Virus kinetics and biochemical derangements among children with Ebolavirus disease

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Summary

Background A paucity of data is available on virologic and biochemical characteristics of paediatric Ebolavirus disease (EVD), compared to adults.

Methods We conducted a retrospective chart review of children (<16 years old) and a comparator group of young adults (16–44 years) from two treatment centres during the 2018–2020 EVD epidemic in Eastern Democratic Republic of the Congo. Statistical methods included chi-squared and Fisher's exact tests (dichotomous and categorical variables), Mann-Whitney U-test (continuous variables), multivariable linear regression (for determinants of admission viral load), linear mixed-effects models (for analysis of longitudinal viral load), and Cox proportional hazard models (to examine risk factors for mortality).

Findings We included 73 children and 234 adults admitted from April to October 2019. Paediatric patients commonly had electrolytes imbalances: hypokalaemia in 26/73 (36%), hyperkalaemia in 38/73 (52%), and hyponatraemia in 54/73 (74%). Hypoglycaemia occurred in 20/73 (27%), acute kidney injury in 43/73 (59%), and rhabdomyolysis in 35/73 (48%). Biochemical abnormalities were detected in a similar proportion of children and adults. The viral load (VL, $\log_{10}$ copies/mL) at admission (7.2 versus 6.5, p=0.0001), the peak viral load (7.5 versus 6.7, p=<0.0001), and the time for viraemia clearance (16 days versus 12 days, p=<0.0001) were significantly different in children. The duration of hospital stay was prolonged in children (20 versus 16 days, p=<0.0001). Risk factors for mortality in children were: VL >7.6 $\log_{10}$copies/mL, alanine transaminase >525 U/L, C-reactive protein >100 mg/L, blood urea nitrogen >7.5 mmol/L, rhabdomyolysis, and.acute kidney injury.

Interpretation Paediatric EVD patients, like adults, experience multiorgan dysfunction with life-threatening electrolyte imbalances, hypoglycaemia, kidney injury, liver injury, and rhabdomyolysis. Paediatric patients have significantly higher VLs throughout the course of EVD than adults.

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Research in context

Evidence before this study

We searched PubMed up to August 2021 inclusive using the search term "ebolavirus disease." Filters used were "human" and all filters that included ages <19 years. There were no language restrictions in the literature search. The search revealed 543 articles. Only two papers were found that described laboratory values in paediatric patients with Ebolavirus disease (EVD). These two papers were small, retrospective studies. In these studies, elevated leukocytes, deranged liver and kidney function, and hypoglycaemia were prominent in the paediatric age group.

Added value of this study

This is the largest study to date of virologic and biochemical abnormalities in paediatric EVD (73 patients and >1700 longitudinal measurements of multiple analytes). The data come from two Ebola Treatment Units (ETUs) in Butembo, North-Kivu, Democratic Republic of the Congo (DRC), during the 10th EVD outbreak (2018–2020). To the best of our knowledge, this study is the first to report the comparison of laboratory analytes between paediatric and adult patients with EVD. It also describes the viral kinetics of the Ebolavirus (EBOV) among the paediatric population.

Implications of all the available evidence

These findings further describe the impact EVD has on the paediatric patients, a vulnerable population with a reported mortality rate ranging from 42 to 86%. This information will help clinicians to better care for the paediatric patients infected with Ebola virus.

Introduction

Zaïre ebolavirus (EBOV) is a zoonotic pathogen that has caused multiple outbreaks since it was first described in 1976.[1] After the largest outbreak occurred in West Africa in 2013–2016, the second largest Ebola Virus Disease (EVD) outbreak occurred in the DRC from August 1 2018 to June 25 2020. In this outbreak, 3 470 confirmed and probable cases and 2 287 deaths were recorded. Of the total cases, 1 002 (29%) were children under 18 years of age.[2]

The West African outbreak provided more data on the impact of the disease on the paediatric population than previous outbreaks. Reported mortality rates for children under 18 years of age were between 42–65%, and as high as 83–86% in children under five years of age in the absence of any specific therapeutics.[3–9] With high mortality and limited laboratory data, there is a need to describe the virologic characteristics and the organ injury in the paediatric population.[3–5] The West African and the three recent DRC outbreaks led to an evolution in EVD patient care from containment and isolation towards supportive management, and provision of specific EVD therapeutics (antivirals and monoclonal antibodies). In addition, vaccination of contacts and contacts of contacts of confirmed case is recommended in response to EVD outbreak, which may have an impact on patients' chance of survival.[10,11] In the 10th EVD outbreak, increased monitoring of critically ill patients within Ebola Treatment Units (ETUs) included longitudinal viral load (VL) and biochemical measurements to guide supportive care and discharge decisions.

The clinical manifestations of EVD in paediatric patients were summarized by Dixit et al.[2] Signs and symptoms include fever (63–100%), anorexia (55–99%), weakness (74–98%), diarrhoea (42–80%), vomiting (36–70%), and abdominal pain (35–60%).[2] Of note, previous retrospective studies looking at children under five years of age have documented lower rates of fever (63–79%), highlighting that children with EVD may not present with fever.[2] In 2015, a WHO report on 7292 patients from the 2014 outbreak, compared the clinical manifestations of three age cohorts (<16, 16–44, and >45) and showed that children under 16 reported fewer symptoms such as abdominal pain, arthralgia, chest pain, headache, and myalgias.[9] This could be related to children's limited ability to self-report symptoms and does not necessarily rule out these manifestations. Due to the multi-organ dysfunction observed in EVD, it is also important to assess the laboratory values in the paediatric population. To our knowledge, there are no prior studies comparing the laboratory parameters of EBOV in adults and children.

