Clinical Presentation, Biochemical and Haematological Parameters and their Association with Outcome in Patients with Ebola Virus Disease: An observational Cohort Study

Short title:

Laboratory abnormalities in EVD

Authors:

Luke Hunt (MBChB)[1], Ankur Gupta-Wright (MSc)[2], Victoria Simms (PhD)[3], Fayia Tamba (HND*)[4], Victoria Knott (MBChB)[1], Kongoneh Tamba (HND*)[4], Saidu Heisenberg-Mansaray (HND*)[4], Emmanuel Tamba (HND*)[4], Alpha Sheriff (Nil)[1], Sulaiman Conteh (MBChB)[1], Tom Smith (MSc)[1], Shelagh Tobin (BSc)[5], Tim Brooks (MBChB)[6], Catherine Houlihan (MSc)[2], Rachael Cummings (MSc)[1] and Tom Fletcher (MRCP)[5, 7]

*Higher National Diploma

Author affiliations:

1. Save the Children International, St. Vincent House, 30 Orange Street, London WC2H 7HH
2. Department of Clinical Research, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT
3. Department of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT
4. Ministry of Health and Sanitation, 4th Floor Youyi Building, Freetown, Sierra Leone
5. Defence Medical Services, Whittington Barracks, Lichfield, Staffordshire WS14 9PY
6. Public Health England, Porton Down, Salisbury, Wiltshire SP4 0JG
7. Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, Merseyside L3 5QA

Correspondence:

Luke Hunt

Luke.hunt@doctors.org.uk

(0044) 7786 397984

Keywords:

Ebola, Zaire Ebolavirus, viral hemorrhagic fever, laboratory abnormalities, haematology, biochemistry, case management, outcomes

Word count: Abstract: 249; Manuscript: 3000.

Abstract

Background

Clinical management of Ebola Virus Disease (EVD) remains challenging. Routine laboratory analytics are often unavailable in the outbreak setting, and few data exist on the associated haematological and biochemical abnormalities. We present laboratory and clinical data from the Kerry Town Ebola Treatment Centre in Sierra Leone to better inform clinical management algorithms, improve understanding of key variables associated with outcome and provide insight into the pathophysiology of EVD.

Methods

150 patients with confirmed EVD were admitted between 8th December 2014 and 9th January 2015. At admission, all patients had clinical presentation recorded and blood taken for Ebola confirmation using reverse-transcriptase–polymerase-chain-reaction (RT-PCR) and for haematological and biochemical analysis. The association between these and clinical outcome was evaluated.

Findings

The mean age of cases was 26 years. Case fatality rate was 35% (55/150). Most patients presented with stage 2 (gastrointestinal involvement, 61%, 72/118) and stage 3 (severe/complicated, 10%, 12/118) disease. Acute Kidney injury (AKI) was common (50%, 52/104), as were abnormal serum potassium (33%, 32/97), severe hepatitis (59%, 54/92) and raised CRP (21%, 21/100). Haematological abnormalities were common, including raised haematocrit (15%, 15/100), thrombocytopaenia (45%, 47/104) and granulocytosis (42%, 44/104). Severe AKI, low RT-PCR cycle threshold (<20 cycles) and severe hepatitis were independently associated with mortality.

Interpretation

EVD is associated with a high prevalence of haematological and biochemical abnormalities, even in mild disease and in the absence of gastrointestinal symptoms. Clinical care targeting hypovolaemia, electrolyte disturbance and AKI are likely to reduce historically high case fatality rates.

Funding

None

Introduction

Ebola virus disease (EVD) is caused by an RNA Filovirus, and is characterised by a febrile illness (87%), often progressing to diarrheoa (67%), vomiting (66%) and, sometimes, haemorrhage (18%).[1] The current outbreak in West Africa is the largest the world has seen, with almost 25,000 cases (25th March 2015).[2] Clinical and public health management is challenging, especially in the worst hit countries of Sierra Leone, Liberia and Guinea, due to severity of clinical presentation, infection control concerns, poor health-system infrastructure and high population density.[3,4]

There are currently no proven treatments for EVD, thus management is supportive, including administration of oral and intravenous fluids and electrolytes, management of co-infections and symptom control.[3-5] EVD is recognised to cause marked biochemical abnormalities which may be amenable to simple interventions, potentially reducing the high case fatality rate (CFR).[4,6-12] Due to the low-resource setting of outbreaks and risks associated with collecting and processing laboratory samples, few data exist on these abnormalities, mostly from animal models, small cohorts or case studies from resource-rich settings.[6-9,11,13]

Kerry Town Ebola Treatment Centre (ETC), operated by Save the Children, opened on November 5th 2014 in Sierra Leone offering supportive care focused on fluid and electrolyte management, in accordance with World Health Organization (WHO) guidelines.[3] This included haematology and biochemistry testing for all admissions from December 8th 2014. We report data on clinical presentation, laboratory abnormalities, and their association with mortality in patients admitted over a one month period. This data will assist in case management in centres without access to laboratory support, provide insight into the disease pathophysiology, corroborate animal models, and help identify priority areas for future research.

Methods

Study Design

We conducted a cohort study of patients consecutively admitted to Kerry Town ETC between 8th December 2014 and 9th January 2015. During this period only patients with EVD confirmed at other isolation or community care centers were admitted. We used a standard case definition as per WHO guidelines.[2] All patients were included in the study except for those who were dead on arrival, or those who had no blood results within 24 hours of admission. Primary outcome measure was discharge from the ETC.

