Relationship between viremia and specific organ damage in Ebola patients: a cohort study

Authors:

Simone Lanini,[1,2] Gina Portella,[3] Francesco Vairo,[1,2] Gary P. Kobinger,[4,2] Antonio Pesenti,[3,5] Martin Langer,[3,6] Soccoh Kabia,[7] Giorgio Brogiato,[3] Jackson Amone,[8] Concetta Castilletti,[1] Rossella Miccio,[3] Maria Rosaria Capobianchi,[1] Gino Strada,[3,2] Alimuddin Zumla,[9,2] Antonino Di Caro,[1,2] Giuseppe Ippolito,[1,2] and INMI-EMERGENCY EBOV Sierra Leone Study group[10]

Institutional affiliations:

1) National Institute for Infectious Diseases "Lazzaro Spallanzani" IRCCS, Rome, Italy. 2) International Public Health Crisis Group (IPHCG); 3) EMERGENCY NGO, Milan, Italy; 4) Research Centre on Infectious Diseases, Faculty of Medicine, Université Laval, Québec Canada; 5) Department of Anesthesia, Critical Care Medicine and Emergency, Fondazione IRCCS Cà Granda-Ospedale Maggiore Policlinico, Milano and Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; 6) Department of Oncology and Onco-Hematology, University of Milan, and Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 7) Connaught Hospital Tower Hill, Freetown, Sierra Leone; 8) Department of Clinical Services, Ministry of Health, Kampala, Uganda; 9) Division of Infection and Immunity, University College London, and NIHR Biomedical Research Centre at UCL Hospitals NHS Foundation Trust, London, United Kingdom; 10) The collaborative group is detailed in the Supplemental Material

Word count: 2914; **References** 38; **Displays:** 3 Tables and Figures: 2; **Keywords:** Ebola virus, Biomarkers, Muscle damage, liver function, renal function, biochemical abnormalities, dose effect response.

Summary

The pathogenesis of Ebola virus disease on specific organ dame remains poorly understood. This study provide evidence to support that Ebola virus may have a direct role in the muscular damage and in the imbalance of the coagulation system.

Abstract

Background.

Pathogenesis of Ebola virus disease remains poorly understood. We used concomitant determination of routine laboratory biomarkers and Ebola viremia to explore the potential role of viral replication in specific organ damage.

Methods

We recruited patients with detectable Ebola viremia admitted to the EMERGENCY ONG ONLUS Ebola Treatment Center in Sierra Leone. Repeated measure of Ebola viremia, ALT, AST, bilirubin, CPK, LDH, aPTT, INR, creatinine and BUN were recorded. Patients were followed-up since admission until death or discharge.

Results

One hundred patients (49 survivors and 51 non-survivors) were included in the analysis. Unadjusted analysis to compare survivors and non-survivors provided evidence that all biomarkers were significantly above the normal range and that the extent of these abnormalities was generally higher in non-survivors than in survivors. Multivariable mixed effect models provided strong evidence for a biological gradient (suggestive of a direct role in organ damage) between the viremia levels and either ALT, AST, CPK LDH, aPTT and INR. In contrast no direct linear association was found between viremia and either creatinine, BUN or bilirubin.

Conclusion

This study provides evidence to support that Ebola virus may have a direct role in muscular damage and imbalance of the coagulation system. We did not found strong evidence suggestive of a direct role of Ebola virus in kidney damage. The role of the virus in liver damage remains unclear, but our evidence suggests that acute severe liver injury is not a typical feature of Ebola virus disease.

Introduction

On June 9, 2016, the World Health Organization officially declared the end of the Ebola virus (EBOV) disease (EVD) epidemic which caused 28,616 confirmed cases with 11,310 deaths. [1] During this outbreak logistical difficulties, lack of political will and weak coordination between partners prevented the rapid implementation of scientifically sound prospective clinical and pathogenesis studies. [2-3] Indeed, good quality trials to assess efficacy of novel treatments [4] and vaccines [5] for EVD came late when the epidemic had already claimed most of its victims.

Understanding clinical aspects of the pathogenesis of EVD may serve to better define targets for developing new therapeutic, preventive and disease monitoring strategies. [6] The only defined biomarker unequivocally associated with patients' outcome is the level of EBOV RNA in the blood (viremia). [7-8-9] However, marked biochemical abnormalities, reflecting the potential capability of the virus to produce damage in different body compartments, have been observed. [10-11] Nevertheless, studies exploring the relationship between EBOV viremia, biochemical abnormalities and specific clinical disorders are scanty. Using prospectively collected repeated measures, we conducted an analysis to explore the potential association between the EBOV viremia and biomarkers reflecting skeletal muscle damage, liver damage, renal impairment and coagulation abnormalities.

METHODS

Study design and aims

The study was based on a cohort of patients with confirmed EVD. The analyses were designed to assess the presence of a biological gradient between EBOV viremia and biomarkers of skeletal muscle damage, liver damage, renal impairment and coagulation abnormalities.

Clinical setting and patients care

The study was set in the EMERGENCY ONG ONLUS Ebola Treatment Center (EMERGENCY-ETC), which was operational between December 2014 and May 2015, in Goderich (Sierra Leone). This center offered advanced supportive care to patients and laboratory monitoring through an internal laboratory, managed by the INMI Lazzaro Spallanzani, where highly infectious biological samples were processed for molecular diagnosis of EVD and standard biochemical assays. Patients were treated with supportive care using a standardized protocol, described elsewhere. [7] In general blood collection (for EBOV molecular testing and standard biochemical assays) was performed within 6 hours since patient's admission and eventually once a day during the acute phase of the disease. Patients with confirmed EVD who recovered were discharged after 2 consecutive undetectable EBOV RNA in blood samples taken at least 2 days apart.

Eligibility criteria

All patients with confirmed EVD (WHO definition [12]) admitted to the EMERGENCY-ETC between 13 December 2014 and 30 May 2015 who had at least one available quantifiable EBOV viremia were enrolled in this study.

Variables studied

For each patient, we collected information about age, sex, dates of renal replacement therapy, clinical outcome (either non-survivors or survivors) and repeated measurements of EBOV viremia, liver function tests, renal function tests, coagulation parameters and muscle enzymes. In details, we collected repeated measure of 10 biomarkers including:

- EBOV viremia in Log(10) copies per ml (continuous);
- total bilirubin in mg/dL (continuous variable);
- international normalized ratio (INR, continuous variable);
- activated protrombin time in seconds (aPTT, continuous variable);
- creatinine in mg/dL (continuous variable);
- blood urea nitrogen in mg/dL as (BUN, continuous variable);
- alanine transaminase in U/L (ALT; right-censored variable with upper limit at 1000 U/L);
- aspartate transaminase in U/L (AST; right-censored variable with upper limit at 1000 U/L);
- creatine phosphokinase in U/L (CPK; right-censored variable with upper limit at 2000 U/L)
- lactate dehydrogenase (LDH, right-censored variable with upper limit at 4000 U/L).

