**Evaluation of convalescent plasma for the treatment of Ebola Virus Disease in Guinea**

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**Word count (Background to Conclusions) : 2698 words**

**Background**

The safety and efficacy of convalescent plasma (CP) to treat Ebola Virus Disease (EVD) was evaluated in Guinea.

**Methods**

In this non-randomized comparative study, patients with confirmed EVD of any age (including pregnant women) received two consecutive ABO-compatible CP units of 200-250ml from different convalescent donors, initiated on the day of diagnosis or up to two days later. The concentration of Ebola virus neutralizing antibodies in CP was unknown at administration (unselected CP). The comparator group was patients with EVD at the same treatment center in the previous five months. The primary analysis compared survival (discharged cured 3-16 days after diagnosis) with adjustments for age and baseline PCR Cycle Threshold (CT) value, excluding patients who died before day 3. A 20% lower mortality in the CP group was considered a clinically important difference.

R**esults**

Eighty-four CP treated patients and 418 comparator patients were included in the primary analysis. CP patients, on average, had slightly higher CT values, shorter duration of symptoms and a higher proportion had eye redness and difficulty in swallowing. Mortality from the 3rd to 16th day after diagnosis was 31.0% in CP patients and 37.8% in comparator patients (risk difference -6.9%; 95% confidence interval (CI): -17.8% to 4.1%). The difference was reduced after adjusting for age and CT value (adjusted risk difference -2.6%, 95% CI: -13.1 to 8.0%). No serious adverse reactions to CP were observed.

**Conclusion**

Transfusion of up to 500ml of unselected CP was well tolerated but not found to significantly improve survival.

**BACKGROUND**

The outbreak of Ebola Virus Disease (EVD) in West-Africa is the worst ever witnessed. By September 9th, 2015, 28,183 cases and 11,306 deaths had been reported.1 The high case fatality rate (40-60%)2, 3 highlights the need for effective EVD specific treatments, which would also provide an incentive to patients to present to treatment centers early. This would facilitate rapid contact tracing and the implementation of measures to control the spread of an outbreak.

Evaluation of treatment with convalescent whole blood (CWB) or plasma (CP), derived from recovered EVD patients, was prioritized by the World Health Organization (WHO).4 Such treatment has been successfully used for other serious infectious diseases and, with appropriate safeguards, is generally considered safe.5, 6 Previous use of CWB and CP for the treatment of EVD is very limited. The largest case series reports on eight patients treated with CWB during the Kikwit outbreak of EVD in 1995, with seven surviving.7 However, it was not possible to assess if the low case-fatality rate was due to treatment with CWB or other factors, such as patient characteristics or the time in the illness at which treatment was given.7 Because of uncertainty about the therapeutic value of convalescent blood products in EVD, the Ebola-Tx trial was implemented to assess the safety and efficacy of CP for the treatment of EVD in Conakry, Guinea.CWB was not evaluated since CP was available at the onset of the trial.

**METHODS**

**Study design, participants and intervention**

Randomization was locally unacceptable in the volatile setting of the Ebola outbreak,16 thus the study had a non-randomized comparative design. It was conducted at the Médecins Sans Frontières (MSF) supported Ebola Treatment Unit (ETU) in Conakry, Guinea between February 17th and August 3rd, 2015. All eligible consenting patients (of any age and including pregnant women), with symptomatic, laboratory-confirmed EVD were enrolled. Exclusion criteria were 1) a history of allergic reaction to blood or plasma products; 2) a medical condition where infusion of additional fluid may be contraindicated (e.g. decompensated congestive heart failure or renal failure with fluid overload); 3) futility based on consensus by the clinical team. Criteria for futility included the presence of shock unresponsive to fluid challenge; the presence of shock with signs of multi-organ failure, defined as the combination of oliguria/anuria and impaired consciousness or the combination of oliguria/anuria and jaundice; 4) the patient’s condition was such that plasma administration carried a significant risk for staff (e.g. an agitated patient).

Eligible consenting patients were given CP as soon as ABO-compatible CP was available to the treatment center. It was planned that a control group would comprise patients admitted during the preparatory period for the study while apheresis and pathogen reduction were being set up and those for whom ABO-compatible CP was not available during the study. At the start of recruitment, there was sufficient CP available to treat all patients and thus a protocol amendment was approved for the comparison group to comprise patients treated prior to the trial at the same ETU.