Blood Analysis

Samples were analysed at the on-site laboratory run by Public Health England and the UK Defence Medical Services (UK-DMS). The Piccolo Express® system (Abaxis, California, USA) was used to generate metabolic and liver function profiles. An assay with biochemistry and liver function profile was primarily used

(Amylyte 13), though some samples were processed on a separate assay that included lactate, magnesium, calcium and phosphate but excluded liver function profile (Metlac 12). Laboratory scientists selected the assay based on availability, unless clinically requested. Haematology samples were processed using a Horiba ABX Micros ES60® analyser (Horiba, Montpellier, France). Reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays for diagnosis of EBOV were performed using the Altona RealStar® Filovirus RT-PCR Kit (Altona, Hamburg, Germany), following inactivation and manual RNA extraction. Positive results were reported as Ct (cycle threshold) values. All patients had a repeat RT-PCR on admission.

Data Collection

All data was collected as part of routine patient care, and recorded on standardised forms, which were kept securely. Data extracted for research purposes was anonymised and stored on a password-protected database. The Sierra Leone Ethics and Scientific Review Committee provided study approval.

Clinical Staging and management

All patients were assigned a disease stage on admission according to their clinical features. The staging system was developed by the UK-DMS in reference to WHO guidelines and the proposed pathogenesis of EVD and is outlined in Table 1.[3,12-15] Children were staged using a similar system, informally adapted to include paediatric indicators of dehydration as per WHO.[3] Standardised therapy was administered to all patients based on disease stage. Targeted electrolyte replacement was provided based on biochemistry results. Patients were discharged once asymptomatic for 72 hours with negative repeat blood Ebola RT-PCR. Acute Kidney Injury (AKI) was defined according to RIFLE criteria.[16] To estimate baseline creatinine, we used the Modification of Diet in Renal Disease (MDRD) for adults,[17] and the Schwartz calculation for children.[18] For ALT and AST, 'high' was defined as 5-15 times the upper limit of normal (ULN), and 'very high/severe' was defined as >15 times ULN. EBOV RT-PCR Ct value (Ct) was categorised as low (<20 cycles) or high (≥20 cycles) with low Ct indicating high viral load.

Statistical Analysis

Descriptive analyses are reported as frequencies, proportions, means or medians. We used χ^2, t-test and Wilcoxon rank-sum for comparisons. Risk factors for mortality were assessed using logistic regression. *A priori* variables, and variables associated with mortality in univariate analysis (at $p<0.1$) were assessed in a multivariate model. Interactions were assessed using likelihood ratio tests. Adjustment was not made for missing data since biochemical and haemotological variables were missing at random. Hypothesis tests were two-tailed (at $p<0{\cdot}05$). Data was analysed using Stata (Statacorp, Texas, USA), v13.

Role of the Funding Source

The sponsor 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 the data in the study and had final responsibility for the decision to submit for publication.

Results

During the study period, 150 patients were admitted with confirmed EVD. Additionally 6 non-EVD cases were referred and 4 patients died in transit. Absent laboratory samples were due to no sample being obtained on admission (n=8) and a laboratory isolator fault meaning no samples were processed from 26th-29th December (n=24). Of the 118 included patients, 104 had haematology and 114 had biochemistry analysed. In these patients, absent tests were due to no haematology sample being taken (n=12), haematology sample inadequate for analysis (n=2) or no biochemistry sample taken (n=4). 101 patients had liver function analysed and 18 had lactate measured, with 5 having both.

Baseline characteristics are described in Table 2. Among EVD cases, 52·5% were male. The mean age was 25·9 years (sd 14·7). Children (<18 years) accounted for 32·2%. Median time from admission to discharge amongst survivors was 8 days (inter quartile range (IQR) 5-12) and median time from admission to death was 4 days (IQR 3-5). Patients without laboratory investigations were more likely to be under 5 years (p=0·003) and female (p=0·033) but otherwise had similar baseline characteristics to those with laboratory results.

Mortality

Overall CFR for the study period was 36·7% (55/150). CFR in those included in this study was 34·7% (41/118). CFR was non-significantly higher in men than women (40·3% vs. 28·6%. X^2=1·79, p=0·181) and lower in children (23·7% vs. 40·0%, X^2=3·02, p=0·082). Higher disease stage on admission was strongly associated with mortality (66·7%, 32·7% and 25·8% for stage 3, 2 and 1 respectively, p=0·001). Mean time from symptom onset to admission was shorter in patients who died than in survivors. There was no association between time from symptom onset and disease stage at admission.

RT-PCR Ct values

The mean Ct in positive patients was 21·4 cycles (sd 4·5), with 41·1% (46/112) having a low Ct. Low Ct on admission was strongly associated with mortality (65·2% vs. 13·6%, p<0·001). Fifteen patients were transferred following positive EBOV RT-PCR tests at the referring center, but tested negative at admission, despite remaining symptomatic. Their survival rate was 100%.

Biochemistry

Biochemistry results for the cohort are outlined in Table 3 and Figure 1. Creatinine levels were significantly higher in fatal than non-fatal cases (median 467µmol/L vs. 90µmol/L respectively, p<0·001, n=104). AKI was common, occurring in 50·0% (52/104) of cases. AKI occurred more commonly in fatal than non-fatal cases (81·3% vs. 18·1%, p<0·001) and when it did occur it was always severe (RIFLE-3). Incidence of AKI did not differ significantly by clinical stage (χ^2= 9·83, p=0·610). Notably, 28·6% (9/31) of patients admitted with stage 1 disease had severe AKI.

