# Clinical features and viral kinetics in a rapidly cured patient with Ebola virus disease: a case report

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## Summary

**Background** **A detailed description of viral kinetics, duration of virus shedding, and intraviral evolution in different body sites is warranted to understand Ebola virus pathogenesis. Patients with Ebola virus infections admitted to university hospitals provide a unique opportunity to do such in-depth virological investigations. We describe the clinical, biological, and virological follow-up of a case of Ebola virus disease.**

**Methods** **A 43-year-old medical doctor who contracted an Ebola virus infection in Sierra Leone on Nov 16, 2014 (day 1), was airlifted to Geneva University Hospitals, Geneva, Switzerland, on day 5 after disease onset. The patient received an experimental antiviral treatment of monoclonal antibodies (ZMAb) and favipiravir. We monitored daily viral load kinetics, estimated viral clearance, calculated the half-life of the virus in plasma, and analysed the viral genome via high-throughput sequencing, in addition to clinical and biological signs.**

**Findings** **The patient recovered rapidly, despite an initial high viral load (about $1\times10^7$ RNA copies per mL 24 h after onset of fever). We noted a two-phase viral decay. The virus half-life decreased from about 26 h to 9·5 h after the experimental antiviral treatment. Compared with a consensus sequence of June 18, 2014, the isolate that infected this patient displayed only five synonymous nucleotide substitutions on the full genome (4901A→C, 7837C→T, 8712A→G, 9947T→C, 16201T→C) despite 5 months of human-to-human transmission.**

**Interpretation** **This study emphasises the importance of virological investigations to fully understand the course of Ebola virus disease and adaptation of the virus. Whether the viral decay was caused by the effects of the immune response alone, an additional benefit from the antiviral treatment, or a combination of both is unclear. In-depth virological analysis and randomised controlled trials are needed before any conclusion on the potential effect of antiviral treatment can be drawn.**

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## Introduction

More than 25 000 cases[1] of Ebola virus disease have been reported in west Africa since the start of the epidemic. The main clinical characteristics and mortality (up to 74%)[2] described in patients from Sierra Leone[2–4] and Guinea[5] show a pattern of disease similar to those of previous outbreaks.[6] Thorough virological investigations have not been done in the field because of little access to technical resources and biosafety constraints.[7] A detailed description of viral kinetics, duration of virus shedding, and intrahost and interhost viral evolution in different body sites is thus warranted. Patients with Ebola virus disease admitted to specialised university hospitals provide a unique opportunity to do these types of investigations.[8–11] These patients are likely to be exposed to experimental antiviral treatment available for compassionate use without formal proof of safety or efficacy in human beings.[12] Such non-controlled observations have serious limitations, but could nevertheless provide information on potentially unexpected side-effects or benefits of new therapeutics. We describe the detailed clinical, biological, and virological follow-up of a case of Ebola virus disease, including Ebola virus sequence analysis with high-throughput sequencing (HTS) and estimation of viral clearance and half-life in plasma before and after experimental treatment is given.

## Patient history

A previously healthy 43-year-old medical doctor working in an Ebola treatment unit in Sierra Leone[13] developed fever on Nov 16, 2014, defined as day 1 of disease onset. Headache and flu-like symptoms were quickly noted during the next few days and the patient was rapidly diagnosed with Ebola virus disease by positive Ebola virus real-time reverse transcription PCR (RT-PCR) done at the Public Health England laboratory in Kerry Town (Freetown), Sierra Leone (day 2 of disease onset). The exact exposure incident leading to contamination remains unidentified. The patient was admitted to the UK Defence Medical Services Ebola Treatment Unit where he received supportive care. A central venous catheter was inserted on day 3 of the disease. On day 4, the patient received fresh frozen plasma, platelets, one dose of tranexamic acid along with vitamin K, and ceftriaxone. On day 5, the patient was airlifted to Geneva University Hospitals, Geneva, Switzerland.