The objective of this study was to describe the viral kinetics and biochemical abnormalities in paediatric patients with EVD and to compare these to young adult EVD patients.

Methods

Study design and patient population

This study was a retrospective review of patient data from the 10th DRC EVD outbreak of 2018–2020. We included EBOV positive patients < 16 years admitted to two ETUs run by the Ministry of Health of the DRC in Butembo and Katwa (North Kivu, DRC) from April to October 2019. In the same period, we also included Ebola positive adolescents and young adults aged 16–44 years old admitted to both ETUs as a comparator group. These age groups were chosen to be consistent with age categories used in a previous WHO study.[9]

During this period, some patients were managed according to clinical guidelines and received investigational therapeutics under compassionate use (Monitored Emergency Use of Unregistered and Investigational Interventions) and others were enrolled

in a clinical trial.[10] Changes in treatment practices reflected an evolving evidence base. Before 19 August 2019, one of the following Ebola-specific treatments were administered to patients, if possible: Z-mapp, MAb114 (Ebanga®), REGN-EB3, and Remdesivir.[10] After 19 August 2019, only MAb114 or REGN-EB3 were used based on the interim results of a Randomized Controlled Trial (RCT) conducted concurrently during the same outbreak.[10]

Data collection and laboratory methods

Patient data including demographics (age, sex, treatment centre, prior vaccination status, known EVD contact, and timing of symptom onset), management (specific antiviral therapy), and outcomes (mortality and duration of hospitalization among survivors) were extracted from patient records within the ETUs throughout admission. The confirmation of EVD was made within INRB (Institut National de Recherche Biomédicale, the DRC National Public Health Laboratory) field laboratories in Butembo and Katwa with Cepheid GeneXpert® instrument, an automated real-time reverse-transcription PCR (rt RT-PCR), as per WHO recommendations. The Xpert Ebola system assay uses human specimens such as whole blood, and other bodily fluids (e.g., semen[14]) to detect Ebola Glycoprotein (GP) and Nucleoprotein (NP) genes. The GeneXpert® cycle threshold values (Ct-values) were used as a proxy to determine the VL.[12] To estimate the VL from Ct-values for the NP gene, we used a published standard curve ($Ct = -3.44 \times VL + 46.11$).[13]

Blood chemistry values for sodium, potassium, calcium, glucose, creatinine, alanine aminotransferase (ALT), aspartate transaminase (AST), bilirubin, albumin, C-reactive protein, and creatine kinase were determined using the Piccolo Amlyte13® discs run on the Piccolo Xpress Chemistry Analyzer® (Abaxis, Union City, CA) within INRB field laboratories as per manufacturer's instructions. In addition, point-of-care glucometer measurements were frequently taken into the ETUs and abstracted from the chart record for this study. All data were collected as part of routine patient care.

Clinical definitions

Vaccination with the rVSVΔG-ZEBOV-GP vaccine was documented based on report from the patient or family members. Moderate and severe hypoglycaemia were defined as a blood glucose level of 2.2 to <4 mmol/L, and <2.2 mmol/L on at least one measurement taken during the hospitalization, respectively. Hyperglycaemia was defined as glucose ≥11.1 mmol/L on at least one measurement.

Hypalbuminaemia was defined as albumin level < 35 g/L on at least one measurement. Rhabdomyolysis was defined as creatine kinase level >1000 U/L on at least one measurement. Hyponatremia and hypernatremia were defined as sodium levels of <135 mmol/L and >145 mmol/L, respectively, on at least one measurement. Hypokalaemia and hyperkalaemia were defined as potassium levels of <3.5 mmol/L and >5 mmol/L, respectively, on at least one measurement. Hypocalcaemia and hypercalcaemia were defined as calcium levels of < 2.0 mmol/L and > 2.5 mmol/L, respectively, on at least one measurement.

Acute kidney injury (AKI) was defined using the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. AKI was defined as an increase in creatinine by ≥26.5 μmol/L within 48 h or an increase in Cr to ≥ 1.5 × baseline which is known or presumed to have occurred within the prior 7 days. To calculate the baseline Cr, we assumed a baseline GFR of 120 mL/min/1.73 m^2 for children (< 18 years of age), 100 mL/min/1.73 m^2in young adults (≥ 18 years and < 40 years).