Abnormal potassium occurred in 33·0% (32/97) of admissions, with similar proportions having hypokalaemia and hyperkalaemia (19·6%, 19/97 and 13·4%, 13/97 respectively). Patients that died were more likely to have hyperkalaemia (35·7% vs. 4·4% of survivors, p<0·001), as were patients with AKI (26·7% with RIFLE-3 AKI vs. 6·5% with no AKI or RIFLE 1-2 AKI, p=0·007). Hyponatraemia was common (31·9%, 36/113), but was not associated with outcome. Abnormal liver function was common, with 70·3% (71/101) having alanine transaminase (ALT) or aspartate transaminase (AST) >5 times ULN. Severe hepatitis (AST >15 ULN) was more common in fatal than non-fatal cases (92·9% vs. 43·8% respectively, p<0·001). Median AST:ALT ratio was 2·3, and did not differ significantly between outcomes. Bilirubin was normal in 87·0% of cases.

Raised creatinine kinase (CK) was common, and higher in fatal than non-fatal cases (median CK 2938 IU/L versus (vs.) 1519 IU/L, p=0·019, n=100). All cases with a normal CK survived. Fourteen cases had CK>5000 IU/L (upper limit of our assay). Almost all cases with RIFLE-3 AKI had raised CK (97·1% vs. 78·3% with no AKI, p=0·014). Median C-reactive protein (CRP) was higher in fatal than non-fatal cases (median CRP 12.9 vs. 69.5, p<0.001, n=100), and more fatal cases had CRP >100mg/L (46·9% vs. 8·8% in survivors, p<0·001). Lactate was raised in 91·7% (16/17) of cases.

Haematology

Haematology results for the cohort are outlined in Table 3 and Figure 1. Higher mean haemoglobin (Hb), haematocrit (Hct) and median platelet count were associated with mortality. Mean Hb, Hct and platelets in non-survivors versus survivors were 15·5g/dl vs. 13·5g/dl (p<0.001, n=103) 45·1 vs. 39·4% (p<0.001, n=100) and 146 x10^9/L vs. 197 x10^9/L (p=0·005, n=104) respectively. Thrombocytopaenia was more common amongst non-fatal cases (53·7% vs. 29·7%, p=0·019). Severe thrombocytopaenia (<50x10^9/L) was uncommon (2·9%, 3/104).

Low median white cell count (WCC), lymphocyte count and granulocyte count also predicted survival. Median WCC was 15·8 x10^9/L vs. 7·9 x 10^9/L, (p<0·001, n=104) median lymphocytes were 4·2 x10^9/L vs. 2·2x 10^9/L (p<0·001, n=104) and granulocytes were 11·5 x 10^9/L vs. 4·3 x 10^9/L (p=0.001, n=104) in non-survivors

vs. survivors respectively. Granulocytosis was present in 42·3% (44/104) of cases, and lymphocytosis was more common in fatal cases (66·7% vs. 31·0% p=0·002). Malaria co-infection was uncommon with 2·5%, 3/114 having a positive Malaria RDT on admission.

Risk factors for mortality

In unadjusted analyses, the strongest risk factors for mortality were RIFLE-3 AKI (OR 19·7, 95%CI 6·7-57·4, vs. no AKI or RIFLE-1-2 AKI, p<0·001), severely raised AST (OR 16·7, 95%CI 3·7-76·5, vs. not severely raised AST, p<0·001), high haematocrit (OR 13·4, 95%CI 2·7-66·7, vs. normal, p=0·002), low EBOV RT-PCR Ct (OR 11·9, 95%CI 4·7-30·1, vs. high Ct, p<0·001), hyperkalaemia (OR 11·1, 95%CI 2·7-45·7, vs. normal potassium, p=0·001), CRP >100mg/dl (OR 9·1, 95%CI 3·1-27·1, vs. CRP ≤100, p<0·001) and granulocytosis (OR 8·7, 95%CI 2·9-26·5, vs. normal granulocytes, p<0·001).

Although hyperkalaemia appeared to be associated with RIFLE-3 AKI, both remained associated with mortality in bivariate analyses (p<0·001). Similarly, haemoglobin and haematocrit were associated with each other, however haematocrit remained strongly associated with mortality after adjusting for haemoglobin (OR 26·9, 95%CI 2·5-289·3). CRP remained strongly associated with mortality (OR 16·5, 95%CI 3·8-71·2), even after adjustment for granulocytosis.

Multivariate analysis was undertaken using variables associated with mortality *a priori* (age, gender, RT-PCR Ct) or in univariate/bivariate analysis. Age was fitted as a continuous variable. Hyperkalaemia, CRP and granulocytosis appeared to become protective due to collinearity and were removed to improve stability. Hematocrit was removed because it had no effect. No interaction terms contributed to the model. The final model is shown in Table 4. The strongest independent risk factors for mortality were low EBOV RT-PCR Ct (OR 6·7, p=0·013, 95% CI 1·5-30.1) and RIFLE-3 AKI (OR 5·8, p=0·033, 95% CI 1·2-29·6). Disease stage on admission was not associated with mortality after adjustment for other risk factors.