Following WHO guidance,4 400-500 mL of CP from two different donors (2 x 200-250 ml), or 10mL/kg for small adults and children weighing <45kg, was given in two transfusions, each over 20 minutes with a 15 minutes interval.

Five ethics committees approved the study protocol (Supplementary Appendix).

**Procedures**

Donors and EVD patients

Engagement of convalescent donors was organized through the Ebola-survivor association of Conakry (Supplementary Appendix, Table S1). For EVD patients, blood group determination was done according to the Beth-Vincent method or using the MDMulticard (Medion Grifols Diagnostics AG, Düdingen, Switzerland) and ABO-compatible plasma ordered. Supportive care (SC) for all patients was based on MSF-EVD treatment guidelines, including intravenous hydration and shock management (Supplementary Appendix).8

Blood samples were collected from study patients on three occasions: 1) at the time of diagnosis (for real-time Reverse Transcription (RT)-PCR for EBOV, blood group typing; i-STAT); 2) 24-hours after transfusion (for RT-PCR) and 3) at the time of discharge to ascertain EVD cure (RT-PCR). Each EBOV RT-PCR test provided a cycle threshold (CT) value, the number of cycles required for the fluorescent signal to cross the threshold for a positive test. A lower CT value is correlated with a higher viral load. EBOV laboratory testing was done in the Guinean national laboratory for hemorrhagic fever viruses using whole blood samples and the QIAmp viral RNA Kit (Qiagen) for nucleic acids extraction, and the LightMix Ebola Zaire rRT-PCR Test (TIB MOLBIOL) and a Smart Cycler (Cepheid) for genomic amplification, according to the manufacturer's recommendations. Patients were discharged after a negative EBOV RT-PCR result.

**Outcome measures and definitions**

The primary outcome was defined as survival at 14 days after CP administration, counting deaths occurring up to day 16 post PCR confirmation in both groups to allow for CP administration up to and including the second day post PCR confirmation (by which time CP administration for all patients had started). CP+SC patients were contacted by phone post discharge to confirm survival up to day 30. Historic patients discharged cured prior to day 16 had not been followed up and were assumed alive on day 16.

Adverse reactions (ARs) and serious adverse reactions (SARs) – events considered by the treating clinician to be related to CP administration - were recorded from the start of CP treatment, until 4 hours after the end of the intervention (Supplementary Appendix). During CP administration, patients were under continuous supervision, with vital parameters checked every 15 minutes until 15 minutes after the second CP unit and at four hours after the end of the intervention. Safety risks to health workers administering CP were also assessed.

**Sample size**

A 20% absolute risk reduction in mortality for CP+SC, compared to SC alone, was considered clinically important based on discussions by international experts during two WHO organized teleconferences and the minimum effect necessary to justify the significant investment in infrastructure, risk to health care workers and mobilization of resources to organize widespread CP treatment in affected countries (David Wood, WHO, personal communication). To detect an absolute difference of 20% with 90% power, a two-sided alpha of 0.05 and an equal number of CP+SC and SC patients, up to 130 patients per group were required for a range of mortality on SC alone from 40%-80%. As CP was available for all patients and no concurrent controls were enrolled, comparative analyses included historic patient data treated at the same ETU for a period pre-specified in the analysis plan as September 2014 to January 2015. During this period 507 confirmed EVD patients were treated. The Data Safety Monitoring Board advised to close the study on July 7, 2015, due to the low caseload. At this time, 102 patients with confirmed EVD had been enrolled. Despite having fewer than 130 CP+SC patients, the higher number of control patients meant that the study was still powered to detect an overall absolute difference of 20% in survival rates.

**Statistical Analysis**

Baseline clinical and demographic characteristics were summarized by treatment group and compared using chi-squared, Fisher’s exact or Wilcoxon rank sum tests.

The primary analysis population, pre-defined in the analysis plan, excluded patients who died before the third day after RT-PCR confirmation (i.e. on the day of diagnosis or on the two following days) from both groups, in order to provide a comparable starting point for measuring survival, given that CP patients started treatment at variable times up to and including the second day post PCR confirmation. Patients who received other experimental treatments (e.g. favipiravir) were also excluded.