ALT, AST, CPK and LDH are considered as right-censored variables because, to minimize risk of accidental exposure of lab-workers, samples with values exceeding methods upper limit of quantification were not always diluted to obtain a quantitative measure. Thus the measures of these biomarkers were recorded either as a punctual point measure (i.e. samples below the upper limit of quantification and those exceeding the limit but re-analyzed after dilution) or as right-censored measure (i.e. undiluted sample above the upper limit of quantification).

Laboratory methods

Blood samples were collected by trained doctors and nurses according to recommended safety and infection control precautions. [13] Samples were not inactivated before being tested.

AST, ALT, LDH, total bilirubin, CPK, BUN and creatinine were measured by SpotChem EZ clinical chemistry analyser (Woodley Equipment Company Ltd). INR and aPTT were measured by a Hemochron JR Signature plus machine (Whitmire Medical Ltd). EBOV RNA testing was performed using a real-time RT-PCR assay (RealStar Filovirus Screen RT-PCR 1.0 kit, Altona Diagnostics), with a limit of detection of 3.11 log cp/ml of EBOV RNA. Viral RNA quantification was based on a standard reference curve provided by the kit producers, spanning up to 9 log cp/ml of EBOV RNA.

Statistics and modeling

Unadjusted analysis to compare survivors and non-survivors was carried out to describe the study sample and to show the distribution of baseline patients characteristics between survivors and non-survivors. Distribution free statistics including Kruskal-Wallis (for continuous variables) and Pearson chi-square (categorical variable) were used to assess significant differences between survivors and non-survivors. Right-censored variables were transformed into 3-level categorical variable (i.e. level-1 within normal range; level-2 above normal but below censoring cut-of; level-3 above the cut-off of censoring).

Association between biomarkers and EBOV viremia was assessed in nine separate multilevel mixed effect (MME) regression models using concomitant determinations. We considered a biomarkers determination to be concomitant to and EBOV viremia determination when the two determinations were made no more than one day apart from each other. All MME regression models were set for allowing for random intercept at patient level, random slope at EBOV viremia level and unstructured variance-covariance matrix. Each biomarker served as the dependent variable for one model only. The same set of independent variables (i.e.: EBOV viremia; clinical outcome; dialysis; sex and age) was used in all the MME regression models. Continuous dependent variables (i.e.: bilirubin, BUN, creatinine, INR and aPTT) were assessed by MME linear regression models.[7,14] Right-censored dependent variables [15-16] (i.e.: ALT, AST, LDH and CPK) were assessed by using MME interval regression models. MME interval regression is a generalization of other censored regression estimators,[17] such as Tobit,[18] and can be used when the dependent variable is measured as point data, interval-censored data, left-censored data, or right-censored data.[17]

Estimates for intercept (baseline) and independent variables coefficients were optimized by using the natural-log transformed dependent variables. Fixed effects measures of the variation of depended variables (biomarkers) according to the level of exposure to independent variables were reported in back-transformed form and interpreted as proportional variation from the baseline (according to standard interpretation for log transformed measures) [19] for providing readers with biomarkers measures conventionally used in medical practice. Full model parameters (in natural log form) including fixed and random effects coefficients were reported in the additional file 1.

A biological gradient suggestive of a potential direct role of EBOV viremia in alteration of individual biomarker was considered present if p-value for log-linear association was less than 0.050.

A further analysis was carried out to assess the potential association between CPK levels and creatinine levels (as proxy of renal damage). In this analysis CPK was added as 5-level independent categorical variable (according to Common Terminology Criteria for Adverse Events version 4.03) [20] to the MME linear model for association between creatinine and EBOV viremia levels.

All analyses and plots were implemented by STATA 13.1 statistical package.

Results

Descriptive and unadjusted analysis to compare survivors and non-survivors

One hundred-six patients with EVD confirmed diagnosis were admitted to the Center. Of these, 6 were excluded from the study for the following reasons: 2 were referred to the Center without an available EBOV viremia result and died soon after arrival (with no additional testing) and 4 had already unquantifiable level of EBOV viremia at arrival. Overall, 100 patients were included in the analysis.

Median time between admission and outcome, either EBOV viremia clearance or death, was 8 and 4 days, respectively. Unadjusted analysis to assess the distribution of patients' characteristics according to clinical outcome is shown in **Table 1**. There was no difference between survivors and non-survivors regarding sex and age. The analysis provided good statistical evidence that peak levels of EBOV viremia and those of all biomarkers assessed, apart from LDH, were significantly lower in survivors than in non-survivors ($p<0.050$).

Biological gradient between EBOV viremia and biomarkers levels

MME regression models made on concomitant determinations of EBOV viremia and biomarker levels provided strong statistical evidence for the presence of a biological gradient between EBOV viremia and the levels of AST, ALT, CPK, LDH and INR, aPTT, suggesting that EBOV can directly affect skeletal muscle tissue, coagulation system and the liver (**Table 2** and **Figure 1**). Proportional increases per 1 Log EBOV viremia were: AST 1.67 (95% CI 1.46-1.90; $p<0.001$), ALT 1.24 (95% CI 1.12-1.37; $p=0.001$), CPK 1.21 (95% CI 1.08-1.37; $p=0.001$), LDH 1.27 (95% CI 1.09-1.47; $p=0.002$), INR 1.06 (95% CI 1.03-1.09; $p<0.001$) and aPTT 1.12 (95% CI 1.08-1.17, $p<0.001$). The analysis did not provide any evidence for a direct linear relationship (suggestive of biological gradient) between EBOV viremia levels and either creatinine ($p=0.973$), BUN ($p=0.205$) or bilirubin ($p=0.741$) values.

In addition to the above reported findings, other significant association were observed (see **Table 2**). In particular: A) patients outcome was associated with the levels of all biomarkers ($p<0.050$), apart from CPK and LDH ($p=0.104$ and 0.218, respectively); B) renal dialysis was associated with the level of CPK ($p<0.001$), LDH ($p= 0.043$), bilirubin ($p=0.006$), INR ($p=0.001$) and aPTT ($p=0.001$); C) sex was associated with creatinine ($p=0.037$) and bilirubin ($p=0.020$) levels; D) age was associated with INR ($p=0.011$) and creatinine ($p=0.025$) levels.

Association between creatinine and CPK levels

To explore the potential role of CPK levels on renal damage we set a further model by including CPK as 5-level categorical variable in the MME linear regression model already used to assess the association between creatinine and EBOV viremia level.

