Acute respiratory distress syndrome after convalescent plasma use: treatment of a patient with Ebola virus disease contracted in Madrid, Spain

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Summary

Background In the current epidemic of Ebola virus disease, health-care workers have been transferred to Europe and the USA for optimised supportive care and experimental treatments. We describe the clinical course of the first case of Ebola virus disease contracted outside of Africa, in Madrid, Spain.

Methods Herein we report clinical, laboratory, and virological findings of the treatment of a female nurse assistant aged 44 years who was infected with Ebola virus around Sept 25–26, 2014, while caring for a Spanish missionary with confirmed Ebola virus disease who had been medically evacuated from Sierra Leone to La Paz-Carlos III University Hospital, Madrid. We also describe the use of experimental treatments for Ebola virus disease in this patient.

Findings The patient was symptomatic for 1 week before first hospital admission on Oct 6, 2014. We used supportive treatment with intravenous fluids, broad-spectrum antibiotics, and experimental treatments with convalescent plasma from two survivors of Ebola virus disease and high-dose favipiravir. On day 10 of illness, she had acute respiratory distress syndrome, possibly caused by transfusion-related acute lung injury, which was managed without mechanical ventilation. Discharge was delayed because of the detection of viral RNA in several bodily fluids despite clearance of viraemia. The patient was discharged on day 34 of illness. At the time of discharge, the patient had possible subacute post-viral thyroiditis. None of the people who had contact with the patient before and after admission became infected with Ebola virus.

Interpretation This report emphasises the uncertainties about the efficacy of experimental treatments for Ebola virus disease. Clinicians should be aware of the possibility of transfusion-related acute lung injury when using convalescent plasma for the treatment of Ebola virus disease.

Funding La Paz-Carlos III University Hospital.

Introduction

The 2014 epidemic of Ebola virus disease in west Africa is the largest outbreak of the disease ever recorded.1 In August, 2014, two health-care workers were evacuated from Liberia to the USA.2 Since then, 27 patients have been treated for Ebola virus disease in high-income settings. At La Paz-Carlos III University Hospital, Madrid, Spain, we treated two missionaries who had been medically evacuated from Liberia and Sierra Leone. Both patients died less than 1 week after evacuation. On Oct 6, 2014, a secondary case, caused by the first known human-to-human transmission of Ebola virus disease outside of Africa, was reported, and the patient was transferred to our isolation unit. Details about diagnosis and initial treatment of this case have been previously reported.3 Here, we provide a detailed description of the subsequent disease course, care, and treatment.

Patient history and findings on admission

The patient was a 44-year-old woman, working as a nurse assistant at La Paz-Carlos III University Hospital. She was a smoker and had a history of hypercholesterolaemia. She had participated in the care of the two missionaries who were medically evacuated from west Africa to Spain because of Ebola virus disease. The first of these patients had been transferred from Liberia to our hospital on Aug 7, and died on Aug 12, 2014. On Aug 30, the nurse assistant completed the 21 days surveillance period after the last interaction with the first patient. The second patient was transferred from Sierra Leone to our hospital on Sept 22, and died on Sept 25, 2014. The nurse assistant had two interactions with the second patient, during which she was wearing personal protective equipment. On Sept 23, she entered the room and changed an adult diaper soiled with diarrhoeic stool. On Sept 25, about 1 h after the corpse had been removed from the room, she entered the room to dispose of medical and non-medical materials (bed linen, gauzes, compresses, and discarded drugs) in a dedicated biosafety container. She stayed in the room for almost 1 h. Two other nurse assistants entered the room with our patient simultaneously to remove the bed mattress.

On Sept 29 (day 1 of the illness), the nurse assistant developed fever (38·5°C) and malaise, and she started...
taking antipyretic drugs (paracetamol and ibuprofen). From days 1 to 7, her axillary temperature oscillated between 36·7°C and 37·9°C. On day 4, she went to see her primary care physician because of isolated low-grade fever. A limited physical examination (throat inspection, her primary care physician was unaware of the patient's potential contact with Ebola virus and recommended treatment paracetamol.

From days 5 to 7, the patient progressively developed headache, myalgias, fatigue, emesis, non-bloody diarrhoea, and dry cough. On day 8, her condition worsened, with temperature increasing up to 38·6°C, frequent vomiting, intense non-bloody diarrhoea, and development of a maculopapular rash. She was transferred by ambulance to the emergency room of Fundación Alcorcón University Hospital (Madrid, Spain) and her blood sample tested positive for Ebola virus on a real-time reverse-transcriptase PCR (RT-PCR) assay (appendix). In the emergency room, she was noted to be hypotensive (90/60 mm Hg) and received treatment with intravenous fluids until she was transferred to our isolation unit at La Paz–Carlos III University Hospital on Oct 7, 2014.

On admission to the isolation unit, the patient complained of increasing malaise, thirst, nausea, sore throat, and diffuse myalgias, as well as episodic, mild, cramp-like abdominal pain and cough that occasionally produced dark sputum. She reported that she did not have dyspnoea, chest pain, epistaxis, gingival
haemorrhage, or rectorrhagia. On admission to the isolation unit she was in the first day of her menstrual cycle. Her temperature was 39·0°C, blood pressure was 105/60 mm Hg, and oxygen saturation was 95% while she was breathing ambient air. She was awake, alert, and fully oriented. Lingual mucosa was dry and she had bilateral conjunctival injection. A diffuse maculopapular rash covered her trunk. Auscultation was not possible because of the personal protective equipment worn by all attending staff. The patient's abdomen was soft and nontender.