Logistic regression methods were used to compare mortality between CP+SC patients and SC patients. Adjustments for age and CT value were pre-specified in the statistical analysis plan, based on published data.9,10 Each patient’s probability of death was estimated by logistic regression and adjusted risk differences and 95% confidence intervals (CIs) were calculated as differences of averages of these probabilities.11 Age was categorized into <5 years, 5-15, 16-44 and 45 years and above.9 Historic patient mortality was tabulated by CT in five CT unit intervals. There were only 1 and 4 CP+SC patients with CT<20 and CT>35 respectively so CT was further categorized into three groups for analysis to avoid sparse data; <25, 25-29.9 and ≥30 cycles. Patients in the CP+SC group receiving incomplete transfusions were included, as per intention-to-treat convention. Sub-group analyses by age group and CT categories were derived from adjusted logistic regression models with interaction terms.

**Results**

Of the 514 individuals assessed at the ETU during the study period, 114 were diagnosed with EVD. Twelve died before enrolment could take place. Of the 102 patients enrolled in the trial, compatible CP was available for all and administered to 99 patients of whom 84 were included in the primary analysis (Figure 1). In total, 19 (16.6%) of the 114 EVD confirmed patients died either before enrollment could take place or CP could be administered. Five children aged < 5 years were treated with CP, including four children aged below one year.

A total of 507 confirmed EVD patients were admitted and treated with SC in the five months preceding the trial with 87 (17.1%) dying before the third day after EVD diagnosis. One patient was excluded due to a missing outcome and one due to missing age, leaving 418 for primary analysis.

On average, CP+SC patients had slightly higher CT values and shorter symptom duration than historic patients. Difficulty swallowing and eye redness was recorded more often for trial patients (Table 1). Otherwise, patient characteristics were generally similar between groups.

Between the 3rd and 16th day after diagnosis, 26 (31.0%) of the 84 patients died in the CP treated group and 158 (37.8%) of the 418 patients in the control group. After adjustment for age and CT value, mortality remained lower in the CP+SC group but the difference was not statistically significant (adjusted OR; 0.88, 95% CI: 0.51-1.51, adjusted risk difference -2.6%, 95% CI: -13.1% to 8.0%, Table 2).

Of the measured factors unbalanced between arms, duration of symptoms and difficulty swallowing were associated with mortality in the historic data (data not shown). Additional adjustment for these factors had little effect on results (OR 0.90; 95% CI 0.50-1.67).

The difference in mortality of patients treated with CP+SC and with SC alone was greater among younger patients (Tables 2 & 3) after adjustment for CT, but subgroups were small and the differences by age were not statistically significant.

Among the 84 primary analysis patients, there was one major protocol deviation whereby a patient received less than 90% of the recommended volume of CP, and 14 minor deviations in 11 patients (Table S2). Exclusion of the patient with the major deviation had no material effect on the primary analysis (data not shown). One day after CP transfusion, a median increase in CT value of 3.5 units was seen (Table S3).

No SARs were observed in the 99 patients receiving CP. Eight (8.1%) patients had an AR during or early after CP. These resolved spontaneously with symptomatic treatment or a reduced rate of transfusion (Table 4, Table S4). There were no safety events in the health care staff relating to CP transfusion.

**Discussion**

This is the largest trial ever conducted of convalescent blood products in EVD. Transfusions were safe and acceptable to both donors and patients. Practical aspects of conducting the study will be described elsewhere. A small but insignificant decrease in mortality was observed in the adjusted analysis in the CP group. A 20% absolute decrease in mortality, which had been pre-specified as clinically relevant, could be excluded (adjusted risk difference -2.6%, 95% CI: -13.1% to 8.0%). Mortality was analyzed up to day 16 as most Ebola patients have either recovered or died before this time. We did not have day 30 follow-up on the historic patients and one patient treated with CP died between day 16 and day 30, after being discharged EVD cured and transferred to another medical facility for the management of another condition.

