BMJ Open

Prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in patients with Lassa fever in Nigeria

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To cite: Erameh C, Edeawe O, Akhideno P, *et al*. Prospective observational study on the pharmacokinetic properties of the Irrua ribavirin regimen used in routine clinical practice in patients with Lassa fever in Nigeria. *BMJ Open* 2020;**10**:e036936. doi:10.1136/bmjopen-2020-036936

► Prepublication history for this paper is available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2020-036936).

Received 14 January 2020
Revised 16 March 2020
Accepted 18 March 2020

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ABSTRACT

Introduction Lassa fever (LF) is a severe and often fatal systemic disease in humans and affects a large number of countries in West Africa. Treatment options are limited to supportive care and the broad-spectrum antiviral agent ribavirin. However, evidence for ribavirin efficacy in patients with LF is poor and pharmacokinetic (PK) data are not available.

Irrua Specialist Teaching Hospital (ISTH) developed an intravenous ribavirin regimen different to the WHO recommendation. Apart from a lower total daily dose the drug is usually administered once per day which reduces the exposure of personnel to patients with LF. The aim of this study is to characterise the PK of the Irrua ribavirin regimen.

Methods and analysis This prospective, observational clinical study will assess PK properties of the Irrua ribavirin regimen on routinely ribavirin-treated patients with LF at ISTH, a referral hospital serving 19 local governmental areas in a LF endemic zone in Nigeria. Participants will be adults with PCR-confirmed LF. The primary objective is to describe classical PK parameters for ribavirin (maximum plasma drug concentration, time to maximum plasma drug concentration, area under the plasma drug concentration vs time curve, half-life time T1/2, volume of distribution). Blood samples will be collected at 0.5, 1, 3, 5, 8, 12 and 24 hours after doses on day 1, day 4 and day 10 of ribavirin treatment. Ribavirin plasma concentrations will be determined using liquid chromatography coupled to tandem mass spectrometry.

Ethics and dissemination The study will be conducted in compliance with the protocol, the Declaration of Helsinki, Good Clinical Practice (GCP) and the Nigerian National Code for Health Research Ethics. The protocol has received approval by the Health Research Ethics Committee of ISTH. Results will be made available to LF survivors, their caregivers, the funders, LF research society and other researchers.

Registration details ISRCTN11104750

Strengths and limitations of this study

- Pharmacological analysis of the Irrua ribavirin regimen will provide pharmacokinetic data on intravenous ribavirin treatment, the current standard treatment in patients with Lassa fever (LF).
- The results of this study will provide the basis for future dose optimisation studies with the ultimate goal of improving patient care.
- A limitation of the study is that due to ethical reasons only patients will be included who are able to give written or oral informed consent themselves.
- Therefore, unconscious patients or patients with impaired consciousness will not be included, which will result in a study population not fully representative of unselected patients with LF.

INTRODUCTION

Background

Lassa fever (LF) is an acute febrile illness associated with bleeding, organ failure and shock caused by the Lassa virus (LASV) (arenavirus).[1] The virus reservoir is the commensal rodent *Mastomys natalensis*.[2] LASV is also transmitted from human to human and may cause nosocomial outbreaks with case fatality rates of up to 60%.[3]

A large number of low-income and middle-income countries (LMICs) of the West African region is affected by LF: Ghana, Guinea, Mali, Benin, Liberia, Sierra Leone, Togo and Nigeria. The proportion of hospital admissions due to LF may reach 15% in endemic zones.[4–6] Fatal cases are associated with high viraemia, liver damage, renal failure, bleeding, encephalopathy and a shock-like syndrome.[4 7–10] Health systems in areas where

the disease is endemic and in high-inome countries are overwhelmed due to the lack of LF diagnostics, the risk of nosocomial transmission and the limited treatment options.[11]

