Interpretation of Negative Molecular Test Results in Patients With Suspected or Confirmed Ebola Virus Disease: Report of Two Cases

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Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) is the most sensitive quantitative diagnostic assay for detection of Ebola virus in multiple body fluids. Despite the strengths of this assay, we present 2 cases of Ebola virus disease (EVD) and highlight the potential for false-negative results during the early and late stages of EVD. The first case emphasizes the low negative-predictive value of qRT-PCR during incubation and the early febrile stage of EVD, and the second case emphasizes the potential for false-negative results during recovery and late neurologic complications of EVD. Careful interpretation of test results are needed to guide difficult admission and discharge decisions in suspected or confirmed EVD.

Keywords. Ebola; hemorrhagic fever; Liberia; PCR; West Africa.

Presumptive diagnosis of Ebola virus disease (EVD) is dependent upon a combination of epidemiologic risk factors with a compatible clinical syndrome [1–4]. Confirmation of EVD during the 2014–2015 West Africa outbreak has been largely reliant on quantitative reverse-transcription polymerase chain reaction (qRT-PCR) testing, the gold standard diagnostic assay for detecting and quantifying Ebola virus (EBOV) [5]. Between August 2014 and March 2015, the US Food and Drug Administration (FDA) issued Emergency Use Authorizations for 8 molecular assays for EBOV [6]. At present, no FDA-approved molecular assay for the detection of EBOV is available for routine use.

In this study, we report 2 cases of EVD and highlight challenges in admission and discharge decisions despite ready availability of EBOV molecular diagnostic testing. The first case reveals the low negative-predictive value of EBOV PCR in blood during incubation and the early febrile stage of EVD, and the second case highlights the potential for false-negative results during recovery and late complications of EVD. Negative molecular test results cannot be relied upon to rule out infection during the early febrile stage of EVD; in patients with a high-pretest probability, serial testing is recommended [5, 7]. False-negative results can occur late in EVD, and this is attributable to 1 or multiple factors including improper specimen collection, transportation, or storage, errors in specimen preparation, or factors related to the assay or equipment performance. Careful clinical interpretation of results is needed to guide admission and discharge decisions.

CASE 1

On September 25, 2014, a 6-month-old boy presented with his mother to the Médecins Sans Frontières (MSF) ELWA-3 Ebola Management Center (EMC) in Monrovia, Liberia. The mother exhibited symptoms of EVD, and all primary family members died with syndromes consistent with EVD. The child was asymptomatic on presentation and breastfeeding from his mother, but he had no other notable medical history. The mother and child were admitted to the suspect tent in the high-risk zone of the EMC for qRT-PCR EBOV testing from blood, which was performed by an adjacent National Institutes of Health (NIH)-supported laboratory. An effort was made to isolate the child from the mother and other suspect cases during the testing interval. The mother's test returned positive, and the child's test was negative with a PCR cycle threshold (Ct) value of 39 (Figure 1). The local cutoff for a "negative" test result was a Ct value ≥35, and during this time in Monrovia there was no indeterminate range used. The mother was transferred to a confirmed tent in the high-risk zone, and the asymptomatic boy

Received 13 August 2015; accepted 10 September 2015.

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Open Forum Infectious Diseases

DOI: 10.1093/ofid/ofv137

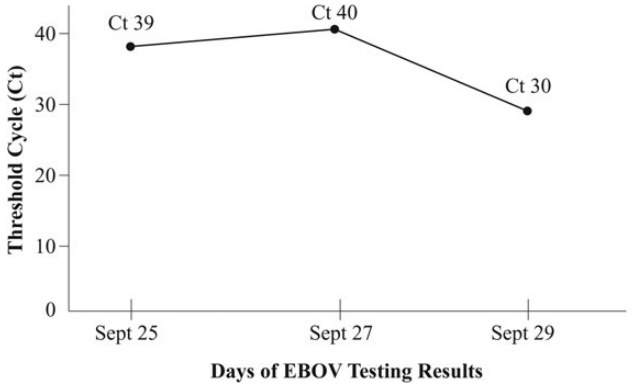

Figure 1. Case 1: Initial results of whole blood testing for Ebola virus with cycle threshold (Ct) in Monrovia, Liberia 2014.

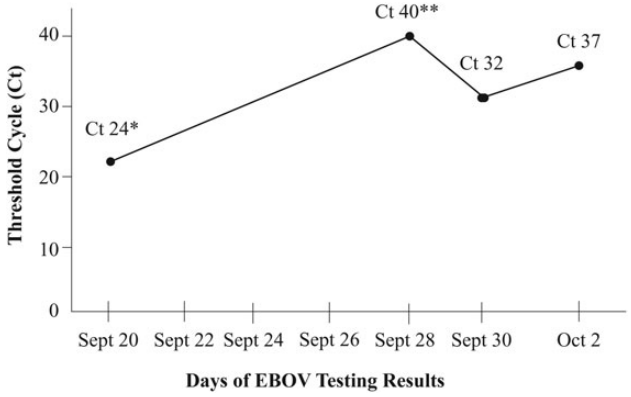

Figure 2. Case 2: Initial results of whole blood testing for Ebola virus with cycle threshold (Ct) in Monrovia, Liberia 2014. *Positive for malaria retrospectively using polymerase chain reaction test. **False-negative quantitative reverse-transcription polymerase chain reaction test based upon follow-up laboratory control and retrospective viral load analysis results.

was discharged under observation. It was normal practice to only hold and retest symptomatic patients.