The concentration of EBOV neutralizing antibodies present in donor plasma is likely to be important for the effectiveness of this intervention, as has been found in studies on non-human primates.12,13 However, the level of neutralizing antibodies present in the CP could not be determined prior to infusion. Ebola virus plaque neutralizing assays require access to biosafety level four laboratories, currently unavailable in the affected countries, and shipment of blood samples abroad for sample testing has not been possible at the time of writing. Consequently, unless CP has been stockpiled during an EVD outbreak, CP will likely be available without information on antibody concentrations, unless simple, field-adapted assays become available. Antibody levels could be low in some patients during early convalescence, which may have diluted an effect of CP. Analyses of the concentration of EBOV neutralizing antibodies in plasma donations, and any correlation with the survival of patients, will be reported later. It is possible that high titer CP or hyperimmune immunoglobulins might be more potent. In addition, the optimal frequency of administration is also not known for CP and repeated administration, with higher total volumes than used in our study, might be required.

We cannot exclude the possibility that some patients will benefit more than others from treatment with CP. Of possible interest is that children below the age of five years are known to have a poor prognosis9 and had the highest mortality rate in the historic patient series (Table 3). However, only one of five patients treated with CP in this age group died. Although pregnant women with EVD have a poor prognosis,14 only 2 of the 8 pregnant women treated with CP died. They might also have benefited from the coagulation factors present in the CP. Unfortunately, pregnancy was incompletely recorded in the historic patients.

The good safety profile of CP administration within an ETU is reassuring, since there were concerns that SARs might occur more commonly. However, it is difficult to distinguish complications such as transfusion related acute lung injury from EVD progression.15

In our comparative design, concurrent controls were to consist of patients presenting before ABO compatible plasma was available. With many survivors volunteering to donate, there was no shortage of CP during the trial and all control patients were historic patients. There are clear limitations with respect to the use of a historic control group and we cannot exclude the possibility of unmeasured confounding factors that may have biased the mortality comparison. We also included CT as a surrogate marker for viral load. There was variation in the mortality rate during the 5-month historic period but there was no clear trend and conclusions remained unchanged when comparison was restricted to patients treated in the three months preceding CP administration. We also conducted an intention-to-treat analysis, comparing the mortality of all patients diagnosed during the period of the CP trial and all historic patients, which yielded similar results (Supplementary Appendix).

The adjusted analyses that we conducted are unlikely to account for any variability in supportive care, such as the introduction of the point of care test or due to differences in case load over time. Moreover, since CP administration requires intravenous access, this could have led to an increased intravenous fluid administration beyond CP. These factors could have contributed to lowering the mortality in the CP group, but despite this we failed to show a significant decreased mortality in CP patients. Assessment for a dose-response between the concentration of neutralizing antibodies in donor plasma and changes in viral load after CP treatment or in survival could be of value in determining any direct effect of antibody therapy.

**Conclusions**

This is the largest trial ever conducted on convalescent blood products in EVD. Treatment with CP was found feasible to organize, safe to administer and acceptable to donors, patients, family and health care providers in the middle of an EVD outbreak. Although uncertainty remains due to the non-randomized nature of the study and the use of historical controls, we could not detect a marked effect on survival of administration of an unselected dose of 2x 200-250 ml of CP. It remains to be assessed whether CP with high concentrations of EBOV neutralizing antibodies, possibly administered repeatedly, would show efficacy, and whether patient subgroups, such as young children or pregnant women are more likely to benefit.

**Role of funding source**

The Ebola-Tx project is funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No 666094. Additional funding is provided by the Department of Economy, Science and Innovation (EWI) of the Flemish government. The mobile plasma unit used in this trial was provided to Guinea by the Bill and Melinda Gates Foundation. The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Acknowledgements**

We would like to thank the following persons for their support from the Institute of Tropical Medicine, Antwerp: Evelyn Depoortere, Severine Caluwaerts, Marc Michiels, Leyla Kjazim, Roeland Scholtalbers, Aine Markham, Anke Kohlenberg, Koen Peeters, Christophe Burm; from the Etablissement Français du Sang: Jean Yves Scotto, Isabelle Azarian, Alain Beauplet, Claire Boulat, Isabelle Brisset, François Charpentier, Jacques Chiaroni, Rachid Djoudi, Christian Gachet, Linda Gimeno, Dominique Legrand, Corinne Kohler, Pascal Morel, Gilbert Sémana, François Toujas; Jean Pierre Cerdan, Alain Chauve and Jean Louis Rodier from Electriciens Sans Frontières, France; Lutgarde Barrez, Belgian Red Cross Flanders; Ashley Jones (University of Liverpool); Delphin Kolie (National Blood Transfusion Service); members of the DSMB (Umberto d’Alessandro, Matthias Egger, Andrew Nunn, Shevin Jacob, Jean-Pierre Allain, Simon Mardel) and the project advisory committee (Kwadwo Koram, Corrah Tumani, André Loua, Michel Van Herp); the entire team of the National Blood Transfusion Service in Guinea and MSF for their fantastic work; the World Health Organization and ISARIC; all national and international stakeholders and partners.