Discussion

The natural history of EVD is well characterised with a gastrointestinal stage leading to significant hypovolaemia, systemic hypoperfusion and shock in inadequately fluid-resuscitated patients.[4] The extent of multi-organ dysfunction syndrome—commonly involving the renal, hepatic, neurological and coagulation systems—varies from mild dysfunction to irreversible organ failure and death, but the pathogenesis of EVD remains poorly understood. Key to improving outcomes is the availability of routine biochemistry and haematological testing. Large observational studies are also required to compare data with animal models and support clinical trials of novel therapeutics.

The majority of our patients were admitted with stage 2 or 3 disease (74/118), and had high levels of organ dysfunction and associated metabolic abnormalities. Fifty percent (52/104) of cases had AKI, and raised creatinine was associated with mortality, supporting earlier published data.[6, 7] Importantly kidney dysfunction was not limited to later disease stage, supporting the role of IV fluid therapy in early disease.

AKI is generally attributed to pre-renal causes.[4,15] However, our results suggest AKI may be multifactorial, given the high prevalence in early disease where dehydration is a less common clinical finding. The role of rhabdomyolysis is unclear and worthy of further study given our almost universal findings of elevated CK (and its association with AKI), and disproportionately elevated AST. However CK was rarely elevated to levels associated with AKI, apart from a discreet number of patients who reached the ceiling of our assay measurement. Subsequent renal injury may also arise due to poor renal perfusion secondary to profound hypovolaemia from gastro-intestinal losses, or viral or secondary bacterial sepsis resulting in acute tubular necrosis.[19] Microvascular damage from disseminated intravascular coagulation may also contribute; raised D-Dimer was a common finding in patients with Sudan Ebolavirus[6] and autopsy of infected primates reported fibrinous cellular debris in glomerulus.[20] Importantly, our data show that EVD-associated AKI can be managed successfully in this setting.

Hepatocellular inflammation has previously been reported.[8,9] Our data showed that most patients had elevated ALT/AST levels, which was associated with mortality. Normal bilirubin levels indicated fulminant hepatic failure is not a major feature consistent with the clinical syndrome observed. Elevated CRP and granulocytosis were also associated with mortality, which may reflect secondary bacterial infection, [20] although all patients were treated with a third-generation cephalosporin from stage 2 of clinical disease.

The data is the first reported on haematological abnormalities, and show that mortality was associated with higher haemoglobin and haematocrit. This may be a surrogate marker for intravascular fluid depletion, widely believed to be associated with poor outcomes.[4] Thrombocytopaenia was common and occurred in 45·2% of patients at presentation, but was rarely severe. It was not possible to measure coagulation in our cohort, and this should be a priority for future research given the frequency and poorly understood nature of haemorrhage in EVD. Semi-quantitative EBOV viral load measurement has been shown to be a useful predictor of mortality [8,12] and was associated with clinical outcome in our series.

We report a number of study limitations. The CFR is lower than others have reported. [7,10,11] While this may be due to a high-standard clinical care, it is possible that there is a survivor bias (i.e. patients with EVD more likely to survive were admitted to this ETC) since only patients confirmed to have Ebola were admitted during the study period, and there were time delays prior to ETC admission. This is supported by the finding

that some patients were RT-PCR negative at admission, suggesting that they may have already cleared the virus. Caution should be used in comparing CFRs between settings. The finding that a shorter time from symptom onset to admission was associated with mortality may have been caused by recall bias and survivor bias; symptom onset date was collected from records sent with the patient, which were not always complete. Patients also presented to the ETC at different stages of illness, and had variable levels of treatment in holding centres prior to admission.

Due to an isolator failure, 24 consecutive patients had no laboratory testing. The significance of this to our results is unclear. A number of the assays (AST, ALT, CK) were limited by their upper limits of detection, potentially underestimating the difference between groups. Additionally, baseline creatinine was estimated based on MDRD scores, which may have over- or under-estimated the prevalence of AKI.

Conclusion

Our data show that the provision of routine laboratory support to an ETC provides opportunity for improved understanding of disease pathophysiology, and correlation with clinical disease staging. We demonstrate a previously unreported high prevalence of electrolyte imbalance and organ dysfunction in all stages of EVD. The ability to diagnose and treat these abnormalities at presentation and follow patients' response to therapy is likely to improve survival.

The most important aspects of supportive care are aggressive management of intravascular volume depletion, correcting electrolyte abnormalities, and preventing the complications of shock.[21] These are underlying tenets of critical care medicine, which can and should be applied in this setting. Improving the provision of good supportive care including laboratory analysis is essential to further clarify the pathogenesis and pathophysiology of EVD in humans. In parallel to ongoing intervention studies, this will inform evidence-based protocols to improve outcomes for this outbreak and the next.

Acknowledgements

We would like to thank Save the Children for enabling us to collect the data, and to all staff at Kerry Town ETC for their contribution towards the successful running of the facility.

Disclosures

None

Author Contribution Statement

LH, AGW, VS, FT, VK, CH, RC and TF contributed towards study design. LH, AGW, VS, FT, VK, KT, SHM, ET,

AS, SC, TS, ST, TB and TF contributed towards data collection. AGW, VS, ST and TB contributed towards data analysis. LH, AGW, VS and TF drafted the manuscript. All authors contributed towards manuscript revision.