Following the Ebola virus disease crisis, WHO has initiated the research and development (R&D) Blueprint as a global strategy and preparedness plan to ensure that targeted R&D brings medical technologies to patients during epidemics.[12] WHO and international experts have agreed on a list of priority diseases for urgent R&D, which also includes LF. WHO recognises that there is insufficient research for epidemic-prone diseases mainly affecting LMICs. The research needs of LMICs span from 'proof of principle/preclinical studies to the implementation and regulation of clinical studies, innovative strategies for the production of health technologies, development of key enabling capacities, such as pathogenesis studies of the priority pathogens and surveillance methodologies and regulatory science needed to enhance regulatory preparedness'.[12]

In Nigeria, LF case management centres are only operational in 3 out of 36 states. LF outbreaks occur annually but have recently started becoming a major threat. At the beginning of 2018, Nigeria experienced the largest outbreak of LF ever with hundreds of recorded clinical cases.[13]

Lassa fever in Irrua

The Irrua Specialist Teaching Hospital in Irrua, Edo State, Nigeria (ISTH) is located in a hyperendemic area for LF.[10 14 15] Molecular LF diagnostics is performed on a daily basis. The laboratory has also been instrumental in the diagnosis of LF outbreaks in several other states of the country. There has been a long-lasting institutional collaboration between Bernhard Nocht Institute of tropical Medicine (BNITM) and ISTH during the past decade with high level of capacity building in laboratory and clinical research, including setting up of a training and research centre. ISTH and BNITM are partners in various networks and projects, such as European and Developing Countries Clinical Trials Partnership-funded projects and the European Commission-funded European Mobile Laboratory project.

Literature review

The only drug with a proven therapeutic effect in humans with LF is the broad-spectrum nucleoside analogue ribavirin. Ribavirin reduces replication of LASV at concentrations between 10 and 50 µg/mL in cell culture, and shows efficacy in LASV-infected monkeys.[16–19] Initiation of treatment within 5 days after inoculation protected all monkeys, while initiation of treatment at day 7 conferred only partial protection. In treated animals, viraemia developed more slowly and peaked at lower titres than in untreated controls.[16 17] The mode of action of ribavirin against LASV is not clear. Recently, it has been shown with other RNA viruses that ribavirin can be incorporated into the RNA strand leading to lethal mutagenesis of progeny genomes.[20 21] It is assumed that, if the mutation rate is too high, the genetic information cannot be maintained and the virus population goes into extinction. This process is called lethal mutagenesis or error catastrophe.

Evidence for the currently recommended ribavirin treatment (30 mg/kg loading dose followed by 15 mg/kg every 6 hours for 4 days followed by 7.5 mg/kg every 8 hours for 6 days) adds up to one clinical study by McCormick *et al* published in 1986.[22] In patients with a high risk of fatal outcome (aspartate aminotransferase/glutamic-oxaloacetic transaminase (AST/GOT) values ≥150 U/L), initiation of treatment within 6 days after onset of fever reduced the case fatality rate from 55% to 5%.[23] Similarly, in patients with high viraemia (≥$10^{3.6}$ 50% tissue culture infection dose per millilitre), treatment reduced the case fatality rate from 76% to 9%. Even if treatment was initiated at day 7 or later, the case fatality could be reduced in these groups to 26% and 47%, respectively. No major differences were seen between oral and intravenous treatment. When, however, reviewing the publication thoroughly, several deficits become apparent. Research participants had not been randomised to either control or treatment group but a historic cohort of untreated patients was taken as control group. The treatment group was further separated into several subgroups with different treatment options (oral ribavirin, intravenous ribavirin, convalescent plasma) and different time points of treatment (within 6 days after onset of symptoms or later). The authors yet did not describe how patients had been allocated to the different subgroups and whether allocation had happened before or after inclusion in the study. There was, furthermore, a questionable deviation from the planned research design as subgroups were merged together after the end of the study. Additionally, total participant numbers in treatment and control groups remain unclear. Still, despite these serious biases this study is taken as reference for LF treatment since >30 years.[23] The dose used in the 1986 study is recommended by WHO for treatment of LF.[24] However, no data exist about the rational for this dose, the achieved ribavirin blood levels under this dose or the efficacy and pharmacokinetics (PK) of other dosing schemes. Clinical experience and expert opinion in the endemic countries agree with the results but scientific evidence is still largely lacking behind. PK assessments of ribavirin are only available for different dosing regimens used for hepatitis C.[25] The multiple dose half-life of ribavirin is estimated to be approximately 300 hours (12.5 days), which would justify less frequent or daily dosing in principle.[26]