Statistical analysis

Comparison of laboratory measurements between the two groups was done with the non-parametric Mann–Whitney U test (continuous data) or the two-tailed Pearson Chi-Square or Fisher's exact test, as appropriate (categorical and dichotomous data). Results with a p-value < 0.05 were considered significant. With respect to the multivariable linear regression analysis, the outcome variable (admission VL) was modeled as a function of age (<16 years versus 16–45 years, dichotomous) and prior vaccination status (dichotomous). The purpose of this analysis was to evaluate the relationships of these variables and adjust for potential confounders; therefore, only variables that were significantly different in the bivariable analyses were included in the multivariable model. With respect to the longitudinal analysis of repeated VL measurements, we followed the method of Lanini *et al.*[11,15] VL as a function of time after admission was modeled with a maximum likelihood linear mixed effects regression model. The model included random intercept at patient level and random slope at time level.[15] As fixed effects, age (<16 years versus 16–45 years, dichotomous), prior vaccination status (dichotomous), treatment category (six-level categorical factor), outcome (fatal versus non-fatal, dichotomous), were included in the model. Fixed effects were considered statistically significant if a likelihood ratio test p-value comparing the model including the factor to the null model without the factor was <0.05. With respect to predictors of mortality, we used Cox proportional hazards regression to model the time-to-death as a function of admission virologic or biochemical parameters, in children and adults separately. Factors that were statistically significantly associated with fatal outcome in the bivariate analyses were included in a multivariable Cox regression model to adjust for

confounding. Forward variable selection was used to build a parsimonious model in which all predictors were statistically significant. For all statistical models, visual inspection of residuals was used to verify model assumptions. Data analyses were performed using GraphPadPrism version 6 (GraphPad Software Inc., La Jolla, CA, USA, 2012), and the R statistical environment (www.r-project.org).

Ethics statement

This study is a part of the Ebola outbreak response conducted under the responsibility of the "Secrétariat Technique du Comité Multisectoriel de Lutte contre la Maladie à Virus Ebola (MVE)" itself under the supervision of the presidency of the Democratic Republic of Congo. The study was approved by the Comité d'Éthique du Nord Kivu, based at the Centre Hospitalier Universitaire du Graben, Butembo, DRC. For this retrospective chart review which did not involve any patient contact, the requirement for written informed consent was waived by the Comité d'Éthique du Nord Kivu.

Role of funder

The funder had no role in study design, data collection, data analysis, data interpretation, writing of the report, or decision to submit the article for publication. The following authors accessed the raw data: KMC and MTH.

Results

Between April 24 and October 14, 2019, 73 children <16 years of age (41% female), were admitted to the Butembo or Katwa ETUs. Their data were reviewed and included in this study (Table 1). An additional 234 young adults, 16–44 years of age (58% female), were admitted over the same period. These patients' data were reviewed and included into the study as a comparator group (Table 1).

A total of 601 estimated VL measurements based on the GeneXpert results, 498 biochemistry panel measurements, and an additional 672 point-of-care glucose measurements were taken for clinical management among the 73 children (Table 2). Of note, the VL at admission (median 7.2 $\log_{10}$copies/mL *versus* 6.5 $\log_{10}$copies/mL, p=0.0001), the peak VL (median 7.5 $\log_{10}$copies/mL *versus* 6.7 $\log_{10}$copies/mL, p<0.0001), the time to clearance of viraemia (median 16 days *versus* 12 days, p<0.0001), and the duration of hospital stay (median 20 days *versus* 16 days, p<0.0001, Table 1), were significantly different in the <16 group compared to the comparator group of young adults (Table 2). Plasma creatinine was lower in children compared to adults (median 62µmol/L *versus* 110µmol/L, p<0.0001), but the frequency of AKI, which adjusts for differences in muscle mass with age and sex, was similar in children (Table 2). There were no other significant differences between groups in other laboratory values (Table 2). With respect to prior reported vaccination status, fewer paediatric patients received the rVSVΔG-ZEBOV-GP

	Overall (N=307)	0 to <16 yr (N=73)	16 to ≤ 44 yr (N=234)	*P*-value
Sex				**0.016**
Male	141 (46)	43 (59)	98 (42)	
Female	166 (54)	30 (41)	136 (58)	
Treatment Centre				0.067
Butembo	171 (67)	55 (75)	154 (66)	
Katwa	86 (33)	18 (25)	80 (34)	
Prior vaccination with rVSVΔG-ZEBOV-GP	45 (15)	4 (5.5)	41 (18)	**0.013**
Known EVD contact, n (%)	189 (62)	47 (64)	142 (61)	0.67
Time from symptom onset to admission [days], median (IQR)	4 (2–7)	4 (2–6)	4 (3–7)	0.28
Treatment				
Z-mapp	34 (11)	9 (12)	25 (11)	reference
MAb114	70 (23)	22 (30)	48 (21)	0.78
REGN-EB3	56 (18)	18 (25)	38 (16)	0.74
Remdesivir	56 (18)	5 (7)	51 (22)	0.054
None	30 (10)	9 (12)	21 (9)	0.97
Missing	61 (20)	10 (14)	51 (22)	0.36
Outcome				
Fatal, n (%)	139 (45)	36 (49)	103 (44)	0.51
Survived, n (%)	168 (55)	37 (51)	131 (56)	
Duration of hospitalization [days], median (range)[1]	16 (13–20)	20 (17–25)	16 (12–18)	**<0.0001**