Laboratory tests showed haemoglobin concentrations, platelet count, and leucocytosis within normal ranges (table). The patient's kidney function was normal with a creatinine concentration of 97·24 μmol/L (1·1 mg/dL). We noted evidence of damage in several tissues: concentrations of liver enzymes were greatly increased, with aspartate aminotransferase at 686 U/L, alanine aminotransferase at 275 U/L, lactate dehydrogenase greater than 4000 U/L, and creatinine phosphokinase at 451 U/L. Magnesium concentration was 0·66 mmol/L (1·6 mg/dL) and total calcium concentration was 1·67 mmol/L (6·7 mg/dL). International normalised ratio was 1·5. The patient's blood group was tested on admission to the isolation unit and was O+.

**Treatment setting**

**Personal protective equipment**

Staff members working in our isolation unit used personal protective equipment certified in accordance with Spanish and European directives. Personal protective equipment did not allow exposure of any skin. The equipment consisted of an impermeable coverall, hood, two pairs of nitrile gloves, two pairs of impermeable waterproof leg coverings, filtering face piece (FFP3) masks, and goggles. Powered air purifying respirators were not used.

**Isolation unit**

The isolation unit was initially designed to care for patients with airborne infectious diseases. The unit had exclusive access controlled with electronic identity cards. The unit includes six individual rooms with airlock anterooms. Rooms were equipped with oxygen, medical air, vacuum intakes, and a mechanical ventilator. Both airlock-room doors have large windows to monitor patients and health-care workers from outside. Use of a negative pressure system—with a minimum of 15 air changes per hour—prevented the opening of both doors simultaneously and air circulating out of the room. The patient was admitted to the same room as the one used for treatment of the second missionary patient. We set up a laboratory unit in one of the negative pressure rooms of the isolation unit. The unit has the facilities for clinical biochemistry, haematology, and blood gas analyses, and coagulation tests were done inside the patient room with a point-of-care system. Laboratory personnel used the same personal protective equipment as the other personnel. Portable radiography was available. The nurse station had an intercom connection with the room and a video monitoring circuit for monitoring the patient and health-care workers in the room and anteroom. We kept a log registering entrances, time spent, and any incidents with the doffing of personal protective equipment.

**Surveillance protocol for health-care workers**

Before the infection of our patient, health-care workers with protected contacts were deemed at low risk of infection. The follow-up protocol consisted of passive surveillance of body temperature. Health-care workers were instructed to take their temperature and call occupational health staff if it exceeded 38·3°C. After our patient became infected, the protocol was changed to active surveillance, including two daily contacts from occupational health staff and a temperature threshold of 37·7°C.

**Ambient surveillance testing**

Ambient surveillance testing for Ebola virus was done on day 18 of illness. 16 samples were collected: three from the airlock anteroom, four from patient room, five from fomites close to the patient, and four from personal protective equipment worn by health-care workers (appendix). All samples tested negative for Ebola virus RNA.

**Clinical course and management**

**Symptomatic and supportive therapy**

Intravenous access was required for treatment. We used metoclopramide, ondansetron, and omeprazole to treat the patient’s emesis and dyspepsia, and paracetamol and metamizole for her fever and pain. For sedation we used haloperidol, lorazepam, and bromazepam as needed. We gave her low-molecular-weight heparin (bemiparin) for deep venous thrombosis once on day 13, but it was stopped because of thrombocytopenia. We used an intra-muscular gonadotropin-releasing hormone agonist (triptorelin) to stop menstruation. We used antibiotic therapy (ceftriaxone followed by piperacillin-tazobactam) empirically; we also used oral vancomycin and metronidazole empirically for suspected *Clostridium difficile* infection (appendix). We used this double treatment for *C difficile* because of the possibility of intestinal ileus that can impair vancomycin distribution in the bowel lumen.

**Baseline fluid and nutrition management**

Replacement of fluid and electrolytes was initially managed with balanced crystalloid solution (Plasmalyte, Baxter, Old Toongabbie, Australia), lactated Ringer’s solution, 5% glucose, isotonic saline solution, and bicarbonate. We estimated rehydration requirements by clinical examination, capillary refill, balance of fluids, blood pressure, respiratory symptoms, oxygen saturation, and laboratory data. Intravenous administration of fluids,
### Table: Clinical variables and laboratory values during the course of illness