Based on the highest case load of patients with LF in any institution in Nigeria, ISTH developed a ribavirin regimen different from the WHO recommendation which is here referred to as 'Irrua regimen' or 'Irrua ribavirin treatment regimen'.[27] Apart from a higher loading dose and a lower total daily dose administered during the

course of the Irrua regimen, the drug is usually administered once per day.

Rationale for this project

LF is a dangerous infection with a high lethality rate. During the past years, cases of LASV infection increased markedly and more evidence on an efficacious therapy of this disease is direly needed. The standard treatment for patients with LF is ribavirin, as the study by McCormick *et al* demonstrated efficacy of ribavirin in reducing the fatality rate of LF; ribavirin also increases survival in in vivo animal models of LASV infection.[28] Ribavirin at ISTH is used at a dose that deviates from the WHO recommendation. From clinical experience during the last decade, the standard Irrua regimen of ribavirin is postulated to be efficacious. Yet it is easier to use and a safer alternative to the McCormick regimen, because the exposure of personnel to LF diseased patients is reduced. However, to our knowledge, the PK properties of the Irrua ribavirin regimen have never been described. It is not known if this dose reaches blood levels that would be sufficient to exert an antiviral effect in the patients. Therefore, in this prospective observational study we aim to obtain evidence on ribavirin PKs in patients who receive the Irrua ribavirin regimen as standard of care at ISTH. The Irrua regimen entails the following ribavirin dosages for intravenous use:

1. Start dose (day 1): 100 mg/kg; if this start dose is >7 g then 2/3 of the dose will be given straight away and the remaining 1/3 is administered 8 hours later. If this dose is ≤7 g than the entire dose will be given at once.
2. Days 2–5: 25 mg/kg/day once daily.
3. Days 6–10: 12.5 mg/kg/day once daily.

Data derived from this observational study will serve as basis for further clinical studies studying the efficacy and safety of ribavirin and provide the possibility to compare the Irrua regimen with alternative treatment candidates such as favipiravir and possible combination regimen of favipiravir and ribavirin. This will serve as basis for further dose optimisation of ribavirin helping to further ameliorate the management of patients with LF in the endemic regions. This work is thus an important cornerstone for the development and implementation of a treatment standard and evidence-based treatment recommendations for LF.

Primary research question

What are the PK properties of ribavirin administered as per the Irrua ribavirin treatment regimen to patients with LF?

Study objectives

General objective

The aim of this study is to describe the PK properties of ribavirin when administered in routine care under the Irrua dosing regimen in patients with PCR-confirmed LF, to generate an evidence base for the use of the said regimen and to inform further studies regarding the efficacy of ribavirin, possibly in comparison and combination with other antiviral agents.

Specific objectives

Primary objective

1. Describe the classical PK parameters for ribavirin (maximum plasma drug concentration (Cmax), time to maximum plasma drug concentration (Tmax), area under the plasma drug concentration vs time curve (AUC), half-life time (T1/2), volume of distribution (Vd)) in patients with LF treated with the Irrua ribavirin regimen.

Secondary objectives

1. Examine the clinical, haematological, biochemical parameters of the patients and correlate them with ribavirin blood levels.
2. Study the kinetics of LASV loads in blood by reverse-transcription PCR (RT-PCR) and describe the association of drug exposure with the viral kinetics.
3. Determine LASV sequences and sequence changes during the treatment that might point towards resistant mutants or to increased error rate induced by the nucleoside analogue ribavirin.

METHODS AND ANALYSIS

Study design

A prospective, observational and descriptive clinical study will be conducted to assess the PK properties of the Irrua ribavirin regimen on routinely ribavirin-treated patients with LF at the Lassa fever isolation ward of ISTH that will be included following provision of written informed consent.