***Table 1*: Clinical characteristics of children and adults with EVD.**
[1] Among survivors.

	Overall (N=307)	0 to <16 yr (N=73)	16 to ≤ 44 yr (N=234)	*P*-value
***Virus kinetics*, median (IQR)**				
Number of tests per patient, median (range)	5 (1–46)	4 (1–33)	5 (1–46)	0.86
Viral load at admission [log_{10}copies/mL]	6.6 (5.1–7.6)	7.2 (6.3–8.1)	6.5 (4.8.0–7.4)	**0.0001**
Peak viral load [log_{10}copies/mL]	6.9 (5.7–7.8)	7.5 (6.7–8.1)	6.7 (5.4–7.6)	**<0.0001**
Time to undetectable viral load [days][1]	12 (8–16)	16 (14–21)	12 (7–14)	**<0.0001**
Biochemistry profile				
Patients with at least one biochemistry measurement	253 (82)	64 (88)	189 (81)	0.24
Number of tests per patient, median (range)	3 (1–27)	3 (1–28)	6 (1–42)	0.85
Renal injury				
Peak creatinine [µmol/L]	97 (71–280)	62 (46–95)	110 (80–340)	**<0.0001**
Peak BUN [mmol/L]	6.4 (3.2–16)	5.7 (3.2–11)	6.4 (3.2–18)	0.33
Acute kidney injury	145 (47)	43 (59)	102 (44)	0.10
Sodium				
Hyponatraemia, n (%)	196 (64)	54 (74)	142 (61)	0.13
Hypernatremia, n (%)	44 (14)	13 (18)	31 (13)	0.59
Potassium				
Hypokalaemia, n (%)	95 (31)	26 (36)	69 (29)	0.91
Hyperkalaemia, n (%)	143 (47)	38 (52)	105 (45)	>0.99
Calcium (corrected for albumin)				
Hypocalcaemia, n (%)	6 (2)	0 (0)	6 (3)	0.34
Hypercalcaemia, n (%)	165 (54)	41 (56)	124 (53)	0.94
Liver injury				
Peak ALT, median (IQR) [IU/L]	260 (120–470)	380 (120–500)	250 (120–430)	0.26
Peak AST, median (IQR) [IU/L]	490 (190–1400)	570 (270–1700)	430 (190–1300)	0.20
Peak bilirubin [µmol/L]	14 (10–21)	12 (9.0–19)	14 (10–24)	0.069
Albumin				
Nadir (g/L)	19 (15–25)	19 (14–23)	20 (16–25)	0.31
Hypalbuminaemia, n (%)	248 (81)	62 (85)	186 (79)	0.27
C-Reactive protein				
Peak CRP (mg/L)	79 (29–190)	62 (25–150)	82 (30–200)	0.21
Peak CRP >40 mg/L, n (%)	161 (53)	39 (53)	122 (52)	0.65
Creatine kinase				
Peak CK [U/L]	1500 (550–3200)	1450 (530–2900)	1500 (590–3400)	0.51
Rhabdomyolysis, n (%)	142 (46)	35 (48)	107 (46)	0.65
Glucose dysregulation				
Patients with at least one glucose measurement	279 (91)	69 (95)	210 (90)	0.25
Number of tests per patient	10 (3–20)	8 (2–23)	10 (3–19)	0.92
Severe hypoglycaemia, n (%)	62 (20)	20 (27)	42 (18)	0.13
Hyperglycaemia, n (%)	132 (43)	33 (45)	99 (42)	0.89

***Table 2*: Viral load and biochemical profile of children and adults with EVD.**
CRP C-reactive protein; CK creatine kinase; ALT alanine transaminase; AST aspartate aminotransferase; BUN blood urea nitrogen; IQR interquartile range.
[1] Among survivors.

vaccine compared to adults (5.5% *versus* 41%, *p*=0.013, Table 1). There was no significant difference in the mortality rate between both groups (Table 1). Further stratification of the findings by paediatric age classes (infants 0 to < 1 years of age, preschool children 1 to < 5 years, and school-aged children 5 to < 16 years) is shown in Supplemental Table S1.

The admission VL was higher among paediatric patients (Table 2) and among those who had not received the rVSVΔG-ZEBOV-GP vaccine. In a multivariable linear regression model, both age <16 (*p*=0.0022) and prior vaccination status (*p*=0.039) remained statistically significant independent predictors of elevated VL at admission (Supplemental Figure S1). Longitudinal trends in the VL are shown in Figure 1. Assuming first-order kinetics, the elimination half-life of EBOV in the blood was similar in paediatric and adult patients [0.82 days (IQR 0.74–0.94) *versus* 0.82 days (IQR 0.70–0.92), *p*=0.72]. In a linear mixed effects model of the VL over time in children and adults, in