Table 3 shows results of the MME linear regression model to assess the proportional variation of creatinine according to CPK levels**.** This analysis highlighted a significant positive association between CPK and creatinine levels ($p<0.001$) independent from EBOV viremia, age, sex, renal dialysis and clinical outcome.

Due to the laboratory methodology for CPK determination with cut off at 2,000 U/L, we could not use CPK values a continuous independent variable, therefore the grading according to Common Terminology Criteria for Adverse Events version 4.03 was adopted. Nevertheless, the variation of creatinine according to the grade of CPK strongly suggests a linear relationship between these 2 biomarkers (see **Figure 2**) which is consistent with a biological gradient.

Discussion

To our knowledge this is the first study to explore the association between the level of EBOV viremia and biomarkers of specific organ damage. This association could be hypothesized from previous evidence showing that EBOV can replicated in several different body compartments. [21]

Previous studies have shown that viremia level is the strongest predictor of clinical outcome in patients with EVD, [7-8-9,22] suggesting a direct role of the EBOV in the host tissue damage. However, the pathogenesis of renal and liver function impairment, coagulation disorders and muscle damage remains poorly understood. In this study, we shed light on EBOV pathogenesis using data from real clinical practice collected during the 2014-2015 EBOV epidemic in Western Africa. There are several important findings from our study.

First, unadjusted analyses to assess distribution of peak values of biomarkers between survivors and non-survivors, confirmed that the highest EBOV viremia levels are strongly associated with unfavorable clinical outcome and that EVD is characterized by a systemic syndrome with a variable degree of clinical severity. [23-24] In fact, we provide evidence that all the assessed biomarkers were above the normal range but also that the observed abnormalities were much more pronounced in non-survivor than in survivors. Similar metabolic alterations either in survivor or non-survivors were reported in clinical study which extensively assessed biomarkers in patient infected with Sudan Ebola virus. [25-26]

Second, the models set using repeated biomarkers measures, provided strong evidence that a biological gradient exists between EBOV viremia and the levels of ALT, AST, LDH, CPK, aPTT and INR, suggesting that the virus may be directly involved in the tissue damage leading to biochemical alterations. [27] The notion that Ebola infection may have a major impact on the coagulation system is old.[28] Though significant hemorrhage were infrequently reported in the recent West Africa EBOV epidemic,[29] clinical studies including extensive monitoring of coagulation system provided evidence that coagulation imbalance was frequent and associated with unfavorable clinical outcome even in absence of evident hemorrhage.[30] In line with these findings, our models predicted that INR and aPTT were mildly to moderately elevated in patients with EVD. Moreover, the levels of INR and aPTT were significantly associated with patient's outcome and showed a direct biological gradient with EBOV viremia level. These observations suggest that EBOV may directly affect the balance of coagulation pathways and that coagulation parameters may have a value as potential prognostic indicator of disease severity in patients with EVD. The hypothesis that EBOV may have a direct role in skeletal muscle damage has been already suggested in previous studies and is consistent with the frequently reported symptoms of myalgia at disease onset and persistent muscular weakness in survivors. Hunt et al. [11] found that median values of CPK was 1949 U/L (i.e. about 5 time above the normal) with AST:ALT ratio >2 in a cohort of 118 patients with confirmed EVD in Sierra Leone. Similar results were reported by Cormac et al.[10] who analyzed a smaller cohort of 22 patients in Guinea. Both studies also suggested an association between CPK and either patient's outcome or the degree of the renal function impairment, but neither of them implemented multivariable analyses to explore potential co-factors. Our study confirms previous results and provides new evidence to support the hypothesis that EBOV is directly involved

in the muscular tissue damage. In fact, our predictions emphasized that CPK levels were always above normal levels in patients with detectable EBOV viremia and that a strong biological gradient exists between EBOV viremia and CPK levels, suggesting that viral replication may have a direct role in muscular damage. The hypothesis that EBOV can cause rhabdomyolysis is also supported by the evidence that a biological gradient exists between EBOV viremia and the level of other biomarkers possibly associated with muscular tissue damage, such as LDH and AST.

Third, our models found no evidence for a biological gradient between the levels of biomarkers of kidney damage (creatinine and BUN) and EBOV viremia. However, similarly to other recent prospective studies, [31] we still found that creatinine and BUN were strongly associated with clinical outcome. These findings suggest that kidney damage may be multifactorial in patients with EVD and that the virus-triggered mechanisms may be complex. One hypothesis is that kidney damage may be the consequence of rhabdomyolysis. In support of this hypothesis we have here shown a strong direct association between the degree of muscular damage and the level of creatinine which is also independent from the EBOV viremia. This finding is in line with post-mortem analyses carried out in patients with EVD and other filovirus infections, showing evidence of acute tubular necrosis with no significant inflammation, suggesting that damage through myoglobin due to rhabdomyolysis is a possible mechanism.[21,32] Nevertheless, the values of CPK we estimated, even at the highest level of EBOV viremia, were not consistent with those expected for acute renal failure due to rhabdomyolysis, suggesting that coexisting conditions such as dehydration and acidosis, nearly always present in African EVD patients, may play a role as cofactors of renal dysfunction.[32] Moreover the hypothesis that EBOV does not have a direct pathogenetic effect on kidney tissues is indirectly supported by the observation that viable EBOV can be found in convalescent (asymptomatic) patients' urine. [33-34] Specific pathogenesis of EBOV on different body compartments could have been investigated, at best, through post-mortem. [21] However, performing autopsy during outbreaks is very challenging, due hazardous nature of EVD corpses. This emphasize the need of including also pathologists and high bio-containment mobile cabinets in networks for preparedness and response to future outbreaks. [21]

Finally, our analyses showed that most patients had raised concentrations of biomarkers suggestive of liver damage including ALT, AST and bilirubin and that increased level of these biomarkers were significantly associated with clinical outcome. However, the mild elevation of bilirubin and the observation that AST levels were steadily much higher than those of ALT suggest that severe acute viral hepatitis is not a typical manifestation of EVD subsequent to EBOV. [35-36] Similar evidence has been reported in clinical studies in patients infected either with Sudan Ebola or [26] Taï Forest Ebola [37] and in animal model investigating pathogenesis of Bundibugyo Virus. [38]

In conclusion, our study provides new evidence to support the hypothesis that EBOV may play a direct role in muscular damage and imbalance of coagulation system. The study did not provide unequivocal evidence about the pathogenesis of kidney damage. In fact, though it is possible that rhabdomyolysis may contribute to the kidney damage, it is also likely that other predisposing conditions should coexist. Finally, our study also demonstrates that merging classical epidemiological study design (i.e. historical cohort) with state-of-art

statistical techniques made it possible to tame the complexity of the data structure (heteroskedastic observations with censored measures) and, thus, to exploit at best the information contained in the clinical datasets.