## Research in context

### Evidence before this study

24 patients with Ebola virus infections have been treated in high-resource hospital settings in Europe and the USA as of Feb 10, 2015. University hospitals provide a unique opportunity to study viral kinetics, duration of virus shedding, and intrahost and interhost viral evolution of the disease in different body sites. Additionally, medically evacuated patients have a higher probability of being exposed to specific antiviral treatments. Specific clinical and biological pictures are still scarce because of limited technical resources in the field. We did a comprehensive scientific literature search of PubMed from Jan 1, 1976, to Jan 15, 2015, using the search terms "Ebola virus", "genetic variation", "sequencing", "sequence analysis", "viral load", "Ebola virus disease", "clinical manifestations", "cutaneous manifestations", "laboratory", "antiviral", and "treatment" in various combinations without any language restrictions. We found three case reports describing the clinical and virological course of evacuated patients with Ebola virus disease. All report prolonged shedding of virus in different body fluids. Use of experimental treatment was described in two patients treated with ZMapp; the effect on viral load was difficult to assess because by the time the patients arrived in the USA, the viral load was already low. Cutaneous manifestations of Ebola virus disease are known and reported in 3–20% of patients. The Ebola virus mutation rate is estimated to be about $9{\cdot}6 \times 10^{-4}$ substitutions per site per year. Gire and colleagues suspected a mutation rate higher than this figure during the outbreak after analysis of 99 Ebola virus sequences, and Scheuerman and colleagues described only 51 nucleotide changes in 100 full-length genome sequences collected for 3 months.

### Added value of this study

In our patient with an Ebola virus infection, we characterised viral decay. We showed a two-phase decay, with a strong acceleration after initiation of antiviral treatment, which could suggest a treatment effect. We did high-throughput analysis of viral RNA and noted only five synonymous mutations. 5 months of intense human-to-human transmission had only a small effect on virus adaptation. We monitored viral loads in different body sites on a daily basis. By contrast with other reports, we did not detect the virus in sweat, but we did detect it in the anal swab for a long period, which is important in terms of infection control. We were able to provide illustrations of two specific signs of Ebola virus disease: first, a photograph of the skin rash and, second, a blood smear showing activated lymphoplasmocytes.

### Implications of all the available evidence

Acceleration of viral decay after initiation of specific antiviral treatment suggests a treatment effect, but appropriate randomised controlled trials are needed to substantiate the effect of any treatment. However, if the number of affected patients continues to decrease, not enough patients might be available to include in this type of investigation and so observations as presented here will remain the only evidence. Although our results suggest that Ebola virus does not evolve substantially, more viral RNA sequencing than was done in this study is still needed to draw any conclusion on viral evolution at the population level.

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## Methods

The patient was treated in a negative-pressure isolation room located in the intensive care unit of Geneva University Hospitals. A team of dedicated nurses was assigned to patient care and supervised by senior infectious diseases, infection control, and intensive care specialists.

ZMAb, a combination of three human–mouse chimeric monoclonal antibodies recognising distinct Ebola virus glycoprotein epitopes named c1H3, c2G4, and c4G7, was provided by the Public Health Agency of Canada (MB, Canada). ZMAb differs from ZMapp, another experimental treatment for Ebola virus disease, in replacement of c1H3 with another chimeric monoclonal antibody called c13C6 and a different manufacturing process.[14] Favipiravir, provided by Toyama Chemical (Tokyo, Japan), is a potential RNA polymerase inhibitor, which might also act as a mutagen.[15]

On admission to Geneva University Hospitals, we started saline and Ringer lactate infusions, low-dose heparin, and parenteral nutrition. Less than 3 h after the patient's arrival, we gave a ZMAb infusion at the dose of 50 mg/kg for 12 h, followed by a second infusion on day 8 (same dose, given for 15 h). We started favipiravir after the first infusion for 6 days at a dose of 6000 mg during the first 24 h (2400 mg–2400 mg–1200 mg every 8 h), followed by 1200 mg twice daily. We empirically switched ceftriaxone to imipenem on day 6 and stopped on day 7.

For RT-PCR, we collected blood in $K_2$ EDTA (edetic acid)-containing plastic plasma tubes and urine in plastic urinalysis tubes without additives. We collected saliva, skin, and conjunctival and rectal swabs in 3 mL universal transport medium tubes (Copan, Brescia, Italy). We inactivated 400 µL of specimens (plasma, native urine, or swabs) with 1 mL of lysis buffer (EasyMAG Lysis Buffer; bioMérieux, Geneva, Switzerland), after which we extracted nucleic acids (NucliSENS easyMAG; bioMérieux), with a final elution volume of 50 µL. We did Ebola virus real-time RT-PCR with the Altona RealStar Filovirus Screen RT-PCR Kit 1.0 (Altona, Hamburg, Germany) and LightMix Modular Ebola Virus Zaire assay (Roche, Rotkreuz, Switzerland), according to the manufacturers' recommendations. All of these assays were done at the Swiss Reference Centre for Emerging Viral Diseases located at the Laboratory of Virology, Geneva University Hospitals. We did procedures in an enhanced P3 facility according to local regulations. We screened for presence of other pathogens with multiplex PCR (Fast-track Diagnostics tropical fever core;