<table>
<thead>
<tr>
<th>Day of illness</th>
<th>Clinical variables</th>
<th>Laboratory values</th>
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<tr>
<td></td>
<td>Temperature (°C)</td>
<td>Haemoglobin (g/L)</td>
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<td></td>
<td>Systolic BP (mm Hg)</td>
<td>Haematocrit (%)</td>
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<td></td>
<td>Diastolic BP (mm Hg)</td>
<td>White cells (×10⁹ per L)</td>
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<td>Respiratory rate (breaths per min)</td>
<td>Platelets (×10⁹ per L)</td>
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<td></td>
<td>Oxygen saturation (%)</td>
<td>International normalised ratio</td>
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<td></td>
<td>Heart rate (beats per min)</td>
<td>AST (U/L)</td>
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<td></td>
<td>Oxygen saturation (%)</td>
<td>ALT (U/L)</td>
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<td></td>
<td>Heart rate (beats per min)</td>
<td>Total bilirubin (μmol/L)</td>
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<td></td>
<td>Oxygen saturation (%)</td>
<td>LDH (U/L)</td>
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<td>Heart rate (beats per min)</td>
<td>Creatinine (μmol/L)</td>
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<td>Oxygen saturation (%)</td>
<td>Sodium (mmol/L)</td>
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<td>Heart rate (beats per min)</td>
<td>Potassium (mmol/L)</td>
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<td>Oxygen saturation (%)</td>
<td>Chloride (mmol/L)</td>
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<td>Heart rate (beats per min)</td>
<td>Calcium (mmol/L)</td>
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<td>Oxygen saturation (%)</td>
<td>pH</td>
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<td>Heart rate (beats per min)</td>
<td>Bicarbonate (mmol/L)</td>
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<td></td>
<td>Oxygen saturation (%)</td>
<td>Creatinine (μmol/L)</td>
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<td></td>
<td>Heart rate (beats per min)</td>
<td>Lactate (mmol/L)</td>
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Supplementary oxygen, when required, was given by nasal cannulas (C), high-concentration oxygen mask (H), or Inspiron humidifier (I). The daily maximum respiratory rate, minimum oxygen saturation (as measured by pulse oximetry), and maximum heart rate were assessed by means of continuous measurement on a medical monitor. BP=blood pressure. ND=not determined. AST=aspartate aminotransferase. ALT=alanine aminotransferase. *Difference between input and output volumes.
Electrolytes, and drugs required a peripherally inserted central venous catheter. Low potassium concentrations were corrected with supplements of 40–60 mmol/L per day of potassium chloride.

Oral nutrition was stopped on admission because of gastrointestinal symptoms. From days 12 to 19 of the illness, we gave the patient parenteral nutrition with a three-compartment bag (Oliclinomel Perif N4-550 E 2·5 L IV, Baxter, Old Toongabbie, Australia) containing a lipid emulsion, aminoacids, and glucose solutions. Oral liquid intake was restarted on day 13, and enteral nutrition was reinitiated on day 19, progressing from astringent to normal diet (appendix).

Experimental treatments
Overview
We considered the use of convalescent frozen plasma, favipiravir (Toyama Chemical, Toyama, Japan), and ZMAB (Mapp Biopharmaceutical and LeafBio, San Diego, USA). After obtaining informed consent from the patient for compassionate use, we decided to start treatment with both convalescent plasma and favipiravir.

The appendix shows details about the assessment of the risks and benefits of these experimental treatments. The patient also provided written informed consent for publication of this report.

Convalescent plasma
We obtained convalescent plasma from two different donors (the only two available at that time). Both donors had travelled to Spain to donate convalescent plasma for the second missionary patient.

The first donor was a black woman, aged 36 years, also a missionary, and without previous pregnancies or blood transfusions, who had been diagnosed with Ebola virus disease on Aug 5, 2014, in Liberia. She was discharged from an Ebola treatment centre on Aug 20, 2014. Her blood group was B+. Three units (200 mL each) of plasma were obtained by aphaeresis on Oct 6, 2014. At that time, serological analyses showed positive titres for VP40 viral matrix protein (1:18,000), glycoprotein (1:20,000), and nucleoprotein (1:85,000).

The second donor was a black woman, aged 45 years, also a missionary, and without previous pregnancies or blood transfusions, who had been diagnosed with Ebola virus disease on Aug 7, 2014, in Liberia. She was discharged from an Ebola treatment centre on Aug 25, 2014. Her blood group was O+. Two units (200 mL each) of plasma were obtained by aphaeresis on Oct 6, 2014. At that time, serological analyses showed positive titres for VP40 (1:4000), glycoprotein (1:20,000), and nucleoprotein (1:34,000).

Aphaeresis was done at the haematology department of the La Paz–Carlos III University Hospital. Plasma was not pathogen-inactivated. Tests for Ebola virus, HIV, hepatitis C virus, hepatitis B virus, malaria (all by PCR), and syphilis serology were all negative for both donors. Tests for HLA and human neutrophil antigen (HNA; done retrospectively by flow cytometry and ELISA) were negative for both donors and for the patient.

Plasma was given in accordance with WHO guidance. Before infusion of each 200 mL plasma unit, we pre-medicated the plasma with dextchlorpheniramine maleate and hydrocortisone. Convalescent plasma was given slowly in 1 h infusions. Two units of plasma from the first donor were given 7 h apart on day 9 of illness, and the third unit was given on day 10. The plasma units from the second donor were given on days 11 and 12 (figure 1).

Favipiravir
Favipiravir (200 mg tablets) was given orally mostly in water suspension after crushing the tablets and instructing the patient to drink the whole suspension.

Figure 1 and the appendix show the administration schedule. Three loading doses—8 h apart—were scheduled, but the third loading dose on day 10 was not given because of worsening of previous abdominal pain, without recurrence after restarting favipiravir on day 11.

Due to stock-out, favipiravir was interrupted for 36 h on day 15 of illness; a new loading dose was started on day 16. Favipiravir was finally stopped on day 20 of illness after two consecutive tests showing undetectable Ebola virus plasma viral loads (figure 1).

Favipiravir was well tolerated and no clearly related adverse events were noted. Abdominal pain and hepatitis were deemed related to Ebola virus disease and improved despite favipiravir maintenance.