Tables

Variable		All (N=100)	Survivors (N=49)	Non survivor (N=51)	P-value [A]
Sex	*female (%)* *male (%)*	50 (50.00) 50 (50.00)	23 (46.94) 26 (53.06)	27 (52.94) 24 (47.06)	0.548
Age in years	*median (iqr)*	29 (20-40)	28 (20-35)	30 (22-45)	0.245
Renal replacement *(at least 1 day after admission)*	*no (%)* *yes (%)*	81 (81.00) 19 (19.00)	47 (95.92) 2 (4.08)	34 (66.67) 17 (33.33)	<0.001
AST peak level in U/L *(cut off limit =1,000)* *Normal range: 10-27 U/L*	*≤27 (%)* *28-999 (%)* *≥1000 (%)*	0 (0.00) 34 (34.00) 66 (66.00)	0 (0.00) 27 (55.10) 22 (44.90)	0 (0.00) 7 (13.73) 44 (86.27)	<0.001
ALT peak level in U/L *(cut off limit =1,000 U/L)* *Normal range :0-33 U/L*	*≤33 (%)* *34-999 (%)* *≥1000 (%)*	0 (0.00) 83 (83.00) 17 (17.00)	0 (0.00) 46 (93.88) 3 (6.12)	0 (0.00) 37 (72.55) 14 (27.45)	0.005
CPK peak level in U/L *(cut off limit =2,000 U/L)* *Normal range : 56-244 U/L*	*≤244 (%)* *245-999 (%)* *≥2000 (%)*	2 (2.00) 30 (30.00) 68 (68.00)	2 (4.08) 20 (40.82) 27 (55.10)	0 (0.00) 10 (19.61) 41 (80.39)	0.017
LDH peak level in U/L *(cut off limit =2,000 U/L)* *Normal range: 230-460 U/L*	*≤460 (%)* *461-1999 (%)* *≥2000 (%)*	0 (0.00) 4 (4.00) 96 (96.00)	0 (0.00) 3 (6.12) 46 (93.88)	0 (0.00) 1 (1.96) 50 (98.04)	0.288
Bilirubin (mg/dL) peak value *Normal range: 0.2- 1.0 mg/dL*	*median (iqr)*	2.5 (1.4 -3.9)	1.5 (1.0-3.6)	2.7 (1.8-4.2)	0.001
Creatinine (mg/dL) peak value *Normal range: 0.8-1.2 mg/dL (male)* *Normal range :0.6-0.9 md/dL (female)*	*median (iqr)*	3.8 (2.2-7.0)	2.2 (1.7-3.7)	5.4 (3.7-8.4)	<0.001
BUN (mg/dL) peak value *Normal range (8-20 mg/dL)*	*median (iqr)*	32 (19-58)	20 (12-32)	50 (32-83)	<0.001
INR peak value [B] *Normal range (0.8-1.2)*	*median (iqr)*	1.9 (1.5-2.8)	1.6 (1.3-1.9)	2.6 (2.0-3.9)	<0.001
aPTT (sec.) peak value [B] *Normal range (21-34 sec)*	*median (iqr)*	68.4 (48.1-89.9)	49.6 (40.7-67.9)	84.9 (68.8-103.7)	<0.001
EBOV RNA (Log cp/mL) peak value	*median (iqr)*	8.21 (7.20-8.90)	7.61 (6.42-8.13)	8.71 (8.18-9.47)	<0.001

Table 1 Unadjusted analysis to compare survivors and non-survivors.

Normal range: values are reported according SpotChem EZ and Hemochron JR user's manuals**; SD**=standard deviation; **ALT**= alanine transaminase; **AST**=aspartate transaminase; **CPK**= creatine phosphokinase; **INR**= international normalized ratio; **BUN=** blood urea nitrogen; **aPTT**= activated protrombin time; **sec.**= seconds; **mg/dL**= milligram per deciliter; **U/L** = international units per liter; **Log cp/mL=** decimal logarithm of copies per milliliter; **iqr**= interquartile range.

A: P values are calculated either by Pearson's chi-squared (for proportions) or by Kruskal Wallis (for medians);
B: Value for 96 patients (i.e. 2 survivors and 2 non-survivors) had no available results on coagulation parameters.