Table 1

Stage		Clinical Features	Typical Patient	Standard Therapy
1	Early/Mild	Non-specific features. Pyrexia, weakness, lethargy, myalgia, arthritis	Ambulatory, able to compensate for fluid losses via oral intake	Oral Rehydration Solution Symptomatic treatment Zinc / Multivitamins Anti-malarials if RDT positive Targeted electrolyte replacement[+] Treatment of hypoglycemia
2	Gastrointestinal Involvement	As above plus: diarrhoea, vomiting and/or abdominal pain	Unable to compensate for fluid losses via oral intake due to emesis or loss of large volumes	*As per stage 1, plus:* IV fluid therapy (3000-6000ml/24h for adults, guided by fluid and electrolyte balance) IV Ceftriaxone*
3	Complicated	As above plus: hemorrhage, shock, neurological involvement and/or signs of organ failure	Critically ill, usually hypovolaemic, often with confusion and/or seizures, bleeding.	*As per stage 1+2, plus:* As clinically indicated: Sedation/anti-epileptics Vitamin K Fresh Frozen Plasma[#]

RDT rapid malaria diagnostic test; IV intravenous. [+] Sodium, potassium, magnesium, calcium and phosphate replaced according to biochemistry results. *Ceftriaxone (2g once per day for adults) was given as an empirical 5 day course in all stage 2 and 3 patients. It was continued for longer or given to stage 1 patients if clinically indicated. # Fresh frozen plasma was given when hemorrhage occurred and when available.

Table 1 Clinical staging system for Ebola virus disease used at Kerry Town ETC and subsequent standard clinical management.

Table 2

		Included in study			No laboratory tests sent
		Survived	Died	Total	
	Total number	77	41	118	32
Gender	Male	37 (48·1)	25 (61·0)	62 (52·5)	10
	Female	40 (51·9)	16 (39·0)	56 (47·5)	22
Age (years)	Mean (sd)	23·2 (13·1)	31·0 (16·2)	25·9 (14·7)	25·1 (18·5)
	0-4	3 (3·9)	0	3 (2·5)	5
	5-14	20 (26·0)	8 (19·5)	28 (23·7)	5
	15-24	20 (26·0)	5 (12·2)	25 (21·2)	4
	25-34	20 (26·0)	11 (26·8)	31 (26·3)	9
	35-44	7 (9·1)	8 (19·5)	15 (12·7)	5
	45-80	7 (9·1)	9 (22·0)	16 (13·6)	4
Time from symptom onset to admission (days)	Mean (sd)	5·3 (3·2)	4·4 (2·9)	5·0 (3·1)	5·6 (2·2)
	1-3	16 (20·8)	13 (31·7)	29 (24·6)	4
	4-6	23 (29·9)	14 (34·1)	37 (31·4)	12
	≥7	14 (18·2)	3 (7·3)	17 (14·4)	7
	Unknown	24 (31·2)	11 (26·8)	35 (29·6)	9
Stage at admission	Stage 1	23 (29·9)	8 (19·5)	31 (26·3)	7
	Stage 2	47 (61·0)	25 (61·0)	72 (61·0)	16
	Stage 3	4 (5·2)	8 (19·5)	12 (10·2)	7
	Unknown	3 (3·9)	0	3 (2·5)	2
Ebola RT-PCR at admission	Positive	62 (80·5)	41 (100·0)	103 (87·3)	30
	Negative	15 (19·5)	0	15 (12·7)	1
	No result	0	0	0	1
Malaria RDT	Positive	1 (1·3)	2 (4·9)	3 (2·5)	1
	Negative	72 (93·5)	39 (95·1)	111 (94·1)	29
	Unknown	4 (5·2)	0	4 (3·4)	2

Data are numbers (column %) unless otherwise stated. RT-PCR reverse-transcriptase-polymerase-chain-reaction; RDT rapid malaria diagnostic test.

Table 2 Characteristics of patients with confirmed Ebola virus disease admitted to Kerry Town ETC during the study period