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**Table 1. Baseline characteristics of patients included in primary analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | **CP+SC**  **(n=84)** | **SC**  **(n=418)** | ***P*-value** |
| Sex | |  |  |  |
| Male | 36 (42.9) | 210 (50.2) |  |
| Female | 48 (57.1) | 208 (49.8) | 0.25 |
| Age (years); median (range) | | 29 (0-75) | 28 (0-87) |  |
| < 5 years | 5 (5.9) | 23 (5.5) |  |
| 5-15 years | 8 (9.5) | 53 (12.7) |  |
| 16-44 years | 57 (66.7) | 258 (61.7) |  |
| 45+ years | 15 (17.9) | 84 (20.1) | 0.79 |
| PCR CT value at diagnosis (cycles); median(range) a | | 27.3 (19.2-35.8) | 26.0 (15.2-39.4) | 0.007 |
| <25 | 21 (25.0) | 159 (38.0) |  |
| 25.0-29.9 | 41 (48.8) | 183 (43.8) |  |
| ≥30 | 22 (26.2) | 76 (18.2) | 0.05 |
| Symptoms on admission | |  |  |  |
| Nausea/vomiting | 42 (50.0) | 203 (48.6) | 0.81 |
| Diarrhea | 29 (34.5) | 155 (37.1) | 0.66 |
| Weakness/asthenia | 77 (91.7) | 353 (84.4) | 0.09 |
| Pain | 73 (86.9) | 342 (81.8) | 0.26 |
| Cough | 11 (13.1) | 40 (9.6) | 0.33 |
| Difficulty breathing | 4 (4.8) | 11 (2.6) | 0.29 |
| Difficulty swallowing | 15 (17.9) | 39 (9.3) | 0.02 |
| Hiccups | 7 (8.3) | 38 (9.1) | 1.00 |
| Eye rednessa | 34 (40.0) | 83 (19.9) | <0.001 |
| Unusual bleedingb | 5 (6.0) | 21 (5.0) | 0.79 |
| Disorientation/agitation | 0 (0) | 2 (0.5) | 1.00 |
| Anuria | 1 (1.2) | 1 (0.2) | 0.31 |
| Seizures | 0 (0) | 1 (0.2) | 1.00 |
| Duration of symptomsc > 6 days | 14 (18.9) | 203 (49.3) | <0.001 |
| Chronic comorbidities | |  |  |  |
| Infectiousd | 1 (1.2) | 2 (0.5) | 0.42 |
| Non-infectiouse | 1 (1.2) | 3 (0.7) | 0.52 |
|  | |  |  |  |

a Eye redness refers to conjunctivitis and conjunctival bleeding (data were not captured separately)

b Excluding conjunctival bleeding

c Binary categorization of duration of symptoms was based on mean duration from published data2

d Tuberculosis or HIV

e Diabetes mellitus, chronic cardiac, pulmonary or renal disease

CP: convalescent plasma; CT: cycle threshold; PCR: polymerase chain reaction; SC: supportive care