Biomarker and study sample	Patients' characteristics	Variation [D]. (95% CI)	P
CPK [A] Pat.= 100 Obs.=335 204 obs. uncensored below 2000 U/L 107 obs. censored at 2000 U/L 24 obs. uncensored above 2000 U/L	EBOV viremia (per 1 Log10)	1.21 (1.08-1.37)	0.001
	outcome (if non-survivors)	1.49 (0.92-2.39)	0.104
	Dialysis (if dialyzed)	2.31 (1.54-3.47)	<0.001
	Sex (if female)	0.65 (0.43-1.00)	0.052
	Age (per 10 years)	0.97 (0.85-1.12)	0.714
	Baseline in U/L (intercept) [C]	***603.68(298.50-1220.88)***	***NA***
AST [A] Pat.= 100 Obs.=335 178 obs. uncensored below 1000 U/L 114 obs. censored at 1000 U/L 43 obs. uncensored above 1000 U/L	EBOV viremia (per 1 Log10)	1.67 (1.46-1.90)	<0.001
	outcome (if non-survivors)	1.67 (1.05-2.65)	0.029
	Dialysis (if dialyzed)	1.62 (0.99-2.62)	0.052
	Sex (if female)	0.87 (0.59-1.27)	0.466
	Age (per 10 years)	1.03 (0.90-1.17)	0.686
	Baseline in U/L (intercept) [C]	***66.08(34.27-127.42)***	***NA***
ALT [A] Pat.= 100 Obs.=335 308 obs. uncensored below 1000 U/L 16 obs. censored at 1000 U/L 11 obs. uncensored above 1000 U/L	EBOV viremia (per 1 Log10)	1.24 (1.12-1.37)	<0.001
	outcome (if non-survivors)	1.59 (1.09-2.31)	0.016
	Dialysis (if dialyzed)	1.01 (0.73-1.40)	0.955
	Sex (if female)	0.78 (0.56-1.09)	0.145
	Age (per 10 years)	0.99 (0.89-1.10)	0.840
	Baseline in U/L (intercept) [C]	***102.86 (56.97-185.70)***	***NA***
LDH [A] Pat.= 100 Obs.=339 79 obs. uncensored below 4000 U/L 201 obs. censored at 4000 U/L 59 obs. uncensored above 4000 U/L	EBOV viremia (per 1 Log10)	1.27 (1.09-1.47)	0.002
	outcome (if non-survivors)	1.45 (0.80-2.61)	0.217
	Dialysis (if dialyzed)	1.85 (1.02-3.35)	0.043
	Sex (if female)	0.67 (0.41-1.09)	0.104
	Age (per 10 years)	0.94 (0.80-1.10)	0.438
	Baseline in U/L (intercept) [C]	***4392.94 (2042.45-9448.40)***	***NA***
Bilirubin [B] Pat.= 100 Obs.=335	EBOV viremia (per 1 Log10)	1.02 (0.93-1.11)	0.741
	outcome (non-survivors)	1.60 (1.20-2.11)	0.001
	Dialysis (dialyzed)	1.39 (1.10-1.77)	0.006
	Sex (if female)	0.74 (0.57-0.96)	0.022
	Age (10 years)	1.02 (0.94-1.11)	0.658
	Baseline in mg/dL (intercept) [C]	***1.01 (0.61-1.67)***	***NA***
Creatinine [B] Pat= 100 Obs.=335	EBOV viremia (per 1 Log10)	1.00 (0.96-1.04)	0.973
	outcome (non-survivors)	1.89 (1.47-2.43)	<0.001
	Dialysis (dialyzed)	1.16 (1.01-1.33)	0.038
	Sex (if female)	0.80 (0.63-1.02)	0.070
	Age (10 years)	1.10 (1.02-1.19)	0.016
	Baseline in mg/dL (intercept) [C]	***1.58 (1.09-2.29)***	***NA***
BUN [B] Pat.= 100 Obs.=335	EBOV viremia (per 1 Log10)	0.95 (0.87-1.03)	0.205
	outcome (non-survivors)	2.33 (1.72-3.16)	<0.001
	Dialysis (dialyzed)	1.10 (0.90-1.34)	0.367
	Sex (if female)	0.80 (0.60-1.07)	0.132
	Age (10 years)	1.01(0.92-1.11)	0.809
	Baseline in mg/dL (intercept) [C]	***19.21(11.34-32.54)***	***NA***
aPTT [B] Pat.= 96 Obs.=328	EBOV viremia (per 1 Log10)	1.12 (1.08-1.17)	<0.001
	outcome (if non-survivors)	1.31 (1.14-1.51)	<0.001
	Dialysis (if dialyzed)	1.22 (1.09-1.38)	0.001
	Sex (if female)	0.94 (0.83-1.07)	0.332
	Age (per 10 years)	0.99 (0.95-1.03)	0.690
	Baseline in sec. (intercept) [C]	***28.45 (22.75-35.57)***	***NA***
INR [B] Pat.= 96 Obs.=328	EBOV viremia (per 1 Log10)	1.06 (1.03-1.09)	<0.001
	outcome (if non-survivors)	1.28 (1.16-1.42)	<0.001
	Dialysis (if dialyzed)	1.19 (1.08-1.32)	0.001
	Sex (if female)	0.98 (0.90-1.07)	0.684
	Age (per 10 years)	0.97 (0.94-0.99)	0.011
	Baseline (intercept) [C]	***1.24 (1.07-1.44)***	***NA***

Table 2 Multilevel regression models to assess the proportional variation of biomarkers in patients with detectable EBOV viremia. The analyses provided strong evidence for a significant biological gradient (i.e. p-value for log-linear association <0.050) between EBOV viremia and the level of ALT, AST, CPK INR and

aPTT. All estimates in the table refer to fixed effect only. Random effect parameters are reported in the additional file.

Pat.= patients tested on the day; **Obs.**=observation; **P**: p-value; **ALT**: alanine transaminase; **AST:** aspartate transaminase; **aPTT**: activated protrombin time; **BUN:** blood urea nitrogen; **CPK:** creatine phosphokinase; **INR**: international normalized ratio; **LDH** Lactate dehydrogenase; **NA:** not applicable

A: Interval regression estimator with random intercept at patient's level and random coefficient at EBOV viremia level; **B:** Linear estimator with random intercept at patient's level and random coefficient at EBOV viremia level; **C:** expected value of biomarker at baseline (i.e.: EBOV viremia=3 Log; outcome=survivor, dialysis= non-dialyzed; sex= male; age in years =0); **D:** these coefficients represent the proportional variation of a specific biomarker form baseline (in bold) for each level of exposure to one of the 5 Patients' characteristics. For example CPK is 2.31 times higher in dialyzed than in non dialyzed patients after adjusting for EBOV RNA level, clinical outcome, sex and age.

To calculated expected value of a biomarker for a specific patient:

$$Biomarker = b0 \times b1^{\,EBOV-3} \times b2_{\,(nonsurv)} \times b3_{\,(dialyzied)} \times b4_{\,(female)} \times b5^{\,\frac{age\ in\ years}{10}}$$

Where:

Biomarker= expected value of either CPK, AST, ALT, bilirubin, creatinine, BUN, aPTT or INR**; b0**= baseline; **b1**= coefficient for EBOV viremia (intercept set to limit of detection = $3Log_{10}$); **b2**= coefficient for non survivors; **b3**= coefficient for dialyzed; **b4**= coefficient for male; **b6**= coefficient for age

Study sample	Patients' characteristics		Variation. (95% CI)	P
Patients = 100 Observation=335	CPK [A]	Normal (≤199 U/L)	Base	<0.001
		Grade 1 (200-499 U/L)	1.23 (1.02-1.49)	
		Grade 2 (500-999 U/L)	1.34 (1.10-1.62)	
		Grade 3 (1000-1999 U/L)	1.41 (1.15-1.72)	
		Grade 4 (≥2,000 U/L)	1.57 (1.29-1.92)	
	EBOV viremia		0.99 (0.94-1.03)	0.568
	outcome (non-survivors)		1.85 (1.45-2.36)	<0.001
	dialysis (dialyzed)		1.08 (0.94-1.24)	0.267
	sex (female)		0.82 (0.65-1.04)	0.101
	age (10 years)		1.10 (1.02-1.18)	0.015
	baseline creatinine in mg/dL (intercept) [B]		***1.22 (0.83- 1.80)***	***NA***

Table 3 Multilevel regression models to assess the proportional variation of creatinine according to CPK levels adjusted for all the shown covariates. The analyses provided strong evidence for a significant positive association between creatinine and CPK level independently form EBOV viremia and other patients' characteristics.