Complications and management
Acute respiratory distress syndrome due to possible transfusion-related acute lung injury
The patient remained clinically stable until day 10 of illness. 3 h after the infusion of the third unit of convalescent plasma from the first donor, oxygen saturation decreased to 91% (and to 89% that day). Blood pressure decreased to 95/70 mmHg, which was the lowest recorded during the patient’s admission (table). No fever, itching, or urticarial rash were noted. During the subsequent hours on day 11, the patient complained of dyspnoea and had tachypnoea (38 breaths per min). Chest radiographs done on days 11, 12, and 13 of illness, after the administration of two plasma units from the second donor, showed confluent bilateral pulmonary infiltrates consistent with acute respiratory distress syndrome (figure 2A and appendix). The patient maintained low oxygen saturation for 5 days (lowest oxygen saturation <85%) until day 15 of illness. Arterial blood gas measurements were not available since they are difficult to obtain in our isolation suits.

The patient received oxygen initially by nasal cannula for a very short time and through a high-concentration mask for 7 days (from day 11 to day 16 of illness). For management of possible transfusion-related acute lung injury, we forced a negative balance with furosemide for 2 days (table). We also used intravenous methylprednisolone and low doses of morphine.
At discharge, on day 37 of illness, chest radiography was normal (figure 2B).

**Asymptomatic thrombocytopenia**

The patient’s platelet count, within normal range on admission, decreased to $37 \times 10^9$ cells per L on day 15 of illness (table). Thrombocytopenia could be attributed to Ebola virus disease, low-molecular-weight heparin, piperacillin or tazobactam, favipiravir, omeprazole, or transfusion-related acute lung injury.

**Suspected bacterial superinfection**

On day 21 of illness, the patient had low-grade fever ($37.8^\circ$C) and loose stools. We restarted oral vancomycin for suspected *C difficile* infection. We obtained a blood sample and did a commercial multiplex PCR test for diagnosis of sepsis (Septifast, Roche Diagnostics, Mannheim, Germany) on 2 consecutive days (days 22 and 23 of illness), which showed a low amplification of coagulase-negative staphylococci deemed clinically non-significant. We also did a rapid test for *C difficile* glutamate dehydrogenase antigen and toxin A and B detection in stools (C Diff Quick Check Complete Assay, Alere, Blacksburg, USA), which was also negative. Fever disappeared and loose stools changed to normal during the subsequent 3 days. Vancomycin was stopped on day 29.

**Ebola virus RNA load and serological findings**

From day 8 of illness, the patient was tested daily for Ebola virus RNA plasma viral load, with measurements done at the National Centre of Microbiology (Madrid, Spain) by use of RealStar Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics, Izasa, Spain), in accordance with standards provided by the manufacturer. We used the results of these measurements to generate a standard calibration curve to monitor viral load daily (appendix). Samples were sent to the National Reference Centre for Tropical Infectious Diseases at the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany) for serological and viral culture analysis. The presence of Ebola virus-specific IgG and IgM antibodies was determined by means of an immunofluorescence assay with the use of Ebola virus-infected Vero E6 cells as an antigen. Maximum plasma Ebola virus RNA viral load was recorded on day 8.
of illness and viral RNA became undetectable on day 18. Anti-Ebola virus antibody titres steadily increased, with peak titres of 1:2560 for IgM antibodies and 1:20 480 for IgG antibodies on day 16 (figure 1). Antibody titres were not available in real time and therefore could not be used to guide therapeutic decisions.

Recombinant Human Anti-Zaire Ebolavirus IgG ELISAs (Alpha Diagnostic International, San Antonio, TX, USA) for VP40, glycoprotein, and nucleoprotein were done on day 36 of illness. VP40 IgG titres were 1:50 000, glycoprotein IgG titres were 1:4500, and nucleoprotein IgG titres were 1:4500.

Discharge from isolation unit and follow-up

From day 22 to day 34 of illness, we tested saliva, conjunctiva, axillary-region sweat, vaginal fluid, stool, and urine for Ebolavirus RNA with RT-PCR, and repeated the testing every 48–72 h. We discharged the patient from the isolation unit on day 34, when all surveillance samples were negative by PCR in at least two consecutive samples separated by a minimum of 48 h. The last sample that tested negative was sweat (appendix). All positive surveillance samples on day 22 were negative for viable Ebolavirus on culture (30 days incubation).

Laboratory tests done immediately before discharge revealed near normal concentrations of transaminases and creatine kinases (table). Tests for thyroid hormones showed asymptomatic hyperthyroidism (thyroid-stimulating hormone [TSH] 0·07 mUI/L; free thyroxine [T4] 1·84 ng/dL [23·68 nmol/L]; triiodothyronine [T3] 1·64 ng/dL [2·51 nmol/L]), with negative findings for antithyroid antibodies (anti-thyroglobulin and anti-peroxidase) and thyroid-stimulating immunoglobulin. Total cholesterol was 323 mg/dL (8·4 mmol/L; appendix).