Because the child had no immediate family members, he was cared for by EMC staff in an observation tent for individuals who tested negative for EBOV and were discharged from either the suspect or confirmed tents. The staff wore "light" personal protective equipment (PPE) consisting of scrubs, boots, surgical gown, single pair of exam gloves, surgical mask, and routine use of hand washing with 0.05% chlorine. On September 27, 2014, 2 days after discharge from the suspect tent, the child developed fever to 38.4°C, without other symptoms of EVD. Repeat EBOV testing was again negative (Ct 40; Figure 1), and he continued to be observed in the low-risk area. On September 29, 2014, 4 days after discharge from the suspect tent in the setting of persistent fever, repeat EBOV testing returned positive (Ct 30; Figure 1). The boy was admitted to the confirmed tent in the high-risk zone and subsequently died of EVD. No staff members who cared for the child became infected.

CASE 2

On September 20, 2014, a 9-year-old boy was brought with his mother and 2 siblings to ELWA-3. They met the suspect case definition for EVD and were transferred to the suspect tent in the high-risk zone, and they tested positive for EBOV by qRT-PCR from blood (Ct 24; Figure 2). The mother died in the suspect tent, and the 3 children were transferred to the confirmed tent. One of the 2 siblings died, and the other recovered and was discharged. At the time of presentation, the 9-year-old boy complained of fever for 5 days and progressive weakness, vomiting, and diarrhea. No other significant medical history was reported. On admission to the confirmed tent, he was confused and apathetic. He was treated with oral rehydration solution as well as metoclopramide and loperamide for vomiting and diarrhea. Empiric treatment for malaria and bacterial coinfection was provided with artemether-lumefantrine and cefixime per MSF protocol. Fever and gastrointestinal symptoms gradually resolved, and he began to walk and communicate. However, he continued to have neurocognitive abnormalities with delayed cognition and difficulty formulating complex sentences, which was believed to represent possible long-standing developmental delay. On September 28, 2014, in the absence of other symptoms for 3 days and 13 days postinitial symptom onset, repeat EBOV testing was negative (Ct 40; Figure 2).

The boy was discharged and transported to his community, but he was not recognized there. He returned to the EMC, was placed in the observation tent in the low-risk zone, and cared for by staff in light PPE while further disposition options were considered. On September 30, 2014, 15 days postinitial symptom onset, he developed fever to 38.6°C with associated neck rigidity, severe weakness, and diminished consciousness. A malaria rapid diagnostic test was negative, and empiric treatment for bacterial meningitis was initiated with ceftriaxone. Repeat EBOV testing was performed, which returned positive (Ct 32; Figure 2), and he was readmitted to the confirmed tent in the high-risk zone. Fever and neck rigidity subsided, and on October 2, 2014, 17 days postinitial symptom onset, repeat EBOV testing was performed. Results showed a rising PCR Ct value consistent with a down-trending viral load in blood (Ct 37; Figure 2). The boy survived and was released to a newly opened orphanage. No staff member who cared for the boy subsequently became infected.

Retrospective Quantification of Viral Load

We sought to precisely characterize viral expansion in the blood of these 2 patients by retrospectively quantifying the viral copy number in each blood specimen. Using an external control specimen with known viral copy number, we established a standard curve for comparison to our patients' blood specimens by q-PCR.

Case 1 had no detectable viral copies in blood from September 25 or 27, 2014 during viral incubation and early febrile disease, but he had 6.0×10^5 viral copies/mL detected from the September 29, 2014 specimen, representing greater than a 5-log expansion in 2 days. Case 2 presented to ELWA-3 during the gastrointestinal stage of illness on September 20, 2014, 5 days after initial symptom onset. Retrospective quantification of viral copy number in blood from September 20, 2014 showed 3.9×10^6 viral copies/mL, consistent with high viral load typically observed during the gastrointestinal stage of EVD, and was positive for malaria. A retrospective analysis of the September 28, 2014 specimen obtained after resolution of gastrointestinal symptoms but in the presence of neurocognitive abnormalities showed that no RNA, neither viral nor host, was present, indicating that an error in specimen handling or RNA extraction likely led to a false-negative result. Retrospective analysis of specimens from September 30 and October 2, 2014 show down-trending viral copy number in blood at 3.1×10^4 copies/mL and 2.3×10^2 copies/mL, respectively.

DISCUSSION

Presumptive diagnosis of EVD is based upon a compatible clinical syndrome and epidemiologic risk factors. Early clinical syndrome of EVD consists of fever, typically associated with lethargy and malaise, which overlaps with many commonly observed infectious syndromes in West Africa, most notably malaria [8]. The presence of epidemiologic risk factors assists in risk-stratifying patients under consideration for EVD, but these risk factors are less helpful when Ebola is widespread in the community. Although clinical case definitions for suspected EVD provide useful guidelines for providers making triage decisions, these definitions are imprecise [4].