P: p-value; **CPK**= creatine phosphokinase;**A:** not applicable

A: CPK level are according to Common Terminology Criteria for Adverse Events version 4.03 **B:** expected value of creatinine at baseline (i.e.: EBOV viremia=0;CPK= normal; outcome=survivor, dialysis= non-dialyzed; sex= female; age in years= 0); **C:** these coefficients represent the proportional variation of a creatinine form baseline (in bold) for each level of exposure to one of the 5 Patients' characteristics. For example creatinine is times 1.57 times higher in patients with CPK grade 4 than in those with normal CPK after adjusting for EBOV RNA level, clinical outcome, dialysis, sex and age.

To calculated expected value of creatinine for a specific patient:

$$Creatinine = b0 \times b1^{\,EBOV} \times b2_{\,(CPK\ level)} \times b3_{\,(nonsurv)} \times b4_{\,(dialyzied)} \times b5_{\,(female)} \times b6^{\,\frac{age\ in\ years}{10}}$$

Where:

b0= baseline; **b1**= coefficient for EBOV viremia (intercept set to limit of detection = $3Log_{10}$); **b2**= coefficient for CPK, **b3=**: coefficient for non survivors; **b4**= coefficient for dialyzed; **b5**= coefficient for male; **b6**= coefficient for age

Figures legends

Figure 1. The figure shows association between biomarkers suggestive of specific organ damage and EBOV viremia, according to clinical outcome and use of dialysis. To reproduce a homogeneous picture of observed population all estimates are adjusted for mean age and male gender.

Black lines: survivors. **Red lines:** non-survivors. **Dashed line:** with dialysis. **Solid line:** without dialysis.

ALT: alanine transaminase; **AST:** aspartate transaminase; **aPTT**: activated protrombin time; **BUN:** blood urea nitrogen; **CPK:** creatine phosphokinase; **INR**: international normalized ratio; **LDH** Lactate dehydrogenase.

Figure 2. The figure shows the variation of creatinine levels according to CPK levels in survivors (blue line) and non-survivors (red line). Estimates are according mean population age (29 years). CPK levels are according Common Terminology Criteria for Adverse Events version 4.03.

Black lines: survivors. **Red lines:** non-survivors

Funding

Italian Ministry of Health, Ricerca Corrente IRCCS; Italian Ministry of Foreign Affairs; EMERGENCY's private donations; Royal Engineers for DFID-UK.

Acknowledgments

Goderich ETC has been built by the Royal Engineers as proxy for the Department for International Development – UK Government (DFID) in cooperation with EMERGENCY's Technical Division. The ETC's operations have been cofunded by DFID and EMERGENCY's private donations. The upgrading of the virology laboratory has been supported by a grant from the Italian Ministry of Foreign Affairs — Direzione General per la Cooperazione allo Sviluppo (DGCS). INMI's research activities have been performed thanks to grants from Italian Ministry of Health (Ricerca Corrente IRCCS and Ricerca Finalizzata). Deployment of laboratory personnel was possible thanks to a grant from the Italian Ministry of Foreign Affairs – DGCS. We thank the EMLab European consortium (IFS/2011/272-372; www.emlab.eu), which has been where we have developed the knowledge and experience that has allowed us to establish the virology laboratory at Goderich ETC. We thank the people who cared for at the ETC in Goderich, Sierra Leone, and all members of the INMI-EMERGENCY EBOV Sierra Leone Study group.

Conflict of interest

The authors have declared that no conflict of interest exists.

References

1 World Health Organization. End of the most recent Ebola virus disease outbreak in Liberia. June 2016 http://who.int/mediacentre/news/releases/2016/ebola-liberia/en/

2 Ippolito G, Lanini S, Brouqui P, Di Caro A, Vairo F, Abdulla S, Fusco FM, Krishna S, Capobianchi MR, Kyobe-Bosa H, Lewis DJ, Puro V, Wolfel R, Avsic-Zupanc T, Dar O, Mwaba P, Bates M, Heymann D, Zumla A. Ebola: missed opportunities for Europe-Africa research. Lancet Infect Dis. 2015 Nov;15(11):1254-5. doi: 10.1016/S1473-3099(15)00236-4. Epub 2015 Jul 28. PubMed PMID: 26231479

3 Lanini S, Zumla A, Ioannidis JP, Di Caro A, Krishna S, Gostin L, Girardi E, Pletschette M, Strada G, Baritussio A, Portella G, Apolone G, Cavuto S, Satolli R, Kremsner P, Vairo F, Ippolito G. Are adaptive randomised trials or non-randomised studies the best way to address the Ebola outbreak in west Africa? Lancet Infect Dis. 2015 Jun;15(6):738-45. doi: 10.1016/S1473-3099(15)70106-4. Epub 2015 Apr 14. Review. PubMed PMID: 25881871.

4 PREVAIL II Writing Group.; Multi-National PREVAIL II Study Team., Davey RT Jr, Dodd L, Proschan MA, Neaton J, Neuhaus Nordwall J, Koopmeiners JS, Beigel J, Tierney J, Lane HC, Fauci AS, Massaquoi MBF, Sahr F, Malvy D. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. N Engl J Med. 2016 Oct 13;375(15):1448-1456. PubMed PMID: 27732819; PubMed Central PMCID: PMC5086427.

5 Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, Carroll MW, Dean NE, Diatta I, Doumbia M, Draguez B, Duraffour S, Enwere G, Grais R, Gunther S, Gsell PS, Hossmann S, Watle SV, Kondé MK, Kéïta S, Kone S, Kuisma E, Levine MM, Mandal S, Mauget T, Norheim G, Riveros X, Soumah A, Trelle S, Vicari AS, Røttingen JA, Kieny MP. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet. 2017 Feb 4;389(10068):505-518. doi: 10.1016/S0140-6736(16)32621-6. Epub 2016 Dec 23. PubMed PMID: 28017403; PubMed Central PMCID: PMC5364328.

6 Fisher R, Borio L. Ebola Virus Disease: Therapeutic and Potential Preventative Opportunities. Microbiol Spectr. 2016 Jun;4(3). doi: 10.1128/microbiolspec.EI10-0014-2016. PubMed PMID: 27337455.

7 Lanini S, Portella G, Vairo F, Kobinger GP, Pesenti A, Langer M, Kabia S, Brogiato G, Amone J, Castilletti C, Miccio R, Zumla A, Capobianchi MR, Di Caro A, Strada G, Ippolito G; INMI-EMERGENCY EBOV Sierra Leone Study Group. Blood kinetics of Ebola virus in survivors and nonsurvivors. J Clin Invest. 2015 Dec;125(12):4692-8. doi: 10.1172/JCI83111. Epub 2015 Nov 9. PubMed PMID: 26551684; PubMed Central PMCID: PMC4665798.