Primary end point

PK parameters of the routine care ribavirin regimen at ISTH.

Secondary end points

a. Viral kinetics in patients routinely treated with the Irrua ribavirin regimen.
b. LASV genome changes under the Irrua ribavirin regimen.

Study site

The Lassa Unit of the ISTH in Edo State is one of the three operating LF case management centres with an adjacent laboratory that conducts PCR testing.[13] It is a referral hospital serving 19 local governmental areas in an LF endemic zone and one of the few hospitals in West Africa that feature facilities for diagnosis, research and treatment of LF. The Institute of Lassa fever Research and Control at ISTH performs molecular LF diagnostics on a daily basis since 2008. An LF clinic for appropriate patient management was commissioned in 2010. Up to 2000 suspected patients are tested each year of which 100–200 test positive for LASV. Most of them are treated at the ISTH Lassa ward. BNITM and ISTH established a

Training and Clinical Trial Center that features laboratory facilities for Good Clinical Laboratory Practice conform processing of samples from clinical studies, including real-time RT-PCR for virus load monitoring, serology and sequencing. These facilities shall be further upgraded and used for this study.

Study population and selection criteria

Study population

The study population consists of patients with LF presented and routinely treated for LF on the Lassa isolation ward of ISTH which fit the below detailed selection criteria of the study.

Sample size

A sample size of 20 evaluable patients as defined by sponsor is proposed. The sample size and sampling design was evaluated using stochastic clinical trial simulation and estimation (SSE) in NONMEM (V.7.4, ICON development solutions, Hanover, USA). In the SSE approach, population PK parameters including their intra-individual and inter-individual variability of a published two-compartment model[29] were used and 500 clinical trials with the proposed sampling design (0.5, 1, 3, 5, 8, 12 and 24 hours after the 1st, 4th and 10th dose) were simulated using the Irrua dosing regimen. The population PK parameters were re-estimated and compared against the known values from the simulation step.

For PK the proposed sampling design will allow the determination of the structural PK parameters with low absolute relative bias (<2.4%) and low imprecision (<17%). The design also supports adequate estimation of the PK variability components (inter-individual variability: absolute relative bias <6.8%, imprecision: <33.2%; intra-individual variability: absolute relative bias <−0.3%, imprecision: 2.5%).

To evaluate the potential link between PK and pharmacodynamic (PD) (viral kinetics) in the exploratory analysis, the model used in the SSE was extended by an assumed PD component (ribavirin stimulating a first-order decay of viral load). Daily sampling (where coinciding with the safety assessment, otherwise every other day as outlined in the study flow chart) of viral load will allow to detect even weak exposure response relations. For example, a ribavirin-induced decline in viral load with a viral elimination half-life of 480 hours compared with no effect assuming high interpatient variability in PD response of 70% and a PD measurement error of 30% will be detectable at a power of 99% with adequate accuracy (absolute relative bias <17.3 %) and imprecision (<33.2%).

Selection criteria

Inclusion criteria

- Age ≥18 years.
- LF confirmed by RT-PCR.
- Written informed consent.
- Anticipated treatment with intravenous ribavirin.

Exclusion criteria

- Inability to give consent (eg, unconscious patients/cognitively impaired patients).
- Critical illness (based on investigator's clinical evaluation).
- Severe malnutrition.
- Haemodialysis.
- History of haemophilia/bleeding disorder.
- Haematocrit <30%.
- History of haemoglobinopathies (ie, sickle-cell anaemia or thalassaemia major).
- Known intolerance to ribavirin.
- Known pregnancy (as assessed during routine care through patient's history, physical and ultrasound examination and/or pregnancy test).
- Women who plan to get pregnant within the upcoming 3 months.
- Patients who already received ribavirin within the last 7 days.
- Concomitant administration of didanosine and other contraindicated concomitant medication.