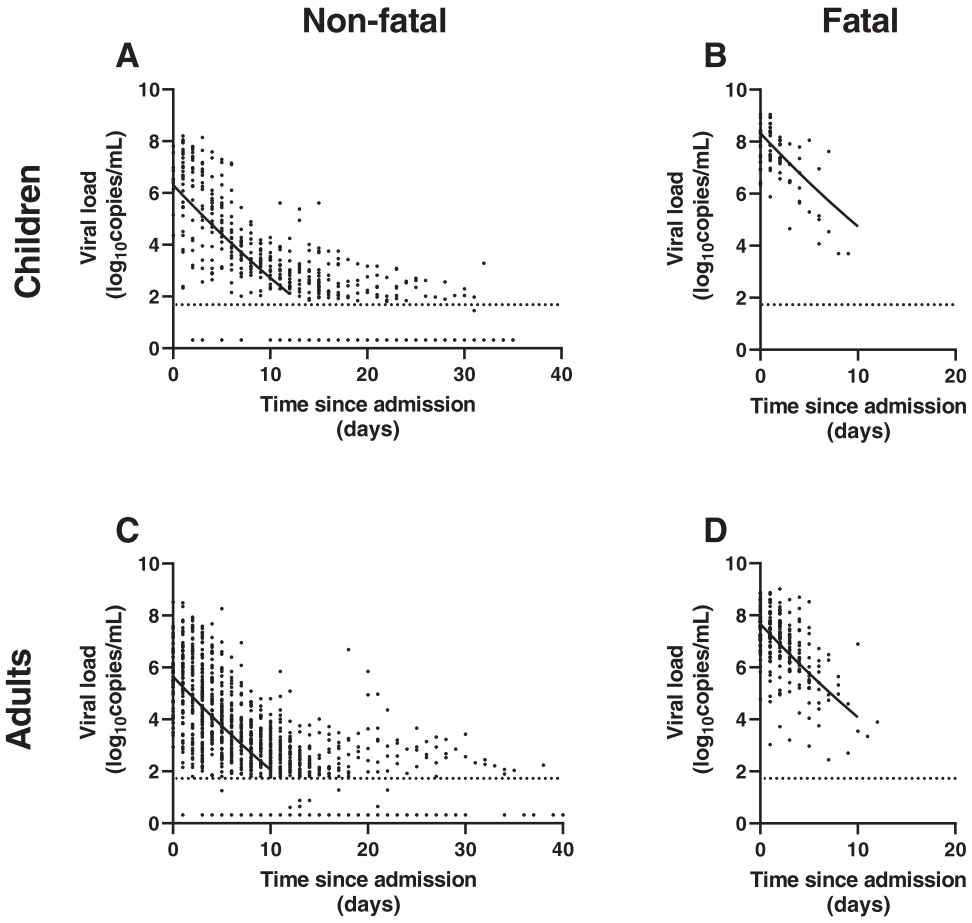

Figure 1. Observed and modelled EBOV RNA blood levels (viral load) according to the time since admission, age category (children versus adults) and clinical outcome. All estimates were made on the full dataset, including 307 patients and 2,268 EBOV RNA determinations. **A.** Children, survivors: 37 patients with 520 EBOV RNA determinations. **B**. Children, fatal outcome: 36 patients with 81 EBOV RNA determinations. **C.** Adult survivors: 131 patients with 1,411 EBOV RNA determinations. **D**. Adults, fatal outcome: 103 patients with 256 EBOV RNA determinations. Dots indicate single EBOV RNA determinations; black line indicates LME model prediction; dashed line indicates the limit of detection of the PCR assay (3.11 $\log_{10}$ copies/ml).

fatal and non-fatal cases, and adjusting for the effect of prior vaccination status and different Ebola specific treatment regimens, children had an adjusted mean difference of 0.56 $\log_{10}$ copies/mL (95%CI 0.23–0.88) higher VL than adults over the course of the infection (Supplemental Table S2, p=0.00080).

Several laboratory parameters at admission to the ETU were associated with subsequent mortality in children (Figure 2). As a measure of the strength of the association, the point estimate of the hazard ratio for each risk factor was generally lower (closer to unity) in children than adults, with wider confidence intervals by virtue of the smaller sample size (Figure 3). The following admission values were associated with fatal outcome in children: ALT >525 U/L, VL >7.6 $\log_{10}$copies/mL, BUN >7.5 mmol/L, CRP >100 mg/L, rhabdomyolysis, and AKI (Table 3). In a multivariable logistic regression model, ALT and VL remained statistically significant independent predictors of the mortality (Table 3).

Discussion

This is the largest study to date of the virologic and biochemical abnormalities in paediatric EVD (73 patients and >1700 longitudinal measurements of multiple analytes) and the first, to our knowledge, to compare laboratory values between paediatric and adult patients with EVD. Although previous studies described clinical manifestations of paediatric EVD, there are limited data on laboratory values, especially in children. Fitzgerald *et al*, in a study of 282 patients under 13 years of age, reported laboratory values on 35 (12%), including blood count, electrolytes, kidney and liver parameters, and inflammatory markers and found that children frequently had

