
261	Vaginal secretions	RT-PCR	Negative ^c
	Urine	RT-PCR	Negative ^c
268	Oral, tears, sweat, rectal fluid	RT-PCR	Negative ^c

Abbreviations: C_t, cycle threshold; US DOD, US Department of Defense; EBOV, Ebola virus; IgG, immunoglobulin G; GP, glycoprotein; IgM, immunoglobulin M; NP, nucleoprotein; PBMCs, peripheral blood mononuclear cells; RT-PCR, real-time reverse transcriptase polymerase chain reaction; SFCs, spot-forming cells.

^aRT-PCR was performed at the Public Health England Makeni laboratory, using methods previously described [20].

^bRT-PCR was performed at the Central Public Health Reference Laboratory–US DOD MEDaC, using methods previously described [4].

^cRT-PCR was performed at the SLE-CHN Bio-safety Lab using a commercial double-channel real-time RT-PCR detection kit (China Registration No.: 20143402058) that targets the GP and NP genes of EBOV (Zaire strain) [3].

^dEnzyme-linked immunosorbent assay was performed by the SLE-C Biosafety Lab for IgG and IgM (Wantai, Beijing, China) [9].

^eVirus isolation was performed by the US CDC in Biosafety Level 4 conditions using Vero cells, as previously described [11].

^fEBOV GP- and NP-specific cellular immune responses were measured using the IFN- γ ELISPOT technique. PBMCs were harvested by Ficoll from the fresh anticoagulant blood of the survivors and stimulated using Ebola GP and NP peptide pools [21]. The unit of measure is SFCs/10⁶PBMCs. Each SFC represents 1 antigen-specific T cell.

to EBOV of the patient presented an ascending trend for IgG after virus infection (Figure 1B). The IgM titer at day 18 was 1600 and decreased to undetectable at day 36. The EBOV-glycoprotein and nucleoprotein-specific T-cell responses (Table 2) were detected at day 36 and retained at a high level at day 57 (Figure 1B).

DISCUSSION

The female participant described in this report was enrolled in the study from the first day of symptom onset during the quarantine after caring for a close relative who died of EBOV infection [1] and was followed up for >200 days after discharge from the ETU, which provided a unique opportunity to assess clinical

manifestations and virus persistence during the acute phase and early stages of convalescence.

From the limited evidence available, there is precedence for detection of EBOV RNA in vaginal fluid after recovery from EVD. During the 2014–2016 West African Ebola epidemic, vaginal swabs from 6 female EVD survivors being treated in the United States and Europe were tested [10], and 2 patients had RT-PCR-negative results at a median of 24 days after symptom onset after previously testing positive (compared with the 17.5 days for duration of viremia), and our findings are consistent with these reports. Other reports, however, have found no evidence for EBOV RNA persistence in vaginal fluid, including 44 vaginal swabs from 20 convalescent females collected at 12 to 57 days after symptom onset that were all negative for

viral antigen by enzyme-linked immunosorbent assay and virus isolation [11], and 21 convalescent females who tested negative by RT-PCR at 40 to 190 days after symptom onset [12].

These findings confirm that short-term EBOV RNA persistence is possible in female survivors and call for closer attention to possible sexual transmission of Ebola virus in the immediate convalescent period. There is not currently sufficient information to quantify the risk of EBOV transmission through sexual intercourse from women with virus RNA detected in vaginal fluid. During the 2014–2016 epidemic, there were not any known cases of EVD linked to sexual activity with a recently recovered female survivor, which suggests that this transmission pathway, although plausible, may be uncommon. In any case, the persistence of EBOV RNA in our case and others raises concern for transmission after exposure to vaginal secretions.

After the virus RNA was no longer detectable in the blood, different clinical sequelae could still be observed for the patient reported here, especially from the central nervous system, including depersonalization, nightmares, and insomnia, and the ocular system, including hyperemic sclera and eye pain. Similar clinical sequelae of EVD survivors have been previously described, in some cases persisting for many months or years [13, 14]. It is currently unknown what host or environmental factors, if any, contribute to EBOV persistence and sequelae, although it has been demonstrated that the virus can evade detection and linger in immune-privileged sites like the eyes, testes, and central nervous system, where it may continue to actively replicate for several months after resolution of viremia [14]. EBOV RNA has been demonstrated in vaginal fluid, saliva, sweat, stool, rectal swab, and urine for variable but more limited time periods postrecovery, which may reflect delayed clearance from the body rather than active replication and persistence [12, 15].

EBOV-specific glycoprotein and nucleoprotein T-cell responses were detected at days 36 and 57 in this patient, concordant with previous reports [16, 17], which illuminated unconventional immune memory to EBOV. Six Sudan virus survivors had serum levels of glycoprotein-specific IgG 12 years after disease onset [17]. In 2014, 2 EVD survivors cared for in the United States had Ebola-specific T cells with activation markers 1 month postdischarge and memory CD8⁺ T cells up to 144 days after disease onset [18]. The EBOV-specific immune memory including IgG and T-cell responses of the female Ebola survivor in this study demonstrated continued expression for at least 2 months after disease onset. Evaluation of the immune response during and after EBOV infection is important to elucidate the relationship between activated immune responses and virus RNA persistence in the body fluids of survivors, which need further investigation.

As mentioned, this patient also received convalescent plasma on day 3 after disease onset. However, the IgG level we tested on day 4 was detected at a low level but increased to 1:400 on

day 15 and was maintained at at least this level afterward. These data may indicate that the convalescent plasma intervention did not notably impact IgG response against EBOV nucleoprotein. Furthermore, whether immunotherapy has an effective impact on viral clearance, especially at the immune-privileged sites, needs further evidence-based investigation.

These findings provide a general profile for the clinical and laboratory features of EBOV disease in a female case from the time of acute disease into convalescence. The persistence of EBOV RNA within the vaginal fluids of the female survivor for a short period of time underscores the need to provide all EVD survivors with preventive, gender-sensitive behavioral counseling on safe sex and with a sufficient supply of condoms.

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Ethics approval. The research protocol was approved by institutional review boards or ethics committees of the Sierra Leone Ministry of Health and Sanitation, the World Health Organization, the United States Centers for Disease Control and Prevention, and the Chinese Center for Disease Control and Prevention. The participant gave written informed consent. The procedures in the study were in accordance with the ethical standards of the Helsinki Declaration of the World Medical Association.

Genome accession number in GenBank. The Ebola genome generated in the current study is available at GenBank under the accession number MF326641.

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