2 weeks after discharge, the patient complained about mild arthralgias and asthenia. On physical examination, no tachycardia, thyroid tenderness, or cervical swelling were detected. Laboratory tests showed similar thyroid findings to those recorded before discharge (TSH 0·02 mUI/L; free T4 1·93 ng/dL [24·83 nmol/L]; T3 1·84 ng/dL [23·68 nmol/L]). A thyroid ultrasonogram showed no clinically significant lesions. 6 weeks after discharge, TSH had increased to 2·73 mUI/L and T4 had decreased to 0·79 ng/dL (11·45 nmol/L). A thyroid ultrasonogram showed no clinically significant lesions. 6 weeks after discharge, TSH had increased to 2·73 mUI/L and T4 had decreased to 0·79 ng/dL (11·45 nmol/L).

2 weeks after discharge the patient complained of a foreign body sensation in her left eye, along with floaters and occasional blurred vision. Ophthalmic examination showed unilateral vitreous body detachment without retinal damage, uveitis, or evidence of bleeding. 6 weeks after discharge, ocular symptoms had disappeared without specific treatment.

Patient’s contacts

High-risk contact

15 people (community members and health-care workers) were admitted to our hospital for quarantine because of high-risk contact with our patient during the week preceding admission.5 None of them became infected with Ebolavirus.

Discussion

Here we describe the clinical course of the first case of Ebolavirus disease contracted outside of Africa. This report expands on the information about diagnosis and initial treatment that was reported previously.3 We believe that this case provides important knowledge that could be useful with respect to the treatment of patients with Ebolavirus disease. Our patient became infected despite wearing personal protective equipment that covered all the skin and despite having had experience helping to care for a previous patient with Ebolavirus disease. At the time of contagion, the personal protective equipment used in our unit was more complete than the one originally recommended by WHO, the US Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control, and the Spanish Ministry of Health. We do not know how the contagion happened, but most probably it occurred during donning of the personal protective equipment, which is the riskiest moment. In our centre, one contagion has occurred among 165 exposed workers who have cared for three patients with Ebolavirus disease during 762 exposures. This contagion, along with two others that occurred in a general hospital in Dallas, TX, USA,6 emphasises the need for specialised units with intense training, supervision, and drills to care for patients with Ebolavirus disease and other highly infectious diseases.7

Our patient met criteria for possible transfusion-related acute lung injury.8 The onset of respiratory distress occurred 3 h after completion of transfusion of the third unit of convalescent plasma and no signs of fluid overload were noted. Since tests for HLA and HNA antibodies were negative, transfusion-related acute lung injury was probably non-antibody mediated. We deemed transfusion-related acute lung injury ‘possible’ because Ebolavirus disease was another risk factor for acute lung injury. However, acute lung injury has not been commonly described in Ebolavirus disease. In the current epidemic in west Africa, dyspnoea and other respiratory symptoms have not been reported as frequent occurrences.9–11 Hypoxia is reported only in the terminal phases of multisystem organ failure.12 Besides, research in animal models of Ebolavirus disease showed that in pigs the cytokine response is localised in the lungs, with
very mild systemic involvement, whereas in human beings and non-human primates cytokine response is predominantly systemic.\(^{13}\) A case of severe Ebola virus disease with vascular leakage, pulmonary oedema, and multiorgan failure has been recently reported.\(^{14}\) By contrast with our patient, vascular leakage was accompanied by pleural effusion, severe hypotension, and renal failure. However, although we believe that transfusion-related acute lung injury was the cause of our patient’s acute respiratory distress syndrome, we acknowledge that we cannot exclude other causes such as a direct injury caused by Ebola virus disease with or without a component of vascular leakage.

Clinicians should be aware of the possibility of transfusion-related acute lung injury when they use convalescent plasma for the treatment of Ebola virus disease. In our patient, this diagnosis was not immediately considered and we initially believed that Ebola virus disease was the most likely cause of our patient’s acute respiratory distress syndrome. The effectiveness of convalescent plasma for the treatment of Ebola virus disease is unknown\(^{15,16}\) and clinical trials are ongoing (ClinicalTrials.gov number NCT02295501). Because we also used favipiravir and supportive treatment, it is difficult to ascertain the effect of convalescent plasma in the outcome of our patient. Finally, because of the urgent need, we used plasma that was not pathogen inactivated. Although both donors had been screened for Ebola virus, HIV, hepatitis C virus, hepatitis B virus, and malaria, we recognise that pathogen inactivation should be the standard of care.\(^{17}\)

As well as convalescent plasma, we decided to treat the patient with favipiravir.\(^{18}\) Taking into account the available data (appendix) and the severity of the disease (including the delay in the initiation of the treatment), we decided to increase the dose of favipiravir to maximise the probability of obtaining a minimum plasma concentration above the projected target (60 μg/mL). Despite the high dose used, favipiravir was well tolerated. Thrombocytopenia resolved with the withdrawal of bemiparin and despite continuation of antiviral therapy. Also, abdominal pain and hepatitis seemed more probably related to the disease since these events were present before favipiravir administration and resolved before the drug was stopped. The contribution of favipiravir to disease resolution is difficult to ascertain because we also treated our patient with convalescent plasma and supportive measures. Recent preliminary data from the JIKI trial\(^ {19}\) suggest that favipiravir could be effective in a subgroup of patients with low viral loads, although the study design does not allow a definitive conclusion.