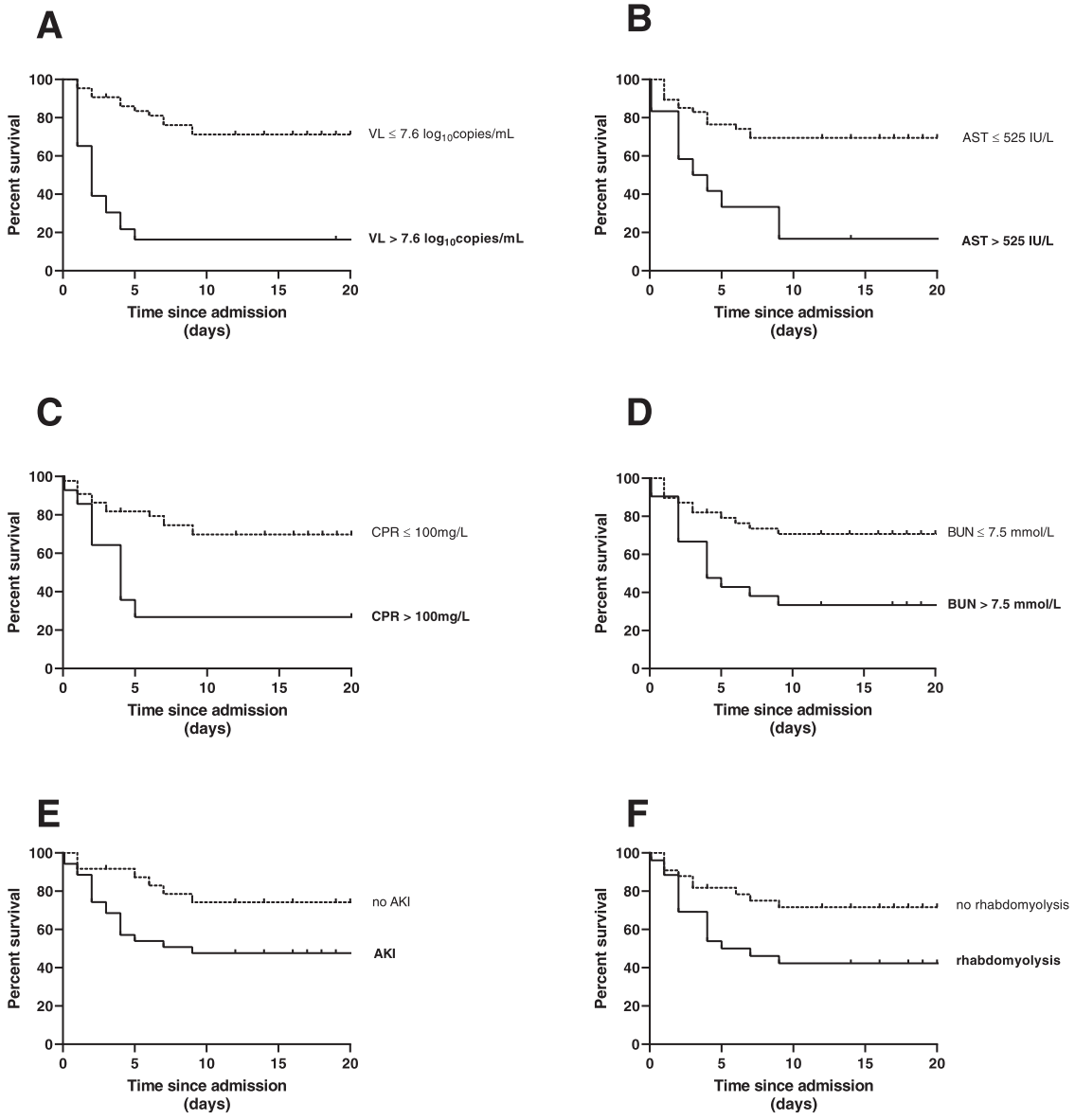

Figure 2. Laboratory predictors of mortality in children (n=73) with Ebola virus disease. The survival curves, stratified by selected virological and biochemical parameters, measured at admission to the ETU, are shown. **A.** Viral load (VL) > 7.6 log_{10}copies/mL was associated with an increased hazard of death (HR 5.8 (95%CI 2.8 to 12), $p<0.0001$), as was **B.** Alanine transaminase (ALT) > 7525 IU/L (HR 4.0 (95%CI 1.8 to 9.1), $p=0.00086$); **C.** C-reactive protein (CRP) > 100 mg/L (HR 3.7 (95%CI 1.6 to 8.6), $p=0.0024$); **D.** Blood urea nitrogen (BUN) > 7.5 mmol/L (HR 3.0 (95%CI 1.4 to 6.7), $p=0.0062$); **E.** Acute kidney injury (AKI, HR 2.7 (95%CI 1.1 to 6.7), $p=0.027$); and **F.** Rhabdomyolysis (HR 2.7 (95%CI 1.1 to 5.8), $p=0.028$).

elevated leukocytes, derangement of liver and kidney function, as well as hypoglycaemia.[5] McElroy *et al.* performed several multiplex assays to measure the concentrations of 55 serum analytes among 55 paediatric patients (≤ 21 years old) with Sudan ebolavirus and found no age-specific associations between serum chemistry results and mortality.[16] Multiple studies have reported laboratory values in both adults and children; however, paediatric and adult patients were not considered separately in these studies.[17–19]

Children with EVD in our study commonly had life-threatening electrolyte abnormalities: hypokalaemia (36%), hyperkalaemia (52%), and hyponatraemia (74%). The prevalence of these metabolic derangements was not significantly different between children and adults. Hunt *et al.* examined laboratory values in 118 patients