8 Faye O, Andronico A, Faye O, Salje H, Boëlle PY, Magassouba N, Bah EI,Koivogui L, Diallo B, Diallo AA, Keita S, Konde MK, Fowler R, Fall G, Cauchemez S, Sall AA. Use of Viremia to Evaluate the Baseline Case

Fatality Ratio of Ebola Virus Disease and Inform Treatment Studies: A Retrospective Cohort Study. PLoS Med. 2015 Dec 1;12(12):e1001908. doi: 10.1371/journal.pmed.1001908. PMID: 26625118.

9 Crowe SJ, Maenner MJ, Kuah S, Erickson BR, Coffee M, Knust B, Klena J, Foday J, Hertz D, Hermans V, Achar J, Caleo GM, Van Herp M, Albariño CG, Amman B, Basile AJ, Bearden S, Belser JA, Bergeron E, Blau D, Brault AC, Campbell S, Flint M, Gibbons A, Goodman C, McMullan L, Paddock C, Russell B, Salzer JS, Sanchez A, Sealy T, Wang D, Saffa G, Turay A, Nichol ST, Towner JS. Prognostic Indicators for Ebola Patient Survival. Emerg Infect Dis. 2016 Feb;22(2):217-23. doi: 10.3201/eid2202.151250. PubMed PMID: 26812579.

10 Cournac JM, Karkowski L, Bordes J, Aletti M, Duron S, Janvier F, Foissaud V, Savini H, de Greslan T, Rousseau C, Billhot M, Gagnon N, Mac Nab C, Dubrous P, Moroge S, Broto H, Cotte J, Maugey N, Cordier PY, Sagui E, Merens A, Rapp C, Quentin B, Granier H, Carmoi T, Cellarier G. Rhabdomyolysis in Ebola Virus Disease. Results of an Observational Study in a Treatment Center in Guinea. Clin Infect Dis. 2016 Jan 1;62(1):19-23. doi: 10.1093/cid/civ779. Epub 2015 Sep 3. PubMed PMID: 26338789.

11 Hunt L, Gupta-Wright A, Simms V, Tamba F, Knott V, Tamba K, Heisenberg-Mansaray S, Tamba E, Sheriff A, Conteh S, Smith T, Tobin S, Brooks T, Houlihan C, Cummings R, Fletcher T. Clinical presentation, biochemical, and haematological parameters and their association with outcome in patients with Ebola virus disease: an observational cohort study. Lancet Infect Dis. 2015 Nov;15(11):1292-9. doi: 10.1016/S1473-3099(15)00144-9. Epub 2015 Aug 10. PubMed PMID: 26271406.

12 World Health Organization. Case definition recommendations for Ebola or Marburg virus diseases. WHO Web site. http://www.who.int/csr/resources/publications/ebola/ebola-case-definition-contact-en.pdf. Updated August 9, 2014. Accessed on June 29, 2016.

13 World Health Organization Clinical management of patients with viral haemorrhagic fever: a pocket guide for front-line health workers: interim emergency guidance for country adaptation. http://apps.who.int/iris/bitstream/10665/205570/1/9789241549608_eng.pdf?ua=1 accessed on (18 November 2016)

14 Curran PJ, Hussong AM. The use of latent trajectory models in psychopathology research. J Abnorm Psychol. 2003 Nov;112(4):526-44. Review. PubMed PMID: 14674867

15 Conroy, R. M. 2005. Stings in the tails: Detecting and dealing with censored data. Stata Journal 5: 395–404.

16 Cameron, A. C., and P. K. Trivedi. 2010. Microeconometrics Using Stata. Rev. ed. College Station, TX: Stata Press.

17 Dismuke CE, Egede LE, Saunders L, Krause JS. Diabetes increases financial burden of individuals with traumatic spinal cord injury (TSCI). Spinal Cord. 2015 Feb;53(2):135-8. doi: 10.1038/sc.2014.200. Epub 2014 Nov 18. PubMed PMID: 25403503.

18 Twisk J, Rijmen F. Longitudinal tobit regression: a new approach to analyze outcome variables with floor or ceiling effects. J Clin Epidemiol. 2009 Sep;62(9):953-8. doi: 10.1016/j.jclinepi.2008.10.003. PubMed PMID: 19211221.

19 London School of Economics -Methodology Institute- Benoit K Linear Regression Models with Logarithmic Transformations March 2011 available at https://pdfs.semanticscholar.org/169c/c9bbbd77cb7cec23481f6ecb2ce071e4e94e.pdf

20 Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 Published: May 28, 2009 (v4.03: June 14, 2010) U.S.DEPARTMENT OF HEALTH AND HUMAN SERVICES National Institutes of Health National Cancer Institute available at https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

21 Martines RB, Ng DL, Greer PW, Rollin PE, Zaki SR. Tissue and cellular tropism, pathology and pathogenesis of Ebola and Marburg viruses. J Pathol. 2015 Jan;235(2):153-74. doi: 10.1002/path.4456. Review. PMID: 25297522.

22 Towner JS, Rollin PE, Bausch DG, Sanchez A, Crary SM, Vincent M, Lee WF, Spiropoulou CF, Ksiazek TG, Lukwiya M, Kaducu F, Downing R, Nichol ST. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription-PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. J Virol. 2004 Apr;78(8):4330-41. PubMed PMID: 15047846.

23 Leligdowicz A, Fischer WA 2nd, Uyeki TM, Fletcher TE, Adhikari NK, Portella G, Lamontagne F, Clement C, Jacob ST, Rubinson L, Vanderschuren A, Hajek J, Murthy S, Ferri M, Crozier I, Ibrahima E, Lamah MC, Schieffelin JS, Brett-Major D, Bausch DG, Shindo N, Chan AK, O'Dempsey T, Mishra S, Jacobs M, Dickson S, Lyon GM 3rd, Fowler RA. Ebola virus disease and critical illness. Crit Care. 2016 Jul 29;20(1):217. doi: 10.1186/s13054-016-1325-2. Review. PubMed PMID: 27468829.

24 Uyeki TM, Mehta AK, Davey RT Jr, Liddell AM, Wolf T, Vetter P, Schmiedel S, Grünewald T, Jacobs M, Arribas JR, Evans L, Hewlett AL, Brantsaeter AB, Ippolito G, Rapp C, Hoepelman AI, Gutman J; Working Group of the U.S.–European Clinical Network on Clinical Management of Ebola Virus Disease Patients in the U.S. and Europe. Clinical Management of Ebola Virus Disease in the United States and Europe. N Engl J Med. 2016 Feb 18;374(7):636-46. doi: 10.1056/NEJMoa1504874.PubMed PMID: 26886522

25 McElroy AK, Erickson BR, Flietstra TD, Rollin PE, Nichol ST, Towner JS, Spiropoulou CF. Ebola hemorrhagic Fever: novel biomarker correlates of clinical outcome. J Infect Dis. 2014 Aug 15;210(4):558-66. doi: 10.1093/infdis/jiu088. Epub 2014 Feb 12. PubMed PMID: 24526742; PubMed Central PMCID: PMC4172044.