There is limited evidence about the importance of testing bodily fluids after patients with Ebola virus disease have cleared viraemia.\(^{20,21}\) In our patient, testing of bodily fluids for Ebola virus by PCR showed positive results in sweat, saliva, urine, conjunctiva, and vaginal fluid up to 10 days after clearance of viraemia, whereas these samples were culture-negative. By contrast, in a recent study,\(^ {22}\) a patient who contracted Ebola virus disease in Africa and was treated in Germany had culture-positive urine samples 9 days after clearing viraemia. It is still unclear if patients should remain isolated until clearance of bodily fluids by PCR. In Africa, transmission of Ebola virus from convalescent patients once Ebola virus viraemia has cleared has not been reported.\(^ {23}\) However, patients with a high viral load, as in our patient’s, have a very high mortality in Africa.\(^ {7}\) It is possible that excretion of Ebola virus in bodily fluids after clearance of viraemia could happen mainly in patients who have had high viraemias. Therefore, risks of transmission through bodily fluids might be restricted to patients with high viraemias, who only tend to survive in high-income countries because of the availability of advanced supportive care. However, the detection of Ebola virus by PCR in bodily fluids in our case might not represent infectious virus. Guidance for the role of testing for body fluids after disappearance of viraemia and before patient discharge is needed.

Our patient had subclinical hyperthyroidism at the time of discharge and hypothyroidism during follow-up. This is the first time that thyroid impairment has been reported during convalescence of Ebola virus disease. Although we do not have data for thyroid hormone concentrations in our patient from before Ebola virus disease, we believe that post-viral subacute thyroiditis is the most likely diagnosis.

This case also lends support to the belief that risk of transmission of Ebola virus disease is low before patients become fully symptomatic. This low risk is in agreement with the reported estimate of a basic reproduction number for Ebola virus disease of 1·71–2·02 in west Africa.\(^ {24}\) Despite several community contacts during 1 week after contagion, nobody else became infected. However, our case also emphasises the need for active surveillance of health-care workers with protected contacts. Initially, our patient was deemed low risk because she was wearing adequate personal protective equipment and no accidents were reported. This assumption delayed diagnosis. Since this contagion happened, national protocols in Spain have been changed and active surveillance of health-care workers by the occupational health department has been implemented. Additionally, temperature threshold to initiate investigation of health-care workers with protected contact was decreased to 37·7°C.\(^ {25}\)

**Contributors**

MM-R, MA, GR-O, FdlC, ML, and JRA wrote the first draft of the report. PS-S, BF-P, AN, and FL processed all laboratory samples. All authors contributed to clinical care of the patient and reviewed and approved the final version of the report.

**Role of the funding source**

The corresponding author had full access to all of the data and the final responsibility to submit for publication.

**Ethics committee approval**

No ethical committee approval was required.
Declaration of interests
We declare no competing interests.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

SUPPLEMENTARY APPENDIX

Acute Respiratory Distress Syndrome after Convalescent Plasma Use in a Patient With Ebola Virus Disease Contracted in Madrid, Spain.

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References
Investigators and collaborators for the Hospital La Paz Carlos III Isolation Unit

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- **Bernhard-Nocht-Institute for Tropical Medicine**: Jonas Schmidt-Chanasit
- **Centro Nacional de Microbiología**: Pedro Anda, Ana Avellón, Josefa Casas, Ave María Coro, Manuel Cuenca, Raquel Escudero, Leticia Franco, Cristina García-Amil, Lourdes Hernández, Laura Herrero, Isabel Jado, Francisca Molero, Marta Ortiz, Fernando de Ory, Arantzazu Potente, Isabel Rodríguez, José Miguel Rubio, Ana Vázquez,
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- **Radiology Technicians:** José Grau, Rosa Marsal, Carmen Ortega.
**Table S1: Review of systems on admission**

<table>
<thead>
<tr>
<th>System</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONSTITUTIONAL</strong></td>
<td>malaise and intense asthenia and myalgia.</td>
</tr>
<tr>
<td><strong>HEENT</strong></td>
<td>conjunctiva injection. No runny nose or epistaxis. Dry mucosae. Sore throat (&quot;like a tightness in her neck&quot;). Dry tongue.</td>
</tr>
<tr>
<td><strong>SKIN</strong></td>
<td>diffuse non-petechial maculopapular rash covering predominantly her trunk. No itching.</td>
</tr>
<tr>
<td><strong>CARDIOVASCULAR</strong></td>
<td>No chest pain or palpitations. No evidence of swelling</td>
</tr>
<tr>
<td><strong>RESPIRATORY</strong></td>
<td>Persistent dry cough.</td>
</tr>
<tr>
<td><strong>GASTROINTESTINAL</strong></td>
<td>Nauseas and anorexia appeared quickly but patient continued to drink some fluids. Non-bloody diarrhea.</td>
</tr>
<tr>
<td><strong>GENITOURINARY</strong></td>
<td>No dysuria or hematuria. First day of her menstrual period</td>
</tr>
<tr>
<td><strong>NEUROLOGICAL</strong></td>
<td>conscious, awake and responsive, fully oriented but drowsy. No dizziness, syncope, paralysis, numbness in the extremities. Moving all extremities, no focal deficit.</td>
</tr>
<tr>
<td><strong>HEMATOLOGICAL</strong></td>
<td>No purpura, petechial or easy bleeding.</td>
</tr>
<tr>
<td><strong>LYMPHATICS</strong></td>
<td>No enlarged nodes</td>
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</table>
### Table S2. Additional Laboratory Parameters