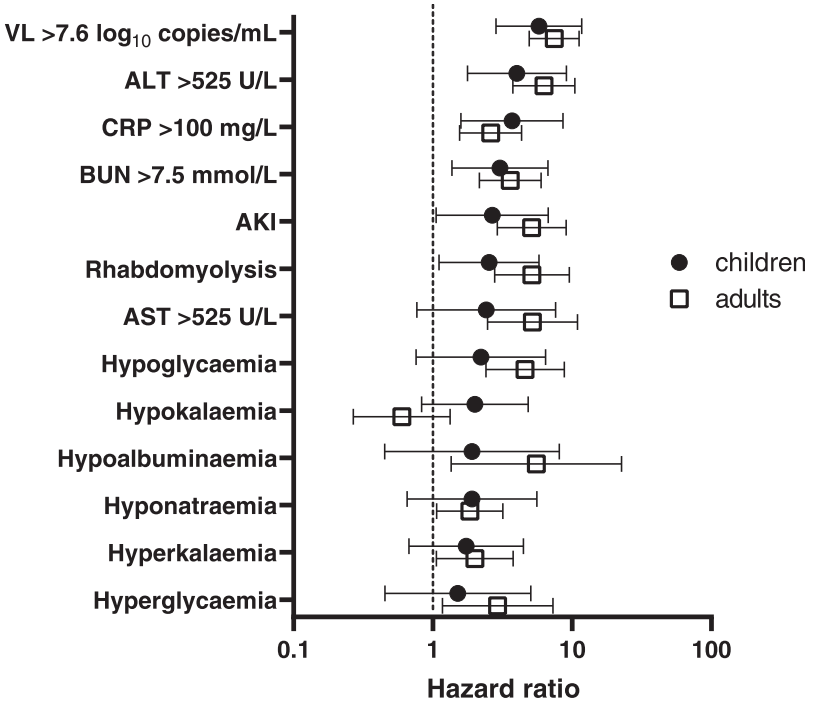

Figure 3. Comparison of laboratory predictors of mortality in children (n=73) and adults (n=234) with Ebola virus disease. The hazard ratios (HRs) are shown as point estimates for children (solid circles) and adults (open squares), with whiskers representing the 95% confidence intervals. Laboratory parameters which did not cross the line of equivalence (HR=1, dashed line) were statistically significant predictors of fatal outcome. For children, these were: viral load >7.6log$_{10}$copies/mL, ALT >525U/L, BUN >7.5 mmol/L, CRP >100 mg/L, rhadomyolysis, and acute kidney injury (AKI).

(74% over 15 years old) and described lower rates of electrolyte abnormalities (20% hypokalaemia, 13% hyperkalaemia, and 32% hyponatraemia) than our study.[18] Gastrointestinal losses leading to hypovolaemia are well documented in EVD, and paediatric patients have a smaller blood volume, which may make them vulnerable to electrolyte imbalances.[6]

Markers of kidney dysfunction and liver injury from EVD have been previously reported in adult or combined paediatric-adult studies.[17–20] Our paediatric group had a median peak ALT of 380 IU/L and AST of 570 IU/L, similar to a previous study in adults (median ALT 253IU/L and median AST 867 IU/L).[18] We observed a significantly higher peak creatinine in adults compared to children but the rate of AKI was not statistically significantly different (59% of children and 44% of young adults). This is possibly explained by a higher muscle mass in adults which is considered in the KDIGO definition of AKI. The rate of AKI in our study was similar to a previous report in adults (50%).[18] Liver and kidney biomarkers were not significantly different in children compared to our young adult comparator group (Table 2). The liver is a target organ for EBOV replication, leading to the hepatocellular damage from

	Univariable		Multivariable	
	HR (95%CI)	P-value	aHR (95%CI)	P-value
VL >7.6 log$_{10}$ copies/mL	5.8 (2.8–12)	<0.0001	7.6 (3.1–19)	<0.0001
ALT >525 IU/L	4.0 (1.8–9.1)	0.00086	5.4 (2.2–13)	0.00076
CRP >100 mg/L	3.7 (1.6–8.6)	0.0024	2.9 (1.2–7.1)	0.020
BUN >7.5 mmol/L	3.0 (1.4–6.7)	0.0062	–	NS
Acute kidney injury	2.7 (1.1–6.7)	0.027	–	NS
Rhabdomyolysis	2.5 (1.1–5.8)	0.028	–	NS

***Table 3*: Laboratory predictors of mortality in 73 children with EVD (age<16 years).**
ALT alanine transaminase; VL viral load; BUN blood urea nitrogen; CRP C-reactive protein; OR odds ratio; aOR adjusted odds ratio.

direct viral cytotoxicity and from the inflammatory response.[21] Causes of kidney dysfunction are multifactorial, including the prerenal azotaemia from volume depletion, as well as direct parenchymal damage from viral proliferation.[22] These pathophysiologic mechanisms appear to be common to both children and adults.

Rhabdomyolysis is a complication of EVD that has been previously reported with rates ranging from 59 to 83%.[18,20,23] In our study, the rate of rhabdomyolysis in the paediatric group was lower (48%), possibly due to the lower muscle mass in children. The cause of rhabdomyolysis in EVD is not completely understood, but it is hypothesized to be from direct viral invasion of muscle tissue, as well as immune-mediated damage from cytokines.[23] It has also been proposed that rhabdomyolysis may further contribute to the renal dysfunction, which was common in our study.[20]

The paediatric group had significantly higher admission and peak VLs, significantly longer time to clear the viremia, but a similar EBOV elimination half-life. Thus, higher VL at the admission, rather than slower elimination, accounted for prolonged viremia in children. Of note, the significantly longer duration of admission for patients <16 years of age may be explained by the longer duration of the viremia because an undetectable VL was a criterion for discharge from the ETU. Higher VLs in paediatric patients compared to adults have been recognized in other viral infections, including HIV and Influenza A.[24,25] This may be due to an immature priming of T helper 1 and CD8+ T cells in children, leading to a reduced ability to control viral replication.[26]