Molecular testing for EBOV may reliably confirm EVD within hours, providing an essential tool for management of the disease. However, a recognized limitation of EBOV PCR is the low negative-predictive value during EBOV incubation and early febrile stage of disease. Difficult triage decisions routinely arose at ELWA-3 at the peak of the outbreak. Case 1 presented a distinct dilemma, high-risk exposure but no fever on presentation. Given that the patient had no alternative caregiver and was at high risk for becoming EBOV PCR positive, he was admitted to the suspect tent of the EMC. The boy's EBOV PCR from blood became positive on September 29, 2014, 4 days after initial presentation to the EMC and 2 days after fever onset. With an average EBOV incubation period of 6–8 days, his presentation is most consistent with infection before EMC admission.

This case reinforces that circulating EBOV remains below the level of detection of sensitive molecular assays during incubation and early febrile illness, and it also highlights the rapid rate of rise of virus in blood over a short time interval. Patients with high-pretest probability of EVD require serial testing and close clinical observation.

Current understanding of EVD pathogenesis suggests that EBOV enters the body through mucosal or skin barrier breaches, encountering the mononuclear phagocyte system. Infected macrophages and dendritic cells migrate to lymph nodes with viral replication, indirectly inducing lymphocyte depletion, and release into blood [9]. Fever, driven by cytokines, typically precedes viral detection in blood. Early after fever onset, viral tropism for multiple cell types results in exponential viral growth [10, 11]. In Case 1, exponential viral growth occurred over a 2-day period in which viral copy number in blood went from undetectable 2 days postfever onset to almost 6 million copies/mL 4 days postfever onset. In the absence of novel diagnostic tests that do not rely upon circulating virus, early diagnosis of EVD depends upon careful interpretation of molecular diagnostic test results in the context of clinical and epidemiologic data. In addition, during early epidemic response with limited resources and overwhelming needs, infection control management of those patients at high risk for EBOV, yet test negative, is especially challenging because of the length of incubation.

The second case highlights that false-negative molecular test results for EBOV may occur in the later stages of EVD, reinforcing the need to carefully interpret molecular test results in the context of clinical factors. Per MSF protocol, this patient underwent PCR testing 3 or more days after resolution of symptoms to determine eligibility for discharge. Despite resolution of symptoms, however, notable neurocognitive abnormalities persisted. These neurological abnormalities progressed to a clinical syndrome most consistent with meningoencephalitis. In review, the persistent neurological symptoms in our patient might have prompted consideration of a false-negative result and the need for repeat testing before discharge.

Retrospective analysis of the September 28, 2014 blood specimen indicated that an error in RNA extraction resulted in a false-negative test result. In Liberia, the epidemic peaked in late September. At this time, staff-patient ratios were low and specimen-testing volume was high. Under these conditions errors can be expected, and issues with suboptimal specimen volume, use of incorrect collection tubes, long specimen transport times, unreliable cold chain, and equipment malfunction all contributed to the potential for false-negative test results [12].

A consensus has not been reached on uniform criteria for safe discharge of EVD patients, and some experts maintain that resolution of clinical symptoms alone without the need for molecular testing is adequate [13, 14]. Others, including the World Health Organization, recommend symptom resolution for 3 days and/or 2 consecutive negative PCR tests 48 hours apart [7, 15]. At this time, MSF maintains recommending discharging EVD patients who are asymptomatic for 3 days and have a single negative PCR result. The likelihood of patients having persisting EBOV infection meeting these discharge criteria is believed to be extremely low [15]. From a public health perspective during an escalating epidemic period, withholding discharge would likely

be counterproductive [16]. However, clinicians must be aware of the potential for false-negative PCR results during resolution of EVD. Therefore, it is recommended that if the clinical context is unclear, 2 negative EBOV PCR tests should be used for discharge eligibility.

CONCLUSIONS

In summary, these cases highlight the need for careful interpretation of EBOV PCR results in the context of clinical and epidemiological factors. In the absence of early diagnostics not reliant on circulating virus, serial testing of patients at high risk for EVD and close clinical observation are recommended. Improved understanding of the kinetics of live virus versus molecularly detectable virus in various body fluids during resolution and late complications of EVD will guide improved decision making at the time of discharge. In the meantime, multiple factors will play a role in discharge decisions, including but not limited to availability of staffing, patient volume, diagnostic capabilities, and bed space in the isolation ward.

Acknowledgments

We thank the staff of ELWA-3 and the National Institutes of Health/Centers for Disease Control and Prevention laboratory in Monrovia, Liberia for hard work and dedication during the Ebola epidemic of 2014–2015.

This case series report used routine program data, which was deidentified; therefore, informed consent was not indicated. The study protocol was evaluated and approved by the ethics committees of Médecins Sans Frontières, Brussels, Belgium and University of Liberia, Monrovia, Liberia.

Financial support. This research was supported in part by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). All other expenses came from the routine budgets of each author's affiliation.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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