<table>
<thead>
<tr>
<th>Day of Illness</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
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<tr>
<td><strong>Laboratory Parameters</strong></td>
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</tr>
<tr>
<td>Red cells (x10^6/mm³)</td>
<td>4.5</td>
<td>4.3</td>
<td>4.5</td>
<td>4.4</td>
<td>2.9</td>
<td>4.0</td>
<td>4.1</td>
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<tr>
<td>Neutrophils (%)</td>
<td>83.2</td>
<td>89.6</td>
<td>89.0</td>
<td>82.5</td>
<td>74.2</td>
<td>78.0</td>
<td>78.0</td>
<td>67.7</td>
<td>69.8</td>
<td>68.3</td>
<td>57.1</td>
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<tr>
<td>Lymphocytes (%)</td>
<td>14.6</td>
<td>7.4</td>
<td>7.9</td>
<td>11.6</td>
<td>17.2</td>
<td>9.3</td>
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<tr>
<td>Monocytes (%)</td>
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<td>5.9</td>
<td>8.6</td>
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<td>Glucose (mg/dL)</td>
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<td>61</td>
<td>52</td>
<td>110</td>
<td>180</td>
<td>97</td>
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<tr>
<td>Total Proteins (g/dL)</td>
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<td>4.9</td>
<td>5.0</td>
<td>5.2</td>
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<td>5.3</td>
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<tr>
<td>Albumin (g/dL)</td>
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<td>2.1</td>
<td>2.4</td>
<td>2.3</td>
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<td>2.3</td>
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<td>Urea (mg/dL)</td>
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<td>29</td>
<td>38</td>
<td>53</td>
<td>62</td>
<td>51</td>
<td>46</td>
<td>46</td>
<td>35</td>
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<td>&lt;11</td>
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<td>Uric Acid (mg/dL)</td>
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<td>2.4</td>
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<td>3.8</td>
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<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>0.7</td>
<td>1.0</td>
<td>1.3</td>
<td>1.9</td>
<td>1.1</td>
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<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>0.8</td>
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<tr>
<td>ALT/GPT (UI/L)</td>
<td>275</td>
<td>462</td>
<td>424</td>
<td>337</td>
<td>290</td>
<td>265</td>
<td>217</td>
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<td>111</td>
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<tr>
<td>Magnesium (mg/dL)</td>
<td>1.6</td>
<td>3.2</td>
<td>2.4</td>
<td>2.0</td>
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<td>2.2</td>
<td>2.3</td>
<td>2.3</td>
<td>2.1</td>
<td>2.0</td>
<td>1.9</td>
<td>1.8</td>
<td>2.0</td>
<td>1.9</td>
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<tr>
<td>Total Calcium (mg/dL)</td>
<td>6.7</td>
<td>6.9</td>
<td>6.4</td>
<td>7.6</td>
<td>6.5</td>
<td>7.6</td>
<td>7.4</td>
<td>6.9</td>
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<tr>
<td>Ionic Calcium (mmol/L)</td>
<td>1.06</td>
<td>1.06</td>
<td>1.01</td>
<td>0.99</td>
<td>1.06</td>
<td>1.07</td>
<td>1.09</td>
<td>1.18</td>
<td>1.16</td>
<td>1.21</td>
<td>1.20</td>
<td>1.21</td>
<td>1.21</td>
<td>1.23</td>
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<tr>
<td>Phosphorus (mg/dL)</td>
<td>-</td>
<td>1.4</td>
<td>1.9</td>
<td>2.2</td>
<td>1.4</td>
<td>1.8</td>
<td>2.5</td>
<td>2.0</td>
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<td>2.9</td>
<td>2.6</td>
<td>2.6</td>
<td>2.8</td>
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</tbody>
</table>
Considerations of experimental therapy

At the time of diagnosis –day of illness 8- we had convalescent frozen plasma available. In the first 24 hours of admission (day of illness 9) we had also available Favipiravir (T705) and at day 11 ZMab (murine antibodies cocktail against EBOV). Neither Brincidofovir (CMX001) nor TKM-Ebola (small-inhibitory RNA targeting three out of seven Zaire-EBOV proteins) were available. The medical team made all decisions regarding experimental treatments with technical support from Hospital La Paz-Carlos III Pharmacology Department. We obtained oral informed consent from the patient.

1) ZMab.

ZMab (Mapp Biopharmaceutical and LeafBio) is a cocktail of three different murine antibodies against EBOV Zaire. In contrast with Zmapp these antibodies are not humanized. Therefore the risk of severe reaction during the infusion could be increased. Given that patient was suffering acute respiratory distress syndrome, the team considered that a severe allergic reaction was not going to be tolerated by the patient. In addition EBOV viral load had already declined at the time of drug reception.

2) EVD convalescent plasma.

Considering immediate availability along with WHO recommendations we decided to use convalescent serum from two different donors on a daily basis (except for the first 2 doses that were 7 hours apart). There were ABO and RhD grouping compatibility. After acute respiratory distress syndrome developed we did not immediately considered the possibility of TRALI and for this reason we administered plasma from the second donor.

3) Favipiravir

Favipiravir (Toyama Chemical) is a new viral RNA polymerase inhibitor already approved in Japan for the treatment of influenza. According to previous recent in vivo and in vitro data of Favipiravir against EBOV in a mouse model [3] and its use in a french patient (personal
communication), together with enough human safety data from Influenza trials (provided by Company), experimental compassionate treatment with FAVI was decided. Favipiravir dose and administration schedule was selected considering mainly pharmacokinetics (PK) data in human volunteers (provided by Company) and pharmacodynamics (PD) and efficacy data in a mouse model.