325 **Table 3**

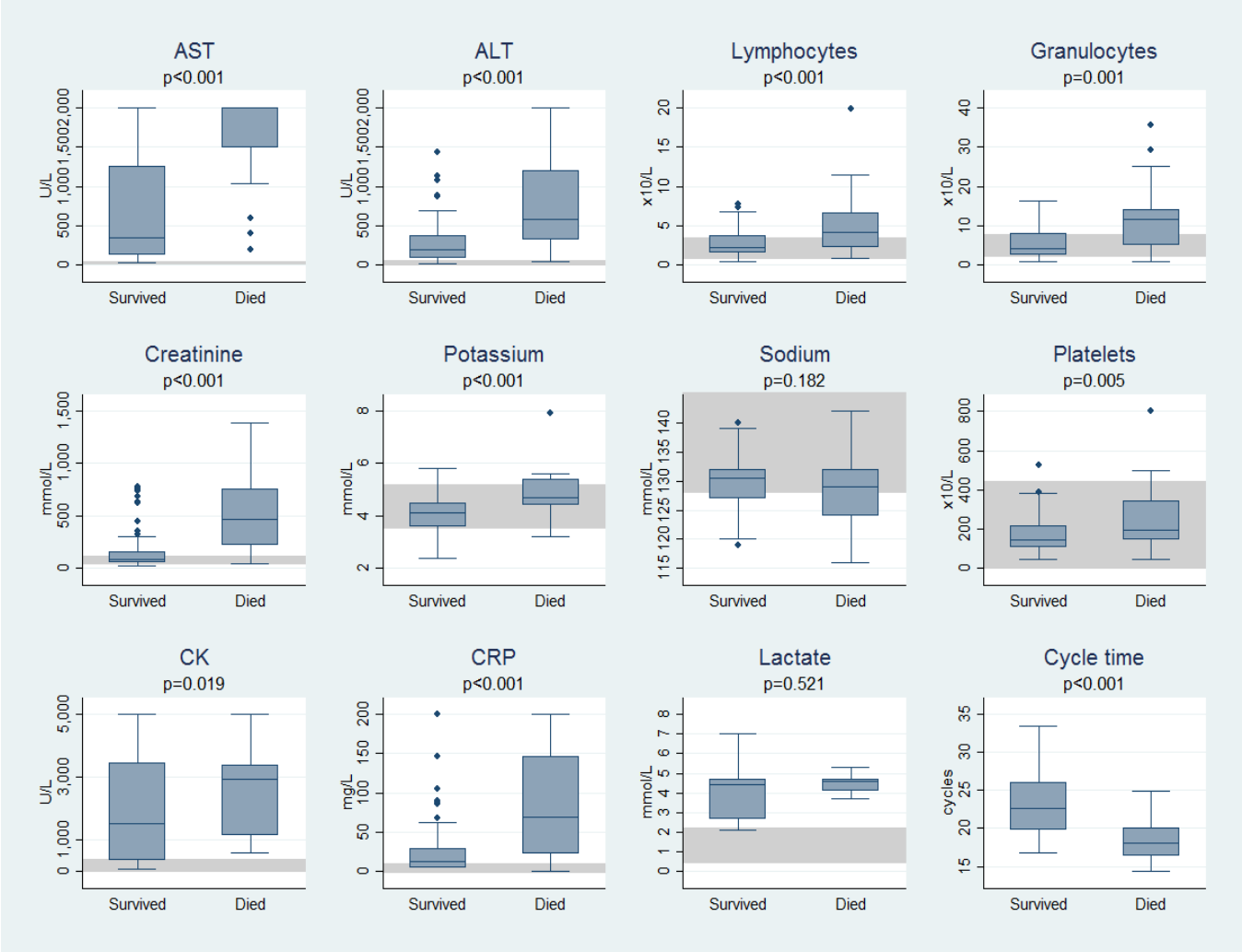
Haematology					
		Survived	**Died**	**Total**	**Test**
Haemoglobin	Mean g/dL (sd)	13·5 (2·1)	15·5 (2·6)	14·2 (2·5)	p<0·001*
	Anemia n (%)	20/66 (30·3)	5/37 (13·5)	25/103 (24·3)	p=0·057#
Haematocrit	Mean % (sd)	39·4 (6·6)	45·1 (7·5)	41·4 (7·5)	p<0·001*
	Raised, n(%)	2/64 (3·1)	13/36 (36·1)	15/100 (15·0)	p<0·001#
	Low, n(%)	29/64 (45·3)	7/36 (19·4)	36/100 (36·0)	
Platelets	Median x10^9/L (IQR)	146 (108-219)	197 (145-346)	155 (119-247)	P=0·005$
	Thrombocytopaenia, n(%)	36/67 (53·7)	11/37 (29·7)	47/104 (45·2)	p=0·019#
White blood cells	Median x10^9/L (IQR)	7·9 (4·8-12·4)	15·8 (8·6-21·7)	10·0 (5·2-16·0)	p<0·001$
	Raised, n(%)	21/67 (31·3)	25/37 (67·6)	46/104 (44·2)	p=0·001#
	Low, n(%)	12/67 (17·7)	5/37 (13·5)	17/104 (16·4)	
Lymphocytes	Median x10^9/L (IQR)	2·2 (1·5-3·7)	4·2 (2·2-6·6)	2·6 (1·6-4·4)	p<0·001$
	Raised, n(%)	19/67 (28·4)	24/37 (64·9)	43/104 (41·4)	p=0·001#
	Low, n(%)	7/67 (10·4)	1/37 (2·7)	8/104 (7·7)	
Granulocytes	Median x10^9/L (IQR)	4·3 (2·5-8·0)	11·5 (4·9-14·0)	5·7 (2·8-11·0)	p=0·001$
	Raised, n(%)	19/67 (28·4)	25/37 (67·6)	44/104 (42·3)	p<0·001#
	Low, n(%)	15/67 (22·4)	7/37 (18·9)	22/104 (21·2)	
Viral Load					
RT-PCR Cycle time	Mean n (sd)*	23·4 (4·3), 60	18·3 (2·7), 39	21·4 (4·5), 99	p<0·001*
	Low, n(%)	16/73 (21·9)	30/39 (76·9)	46/112 (41·1)	p<0·001#
Biochemistry					
Creatinine	Median µmol/L (IQR)	90 (58-161)	467 (225-754)	121 (65-392)	P<0·001$
	RIFLE-1, n(%)	5/72 (6·9)	0/32	5/104 (4·8)	p<0·001#
	RIFLE-2, n(%)	8/72 (11·1)	0/32	8/104 (7·7)	
	RIFLE 3, n(%)	13/72 (18·1)	26/32 (81·3)	39/104 (37·5)	