Enrolment

Recruitment of patients and inclusion in the study will be performed at the study centre during the consultation on day 0 by an investigator who is not involved in the standard of care treatment of the patients. Vulnerable patients such as pregnant women and minors will be excluded from the research because they should not be exposed to the additional burden of drawing more blood than necessary. Cognitively impaired patients will be excluded because they cannot make an informed consent about the study participation. Furthermore, children and pregnant women do not receive ribavirin intravenously but orally and are therefore not eligible for enrolment. Patients who fulfil selection criteria are eligible and will be proposed to participate. The patients will be informed in writing and orally about the study and they will have time to address possible questions to the investigator. They will have time to consider their participation in the study. Inclusion will follow the provision of voluntary written informed consent of the patient or the impartial witness in case of illiteracy.

A unique identifier (unique participant number) will be allocated and demographic baseline data including date of birth, sex, weight and height will be collected and documented in a study-specific electronic case report form (eCRF). Patients then continue the routine standard care. The enrolment period corresponds to the period of hospitalisation at the Lassa isolation ward but is limited to 11 days.Further details are outlined in table 1.

Study limitations

This is an observational study and no interference with medical treatment will take place. Therefore, also no comparison with different treatments or treatment regimens (eg, the WHO recommended ribavirin regimen) will be performed.

Table 1 Time schedule of enrolment and assessments for participants

Study exam	Screening	First dose, day 1	0.5 hours post first dose	1 hour post first dose	3 hours post first dose	5 hours post first dose	8 hours post first dose and administration of second dose of day 1	12 hours post first dose	24 hours post first dose	Second day of dosing	Third day of dosing	Fourth day of dosing	0.5 hours post dose	1 hour post dose day 4	3 hours post dose day 4	5 hours post dose day 4
Visit-ID	D0		D1_h 0.5	D1_h1	D1_h3	D1_h5	D1_h8	D1_h12	D1_h24		D3		D4_h0.5	D4_h1	D4_h3	D4_h5
Written informed consent	X															
Medical history	X															
Previous medication	X															
Baseline characteristics	X															
Body temperature	X		X						X		X		X			
Signs and symptoms	X		X						X		X		X			
Physical examination	X															
Inclusion/Exclusion criteria	X															
Blood sample for haematology/ biochemistry	X										X					
Blood sample for PK/PD	X		X	X	X	X	X	X	X				X	X	X	X
Blood sample for RT-PCR and virological analyses	X								X		X		X			
Adverse events associated with phlebotomy			X						X		X		X			

Study exam	8 hours post dose day 4	12 hours post dose day 4	24 hours post dose day 4	Fifth day of dosing	Sixth day of dosing	Seventh day of dosing	Eighth day of dosing	Ninth day of dosing	Tenth day of dosing	0.5 hours post dose day 10	1 hour post dose day 10	3 hours post dose day 10	5 hours post dose day 10	8 hours post dose day 10	12 hours post dose day 10	24 hours post dose day 10
Visit-ID	D4_h8	D4_h12	D4_h24	D5	D6	D7	D8	D9	D10	D10_h0.5	D10_h1	D10_h3	D10_h5	D10_h8	D10_h12	D10_h24
Written informed consent																
Medical history																
Previous medication																
Baseline characteristics																
Body temperature			X		X	X	X	X		X						X
Signs and symptoms			X		X	X	X	X		X						X
Physical examination																
Inclusion/Exclusion criteria																

Continued

Table 1 Continued

Study exam	8 hours post dose day 4	12 hours post dose day 4	24 hours post dose day 4	Fifth day of dosing	Sixth day of dosing	Seventh day of dosing	Eighth day of dosing	Ninth day of dosing	Tenth day of dosing	0.5 hours post dose day 10	1 hour post dose day 10	3 hours post dose day 10	5 hours post dose day 10	8 hours post dose day 10	12 hours post dose day 10	24 hours post dose day 10
Blood sample for haematology/biochemistry			X			X		X								
Blood sample for PK/PD	X	X	X							X	X	X	X	X	X	X
Blood sample for RT-PCR and virological analyses			X			X		X		X						
Adverse events associated with phlebotomy			X		X	X	X	X		X						X

PD, pharmacodynamic; PK, pharmacokinetic; RT-PCR, reverse-transcription PCR.