**Favipiravir**

**Pharmacokinetics and pharmacodynamics data supporting Favipiravir schedule**

Human Pharmacokinetics (PK) data and mouse model [3] pharmacodynamics (PD) data used to calculate FAVI dose were:

- EBOV-mouse model [3] showed EBOV-IC 90 17 $\mu$ g/mL and therapeutic dose 300 mg/kg/day.

- PK data in volunteers with 1200/600 mg BID doses were: Day 1. Cmax: 59.43 $\mu$ g/mL; Day 6. Cmax: 30.56 $\mu$ g/mL, and t1/2: 3,4-5,8 hr (provided by Company).

- No-observed-adverse-effect level in monkeys was 100 mg/kg/day (provided by Company)

- Plasma albumin binding was 53% (provided by Company)

With this limited information, we aimed to maintain a free Cmin above IC90 (and as close as possible to 60 $\mu$ g/mL of total concentration), with a loading dose of 50mg/kg BID and a maintenance dose of 25mg/kg TID.
Figure S3. Favipiravir detailed dosing. Numbers within columns are number of tablets administered at each time point.
Figure S2. Drugs and supportive therapy. Timeline.

All drugs administered during the admission are listed on the y-axis. Bars represent the duration of therapy in days, using day of illness as timescale. White asterisks on enteral feeding bar represent only water administration, progressively from DOI 19 normal diet was introduced.
Laboratory Methods

Figure S1. PCR results in the screening and confirmatory PCRs on blood samples the day of admission.

A and B represent independent aliquots of nucleic acids extracted from plasma. Dilutions (1/20) of each of them were also assayed. A) Detection using the Real Star Filovirus Screen RT-PCR designed in L gene. B) Detection using the confirmation assay designed in the NP gene. 1: MW, 2: nucleic acids from sample A, 3: nucleic acids from sample A diluted 1/20, 4: nucleic acids from sample B, 3: nucleic acids from sample B diluted 1/20, 6: Neg control, 7 and 8: Positive control (RNA from EBOZ –Yambuku-DRC-76; KM655246.1), 9: Positive control (RNA from EBOZ-GAB-2002-Ilembe; kc 242800.1)

Blood samples in EDTA were received in the BSL3 laboratory of the Centro Nacional de Microbiología and managed as described in Negredo et al [1]. Briefly, 3 aliquots were obtained in AVL buffer (Qiagen, Izasa, Spain). Nucleic acids from two of them were extracted as recommended by the manufacturer. The third one was conserved at -80ºC.

Pure extracts and 1/20 dilutions of the 2 aliquots processed were assayed. We used 10 µl of pure extracts and dilutions, 4 samples in total. RealStar Filovirus Screen RT-PCR Kit 1.0. (Altona Diagnostics, Izasa, Spain) was used. Positive results were obtained in all samples (Fig 1A). For confirmation of the results a second PCR designed in a different genomic
region (NP gene) [2] was used. Positive results were also obtained with all the samples assayed (Fig 1B). Since it was the first case of Ebola infection outside Africa, the third aliquot was also used for an extra confirmation and again the results were confirmed (not shown). The remaining blood was sent to Bernhard Nocht Institute for Tropical Medicine National Reference Centre for Tropical Infectious Diseases (Hamburg, Germany) and results were also confirmed.

**Other samples tested for Ebola virus**

**Table S3. Bodily Fluids testing for Ebola Virus**

<table>
<thead>
<tr>
<th>Sample</th>
<th>22</th>
<th>24</th>
<th>25</th>
<th>28</th>
<th>30</th>
<th>32</th>
<th>34</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweat</td>
<td>Negative</td>
<td>ND</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Saliva</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>Urine</td>
<td>Positive</td>
<td>ND</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>Conjunctival swab</td>
<td>Negative</td>
<td>ND</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>Vaginal swab</td>
<td>Positive</td>
<td>ND</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>Stool</td>
<td>Positive</td>
<td>ND</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND: not done

**Ambient surveillance samples:**

- Personal protective equipment: left glove, goggles, hazmat suit chest area, FFP 3 mask.
- Airlock anteroom: inside-doorknob, floor, and wall
- Patient room: interior doorknob, floor, wall, and disinfectant spray bottle.
- Closest area to patient: bedside table, bed rail, pillow, bed sheet, urine collector.
Radiologic findings

Figure S4. Chest X-ray on day 11 of illness

Bilateral alveolar interstitial infiltrates.
Figure S5. Chest X-ray on day 12 of illness

Bilateral pulmonary infiltrates
Figure S6. Chest X-ray on day 13 of illness

Bilateral alveolar infiltrates and peripherally inserted central catheter in right subclavian vein.
Figure S7. Chest Radiographs on day 37 of illness (immediately before discharge)
Normal chest radiography
Health care workers personnel in the isolation unit

24 hours shifts:
   One Infectious Diseases/Tropical Medicine specialist
   One Intensive Care specialist
   Laboratory physician

8 hours shifts
   Three nurses
   Three nurse-assistants
   One occupational health nurse to supervise personal protective equipment
   One janitor
   Two cleaning services personnel

12 hours shifts
   Two security personnel

A delegate from the hospital administration is available at all times
REFERENCES