Potassium	Mean mmol/L (sd)	4·0 (0·7)	4·8 (0·9)	4·2 (0·9)	p<0·001*
	Raised, n(%)	3/69 (4·4)	10/28 (35·7)	13/97 (13·4)	p<0·001#
	Low, n(%)	16/69 (23·2)	3/28 (10·7)	19/97 (19·6)	
Sodium	Mean mmol/L (sd)	129·8 (4·6)	128·5 (5·8)	129·4 (5·1)	p=0·182*
	Low, n(%)	22/76 (30·0)	14/37 (37·8)	36/113 (31·9)	p=0·341#
Lactate	Mean mmol/L (sd)	4·0 (1·4)	4·5 (0·6)	4·2 (1·2)	p=0·521*
	Raised, n(%)	11/12 (91·7)	5/5 (100·0)	16/17 (94·1)	p=0·506#
Total dissolved CO_2	Median mmol/L (IQR)	22 (17-28)	17 (11-18)	20 (15-23)	P=0·035$
	Low, n(%)	3/12 (25·0)	4/6 (66·7)	7/18 (38·9)	p=0·087#
Liver function tests					
Alanine transaminase (ALT)	Median IU/L (IQR)	192 (86-371)	577 (314-1195)	253 (121-594)	P<0·001$
	High, n(%)	21/68 (30·9)	16/33 (48·5)	37/101 (36·6)	p<0·001#
	Very high, n(%)	5/68 (7·4)	11/33 (33·3)	16/101 (15·8)	
Aspartate transaminase (AST)	Median IU/L (IQR)	348 (129-1254)	>2000 (1491->2000)	867 (168->2000	P<0·001$
	High, n(%)	11/64 (17·2)	2/28 (7·1)	13/92 (14·1)	p<0·001#
	Very high, n(%)	28/64 (43·8)	26/28 (92·9)	54/92 (58·7)	
AST:ALT ratio	Median (IQR), n	2·2 (1·4-3·3)	3·1 (1·6-3·5)	2·4 (1·4-3·4)	p=0·259$
Creatinine kinase	Median IU/L (IQR), n	1519 (367-3454)	2938 (1137-3400)	1949 (589-3400)	P=0·019$
	High, n(%)	51/68 (75·0)	32/32 (100·0)	83/100 (83·0)	p=0·002#
C-reactive protein	Median mg/L (IQR)	12·9 (5·0-29·4)	69·5 (22·5-147·0)	21·4 (6·0-77·0)	P<0·001$
	High, n(%)	6/68 (8·8)	15/32 (46·9)	21/100 (21·0)	p<0·001#

All data are numbers (column %) unless otherwise stated· *median only presented for RT-PCR results that were positive·* denotes p-value from t-test, #deontes p-value from χ^2 test, $deontes p-value from Wilcoxon rank-sum· Definitions are as follows; RT-PCR - reverse-transcriptase-polymerase-chain-reaction, anemia - as per WHO definitions, [22] thrombocytopaenia <150 x $x10^9$/L, raised white cell count >11 $x10^9$/L, low white cell count <4 $x10^9$/L, raised lymphocytes >3·2 $x10^9$/L, low lymphocytes <1 $x10^9$/L, raised granulocytes >7·5 $x10^9$/L, low granulocytes <2·5 $x10^9$/L, low RT-PCR Cycle time <20 cycles, RIFLE-1 AKI 1·5-2 x baseline creatinine, RIFLE 2 AKI 2-3 x baseline creatinine, RIFLE-3 AKI >3 x baseline creatinine, raised potassium >5·1 mmol/L, low potassium <3·6mmol/l, raised Lactate >2·1 mmol/l, low total dissolved CO2 <18 IU/L, high ALT >240 - ≤720 IU/L, very high ALT >720 IU/L, high AST >175 - ≤525 IU/L, very high AST >525 IU/L, high creatinine kinase >380 U/L and high c-reactive protein >100 mg/l·

Table 3 Laboratory results and abnormalities in patients admitted to Kerry Town ETC with confirmed Ebola

Figure 1



ALT alanine transaminase; AST asparate transaminase; CK creatinine kinase; CRP C-reactive protein; Cycle time is Ebolavirus reverse-transcriptase-polymerase-chain reaction cycle time. P-values are from t-tests (potassium and sodium) and Wilcoxon rank-sum tests (all others). Shaded area indicates normal range for the UK based population.

Figure 1 Box plots of laboratory results from patients admitted to Kerry Town ETC; results are presented by outcome.

Table 4

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Female Gender	0·59 (0·27-1·28)	0·182	0·33 (0·07-1·63)	0·174
Age	1·04 (1·01-1·07)	0·008	1·01 (0·95-1·06)	0·819
Disease stage	2.16 (1.08-4.32)	0.029	1.40 (0.41-4.84)	0.593
RT-PCR Cycle threshold <20	11·88 (4·69-30·06)	<0·001	6·72 (1·50-30.07)	0·013
RIFLE-3 AKI	19·67 (6·73-57·43)	<0·001	5·84 (1·15-29·58)	0·033
AST>525 U/L	16·71 (3·65-76·47)	<0·001	6.95 (0·70-68·86)	0·097
Potassium >5·1 mmol/L	11·11 (2·70-45·66)	<0·001	-	
CRP >100mg/dl	9·12 (3·07-27·07)	<0·001	-	
Granulocytes >7·5 x10^9/L	8·68 (2·85-26·45)	<0·001	-	
High haematocrit	13·41 (2·70-66·67)	0·002	-	

OR odds ratio; RT-PCR reverse-transcriptase-polymerase-chain-reaction; AST aspartate transaminase, AKI acute kidney injury. P-values are from likelihood ratio tests. Age was treated as a continuous variable.