26 Rollin PE, Bausch DG, Sanchez A. Blood chemistry measurements and D-Dimer levels associated with fatal and nonfatal outcomes in humans infected with Sudan Ebola virus. J Infect Dis. 2007 Nov 15;196 Suppl 2:S364-71. PubMed PMID:17940972.

27 Lucas RM, McMichael AJ. Association or causation: evaluating links between "environment and disease". Bull World Health Organ. 2005 Oct;83(10):792-5. Epub 2005 Nov 10. PubMed PMID: 16283057; PubMed Central PMCID: PMC2626424.

28 Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, Hensley LE. Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis.2003 Dec 1;188(11):1618-29. Epub 2003 Nov 14. PubMed PMID: 14639531.

29 Schieffelin JS, Shaffer JG, Goba A, Gbakie M, Gire SK, Colubri A, Sealfon RS, Kanneh L, Moigboi A, Momoh M, Fullah M, Moses LM, Brown BL, Andersen KG, Winnicki S, Schaffner SF, Park DJ, Yozwiak NL, Jiang PP, Kargbo D, Jalloh S, Fonnie M, Sinnah V, French I, Kovoma A, Kamara FK, Tucker V, Konuwa E, Sellu J, Mustapha I, Foday M, Yillah M, Kanneh F, Saffa S, Massally JL, Boisen ML, Branco LM, Vandi MA, Grant DS, Happi C, Gevao SM, Fletcher TE, Fowler RA, Bausch DG, Sabeti PC, Khan SH, Garry RF; KGH Lassa Fever Program; Viral Hemorrhagic Fever Consortium; WHO Clinical Response Team. Clinical illness and outcomes in patients with Ebola in Sierra Leone. N Engl J Med. 2014 Nov 27;371(22):2092-100. doi:10.1056/NEJMoa1411680 PMID: 25353969.

30 McElroy AK, Harmon JR, Flietstra TD, Campbell S, Mehta AK, Kraft CS, Lyon MG, Varkey JB, Ribner BS, Kratochvil CJ, Iwen PC, Smith PW, Ahmed R, Nichol ST, Spiropoulou CF. Kinetic Analysis of Biomarkers in a Cohort of US Patients With Ebola Virus Disease. Clin Infect Dis. 2016 Aug 15;63(4):460-7. doi: 10.1093/cid/ciw334. Epub 2016 Jun 27. PubMed PMID: 27353663; PubMed Central PMCID: PMC4967605.

31 van Griensven J, Bah EI, Haba N, Delamou A, Camara, Kadio BS, Olivier JJ, De Clerck H, Nordenstedt H, Semple MG, Van Herp M, Buyze J, De Crop M, Van Den Broucke S, Lynen L, De Weggheleire A, the Ebola-Tx Consortium Electrolyte and Metabolic Disturbances in Ebola Patients during a Clinical Trial, Guinea, 2015 Emerg Infect Dis. 2016 Dec 22;12:2120-2127. DOI: http://dx.doi.org/10.3201/eid2212.161136

32 Bosch X, Poch E, Grau JM. Rhabdomyolysis and acute kidney injury. N Engl J Med. 2009 Jul 2;361(1):62-72. doi: 10.1056/NEJMra0801327. Review. PubMed PMID: 19571284.

33 Chughtai AA, Barnes M, Macintyre CR. Persistence of Ebola virus in various body fluids during convalescence: evidence and implications for disease transmission and control. Epidemiol Infect. 2016 Jun;144(8):1652-60. doi: 10.1017/S0950268816000054.

34 Petrosillo N, Nicastri E, Lanini S, Capobianchi MR, Di Caro A, Antonini M, Puro V, Lauria FN, Shindo N, Magrini N, Kobinger GP, Ippolito G; INMI EBOV Team. Ebola virus disease complicated with viral interstitial pneumonia: a case report. BMC Infect Dis. 2015 Oct 16;15:432. doi: 10.1186/s12879-015-1169-4. PubMed PMID: 26471197; PubMed Central PMCID: PMC4608352.

35 Sharma N, Cappell MS. Gastrointestinal and Hepatic Manifestations of Ebola Virus Infection. Dig Dis Sci. 2015 Sep;60(9):2590-603. doi: 10.1007/s10620-015-3691-z. Epub 2015 May 14. Review. PMID: 25972150.

36 Akmal M, Massry SG. Reversible hepatic dysfunction associated with rhabdomyolysis. Am J Nephrol. 1990;10(1):49-52. PubMed PMID: 2343880.

37 Formenty P, Hatz C, Le Guenno B, Stoll A, Rogenmoser P, Widmer A. Human infection due to Ebola virus, subtype Côte d'Ivoire: clinical and biologic presentation. J Infect Dis. 1999 Feb;179 Suppl 1:S48-53. PubMed PMID: 9988164.

38 Kozak R, He S, Kroeker A, de La Vega MA, Audet J, Wong G, Urfano C, Antonation K, Embury-Hyatt C, Kobinger GP, Qiu X. Ferrets Infected with Bundibugyo Virus or Ebola Virus Recapitulate Important Aspects of Human Filovirus Disease. J Virol. 2016 Sep 29;90(20):9209-23. doi: 10.1128/JVI.01033-16. Print 2016 Oct 15. PubMed PMID: 27489269; PubMed Central PMCID: PMC5044830.

INMI-EMERGENCY EBOV Sierra Leone Study group

Affiliated to "National Institute for Infectious Diseases INMI "Lazzaro Spallanzani", IRCCS. Via Portuense, 292. 00149 Rome, Italy: *Mirella Biava, Angela Cannas, Roberta Chiappini, Sabrina Coen, Francesca Colavita, Germana Grassi, Daniele Lapa, Antonio Mazzarelli, Silvia Meschi, Claudia Minosse, Serena Quartu, Maria Beatrice Valli, Carolina Venditti Antonella Vulcano, Paola Zaccaro*

Affiliated to"EMERGENCY NGO, Via Vida, 11. 20127 Milan, Italy": *Umar Ahmad, Elisabetta Checcarelli, Michela Delli Guanti, Elena Giovanella, Davide Gottardello, Maurizio Guastalegname, Milos Jocic, Giorgio Monti, Clare Parsons, Nicola Rossi, Giampiero Salvati, Giovanna Scaccabarozzi, Erminio Sisillo, Paola Tagliabue, Marta Turella, Caterina Valdatta.*

Giuseppe Ippolito (INMI) and Gino Strada (EMERGENCY) have authorized authorship on behalf of the Study Group