Factors associated with a fatal outcome in the paediatric group were elevated VL, ALT, CRP, BUN, as well as rhabdomyolysis and AKI. Our findings are similar to a previous paediatric study by Fitzgerald et al., who reported similar risk factors for mortality.[5] VL, ALT and CRP were statistically significant independent predictors of death in a multivariable Cox proportional hazard model. Adult studies have reported that increased VL, AST, ALT, CK, bilirubin, creatinine, urea, INR, and PTT were associated with mortality.[17,18,20,27]

Our study demonstrated a high EVD case fatality rate (49%) in children. This is similar to previous reports in which the case fatality rate in children was 42–65%.[3–9,28] Relative to adults in our study, the children had similar mortality, despite a higher VL. However, previous studies have shown that young age is associated with increased mortality, especially in children under five years of age.[6,7] We speculate that the availability of effective monoclonal antibodies treatment (25% received REGN-EB3, 30% received MAb114) may explain the absence of a differential mortality rate in children versus adults, although the absolute case fatality rate (~50%) remains very high.

There were no statistically significant differences in the specific EVD treatment regimens administered to children versus adults in our study; however, a trend toward lower use of remdesivir in children was observed. In the authors' experience in the Butembo ETU, paediatric patients in the "red zone" often had inconsistent supervision and nursing oversight, such that intravenous (IV) lines were challenging to maintain. We speculate that the preference for single dose treatment regimens (e.g., REGN-EB3 and MAb114) over remdesivir, which requires daily IV infusion over 14 days, reflect the difficulty of IV therapy in children in the ETU.

When looking at the vaccination rate in our population, only 5.5% of admitted children had received the rVSVΔG-ZEBOV-GP vaccine compared to 18% of adults. rVSVΔG-ZEBOV-GP was given to high-risk contacts and contacts of contacts of EBOV cases in a ring-vaccination strategy in West Africa, and in the DRC.[11] Individuals who were pregnant, breastfeeding, severely ill, or younger than six years old were not eligible for the vaccine in Guinea during the 2016 outbreak.[29] During the 10th DRC EVD outbreak, pregnant and lactating women as well as children under the age of one year were not consistently included in the vaccination plan due to safety concerns.[30] Since this time, the WHO Strategic Advisory Group of Experts on immunization has recommended expanded off-label use of rVSVΔG-ZEBOV-GP vaccine in children from birth to 17 years of age, as well as pregnant and lactating women. Additional factors may have led to lower childhood vaccination rates (e.g., if children were less likely to be listed as contacts).

A limitation of this study is the retrospective design, based on chart review of data from ETUs. All available laboratory tests were abstracted from the chart record; however, there was no systematic, prospective data collection, which resulted in incomplete data for some patients. The frequency of blood tests depended on the ETU treatment protocol and clinical judgement. In the event of a future outbreak, systematic, prospective data collection may be adopted to address the flaws of the current design. This was the largest study to date focusing on paediatric laboratory values in EVD confirmed cases; however, when the age groups were broken down to further analyse impact of the disease on subgroups within paediatrics, the numbers were small. The <16-year age group is heterogeneous, and a larger sample size with sufficient power to separately examine more specific strata such as neonates, pre-school, school-aged children, and adolescents, would be desirable. Blood cultures were not performed, although concurrent bacterial sepsis is a recognized complication in EVD patients. The vaccination status was assessed by verbal report by the patient or family member, rather than objective evidence (e.g., vaccination card), leaving open the possibility of recall bias or erroneous reporting.

In conclusion, paediatric patients with EVD, like adults, experience multiorgan involvement with life-

threatening electrolyte imbalances, kidney and liver injury, and rhabdomyolysis. They have significantly higher VLs at admission and throughout the course of EVD and longer clearance time than adults. In the era of effective monoclonal antibody therapy and improved supportive care for EVD, mortality in children (~50%) was similar to adults but remained startlingly high. Further research in the subgroups of paediatric age strata would help to further describe elements of the disease that are specific to children.

Contributors

LK wrote the first manuscript draft, critically reviewed the manuscript, and approved the final version. KMC supervised the data collection, obtained ethical and operational approvals, analysed the data, critically reviewed the manuscript, and approved the final version. DMB supervised the data collection, wrote the first manuscript draft, critically reviewed the manuscript, and approved the final version. RKO supervised the data collection, critically reviewed the manuscript, and approved the final version. DD, FEM, MMK, JBP, FMM, OTM, JD, SM, AL, PMK, PF, SAM, and JJMT critically reviewed the manuscript and approved the final version. MTH conceived the study, analysed the data, critically reviewed the manuscript, and approved the final version.

Data sharing statement

Data, including de-identified individual participant data and a data dictionary defining each field in the data set, will be made available upon reasonable request to the study authors (mthawkes@ualberta.ca, richardkitenge2@gmail.com, claudemasumbuko@gmail.com) for five years after the date of publication.

Declaration of interests

SM is employed by Ridgeback Biotherapeutics, and is listed as inventor on the patent application for mAb 114, US Application No.62/087, 087 (PCT Application No. PCT/US2015/060733) related to anti-Ebola virus antibodies and their use. SM receives royalties paid by the NIH Office of Financial Management (OFM).

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Supplementary materials

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