Table 4 Factors associated with mortality in patients with previously confirmed Ebola virus admitted to Kerry Town ETC

References:

1. WHO Ebola Response Team. Ebola virus disease in West Africa—the first 9 months of the epidemic and forward projections. *New England Journal of Medicine.* 2014. 371(16): 1481-1495.
2. World Health Organization. WHO Ebola Situational Report- 25th March 2015. Geneva: 2015.
3. World Health Organization. Clinical Management of Patients with Viral Haemorrhagic Fever: A Pocket Guide for the Front-Line Health Workers. Geneva: 2014.
4. Fowler R, Fletcher T, Fischer W, Lamontagne F, Jacob S, Brett-Major D et al. Caring for Critically Ill Patients with Ebola Virus Disease. Perspectives from West Africa. *American Journal of Respiratory Critical Care Medicine*. 2014; 190(7): 733-737.
5. Yazdanpanah Y, Arribas J, Malvy D. Treatment of Ebola Virus Disease. *Intensive Care Medicine*. 2014; 41(1): 115-117.
6. Rollin P, Bausch D, Sanchez A. Blood Chemistry Measurements and d -Dimer Levels Associated with Fatal and Nonfatal Outcomes in Humans Infected with Sudan Ebola Virus. *Journal of Infectious Diseases*. 2007; 196(s2): S364-S371.
7. Schieffelin J, Shaffer J, Goba A, Gbakie M, Gire S, Colubri A et al. Clinical Illness and Outcomes in Patients with Ebola in Sierra Leone. *New England Journal of Medicine*. 2014; 371(22): 2092-2100.
8. Sanchez A, Lukwiya M, Bausch D, Mahanty S, Sanchez A, Wagoner K et al. Analysis of Human Peripheral Blood Samples from Fatal and Nonfatal Cases of Ebola (Sudan) Hemorrhagic Fever: Cellular Responses, Virus Load, and Nitric Oxide Levels. *Journal of Virology.* 2004; 78(19): 10370-10377.
9. Kortepeter M, Bausch D, Bray M. Basic Clinical and Laboratory Features of Filoviral Hemorrhagic Fever. *Journal of Infectious Diseases*. 2011; 204(s3): S810-S816.
10. Bah E, Lamah M, Fletcher T, Jacob S, Brett-Major D, Sall A et al. Clinical Presentation of Patients with Ebola Virus Disease in Conakry, Guinea. *New England Journal of Medicine*. 2015; 372(1): 40-47.
11. Chertow D, Kleine C, Edwards J, Scaini R, Giuliani R, Sprecher A. Ebola Virus Disease in West Africa — Clinical Manifestations and Management. *New England Journal of Medicine*. 2014; 371(22): 2054-2057.
12. Rees P, Lamb L, Nicholson-Roberts T, Ardley C, Bailey M, Hinsley D et al. Safety and Feasibility of a Strategy of Early Central Venous Catheter Insertion in a Deployed UK Military Ebola Virus Disease Treatment Unit. *Intensive Care Medicine*. 2015; 1-9
13. Davis K, Anderson A, Geisbert T, Steele K, Geisbert J, Vogel P. Pathology of Experimental Ebola Virus Infection in African Green Monkeys: Involvement of Fibroblastic Reticular Cells. *Archives of Pathology & Laboratory Medicine*. 1997; 121: 805-19.
14. Zaki S, Goldsmith C. Pathologic Features of Filovirus Infections in Humans. *Current Topics in Microbiology and Immunology* 1999; 235: 97-116.
15. Fletcher T, Fowler R, Beeching N. Understanding Organ Dysfunction in Ebola Virus Disease. *Intensive Care Medicine*. 2014; 40(12): 1936-1939.
16. Hoste E, Clermont G, Kersten A, Venkataraman R, Angus D, De Bacquer D, Kellum J. RIFLE Criteria for Acute Kidney Injury are Associated with Hospital Mortality in Critically Ill Patients: a Cohort Analysis." *Critical Care*. 2006; 10.3: R73.

17. Levey A, Bosch J, Lewis J, Greene T, Rogers N, Roth D. A more Accurate Method to Estimate Glomerular Filtration Rate from Serum Creatinine: A New Prediction Equation. *Annals of Internal Medicine*. 1999; 130.6: 461-470.

18. Schwartz G, Haycock G, Edelmann C, Spitzer A. A Simple Estimate of Glomerular Filtration Rate in Children Derived from Body Length and Plasma Creatinine. *Pediatics. 1976;* 58.2: 259-263.

19. Schrier R, Wang W. Acute Renal Failure and Sepsis. *New England Journal of Medicine*. 2004; 351(2): 159-169.

20. Kreuels B, Wichmann D, Emmerich P, Schmidt-Chanasit J, de Heer G, Kluge S et al. A Case of Severe Ebola Virus Infection Complicated by Gram-Negative Septicemia. *New England Journal of Medicine*. 2014; 371(25): 2394-2401.

21. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B et al. Early Goal-Directed Therapy in the Treatment of Severe Sepsis and Septic Shock. *New England Journal of Medicine*. 2001; 345(19): 1368-1377.

22. World Health Organisation. Iron deficiency Anaemia : Assessment, Prevention and Control : A Guide for Programme Managers. Geneva: World Health Organisation; 2001.