**Table 2. Primary mortality analysis**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | **CP+SC** | | **SC** | | |
| Number analyzed | | | 84 | | 418 | | |
| Died between 3rd and 16th day after diagnosis, n (%) | | | 26 (31.0) | | 158 (37.8) | | |
| Unadjusted CP effect on mortality, OR (95%CI) | | | 0.74 (0.45 – 1.22) | | 1 | | |
| Absolute risk difference: -6.9% (-17.8 to 4.1) | |  | |  | | |
| Adjusted for age and CT value | | | 0.88 (0.51-1.51) | | 1 | |
| Absolute risk difference: -2.6% (-13.1 to 8.0) |  |  | |
| Models with interactions (Odds ratios shown): | | |  | |  | | |
| (adjusted for CT) | | |  | |  | | |
| CP effect in <5 years | | 0.18 (0.02-2.12) | | 1 | | |
| CP effect in 5-15 years | | 0.75 (0.08-7.41) | | 1 | | |
| CP effect in 16-44 years | | 0.86 (0.44-1.68) | | 1 | | |
| CP effect in 45+ years | | 1.52 (0.48-4.88) | | 1 | | |
| P-value for interaction | | 0.92 | |  | | |
| (adjusted for age) | | |  | |  | | |
| CP effect in CT <25 | | 0.87 (0.34-2.22) | | 1 | | |
| CP effect in CT 25-29.9 | | 0.81 (0.37-1.76) | | 1 | | |
| CP effect in CT ≥30 | | 1.11 (0.31-3.97) | | 1 | | |
| P-value for interaction | | 0.43 | |  | | |
|  | | |  | |  | | |

CP: convalescent plasma; CT: cycle threshold (categories: <25, 25-29.9, ≥30); CI: confidence interval; SC: supportive care; OR: odds ratio; p-value from likelihood ratio test comparing models with and without interaction terms between treatment group and factor of interest

**Table 3. Deaths between the 3rd and 16th day after diagnosis by age and CT value in primary analysis population**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **CP + SC**  **(n=84)** | | **SC**  **(n= 418)**a | |
|  | | Total | Died, n (%) | Total | Died, n (%) |
| Age <5 years | | 5 | 1 (20) | 23 | 15 (65) |
| CT value <25 | 1 | 1 (100) | 12 | 10 (83) |
| CT value 25-29.9 | 3 | 0 (0) | 10 | 5 (50) |
| CT value ≥30 | 1 | 0 (0) | 1 | 0 (0) |
| Age 5-15 years | | 8 | 1 (13) | 53 | 10 (19) |
| CT value <25 | 2 | 0 (0) | 19 | 5 (26) |
| CT value 25-29.9 | 3 | 1 (33) | 23 | 3 (13) |
| CT value ≥30 | 3 | 0 (0) | 11 | 2 (18) |
| Age 16-44 years | | 57 | 16 (28) | 258 | 90 (35) |
| CT value <25 | 15 | 8 (53) | 97 | 50 (52) |
| CT value 25-29.9 | 28 | 5 (18) | 112 | 34 (30) |
| CT value ≥30 | 13 | 3 (23) | 49 | 6 (12) |
| Age 45+ years | | 15 | 8 (53) | 84 | 43 (51) |
| CT value <25 | 3 | 2 (67) | 31 | 25 (81) |
| CT value 25-29.9 | 7 | 5 (71) | 38 | 14 (37) |
| CT value ≥30 | 5 | 1 (20) | 15 | 4 (27) |
| CT; cycles (PCR) | |  |  |  |  |
| CT value <25 | 21 | 11 (52) | 159 | 90 (57) |
| CT value 25-29.9 | 41 | 11 (27) | 183 | 56 (31) |
| CT value ≥30 | 22 | 4 (18) | 76 | 12 (16) |

CP: convalescent plasma; CT: cycle threshold; PCR: polymerase chain reaction; SC: supportive care; OR: odds ratio

**Table 4. Numbers of patients with adverse reactions reported among those receiving convalescent plasma (n=99)**a

|  |  |
| --- | --- |
|  | **n (%)** |
| Serious adverse reaction | 0 (0) |
| Adverse reaction | 8 (8) |
| Raised temperature | 5 (5) |
| Itching/skin rash | 4 (4) |
| Nausea | 1 (1) |
| Adverse reaction requiring reduction in infusion rate | 2 (2) |
| Adverse reaction requiring temporary or permanent interruption of infusion | 0 (0) |

a Two patients experienced two adverse reactions (fever and nausea; fever and itching)



**Figure 1. Disposition of the patients.** During the trial, 19 (16.6%) of the 114 EVD confirmed patients died on the day of diagnosis or on the two following days. In the period preceding the trial, 87 (17.1%) of the 507 confirmed patients died in the corresponding period. aNone of the four early deaths could be attributed to CP. bTen patients who were health care workers were subsequently referred to a center dedicated to health staff and received favipiravir in a different trial, three out of the ten patients treated with favipiravir died.