The potential bias in this study include:

a. missing samples or insufficient sample volume to perform laboratory tests;
b. early withdrawal due to death, medical condition contraindicating the collection of blood or premature termination of intravenous ribavirin administration due to clinical improvement and switch to oral treatment based on physicians' discretion;
c. inclusion of non-severe LF cases, which may not be directly representative of patients with severe organ failure.

Study duration

The enrolment period corresponds to the length of the participant's stay at the study site until they are discharged but is limited to a duration of 11 days. The common duration for LF treatment is 10 days. The study itself is intended to start in January 2020 and it is supposed to end in September 2020.

During the course of a research project, new information may become available that impacts the research. If this happens, the investigator will tell the participants about it and discuss with them whether they want to continue in the research project. If they decide to withdraw, the investigator will make arrangements for their regular healthcare to continue. If they decide to continue in the study, they will be asked to sign an updated consent form.

Also, on receiving new information, the investigator might consider it to be in the participants' best interests to withdraw them from the research project. If this happens, he/she will explain the reasons and arrange for their regular healthcare to continue.

Study procedures

The study procedures are applied by designated trained staff of ISTH, BNITM and University of Hamburg within their usual work scope since this is an observational study and the research team is not involved in decisions regarding the participant's treatment. This study's personnel will receive a reasonable and adequate financial compensation for the time and risk/hazards involved in this research.

Data acquisition

Baseline data (age, sex, medical history, concomitant medication, concomitant treatment, pregnancy status, weight, height, physical examination) will be collected on study-specific eCRFs. Body temperature will be measured and signs and symptoms of LF (such as fatigue, diarrhoea, nausea, vomiting, abdominal pain, bleeding, chest pain, hearing problems and decreased vision) will be assessed daily (during study visits indicated in the study flow) and documented in the eCRF.

Blood sampling and analysis

Blood will be taken by laboratory staff of ISTH. Appropriate training in phlebotomy, with the aim to reduce the burden and risk on participants will be provided to all

personnel involved prior to the commencement of the trial. A peripheral venous catheter will be inserted on days where more than two blood draws are necessary. Before each blood withdrawal, the catheter will be rinsed and the first 3 mL will be discarded to ensure adequate quality of the blood. At the end of the study, leftover material (eg, heparin plasma which is leftover after all biochemistry analyses have been performed), will be stored at ISTH and BNITM.

- 4 mL EDTA blood for PK and RT-PCR analyses.
- 3 mL of EDTA blood for haematology analyses (and RT-PCR analyses in case of routine haematology blood draw).
- 3 mL of heparin blood for biochemistry analyses.
- 2 mL EDTA blood for RT-PCR analyses and serology.
- 2 mL EDTA blood for PK analyses.

In total, approximately 160 mL blood (corresponding to approximately 11 tablespoons of blood) will be withdrawn within the scope of the study whereof 42 mL (approximately 3 tablespoons) are part of the routine practice and 117 mL (approximately 8 tablespoons) are additional withdrawals due to the study participation. This total amount of blood which will be withdrawn does not exceed the maximum allowable total blood draw volumes for clinical research studies.[30]

Bioanalysis/Ribavirin PK

Blood samples will be collected at 0.5, 1, 3, 5, 8, 12 and 24 hours after the doses on day 1, day 4 and day 10 of ribavirin treatment. Additionally, it will be collected during screening before the first dose of ribavirin. Blood samples will be centrifuged and the plasma supernatant will be frozen at −80°C within 2 hours after blood sampling. Plasma samples will be shipped frozen to BNITM for viral heat inactivation using a validated protocol.[31 32] The samples will then be shipped to the bioanalysis site (Department of Clinical Pharmacy, Institute of Pharmacy, University of Hamburg, Germany). There, ribavirin plasma concentrations will be determined using liquid chromatography coupled to tandem mass spectrometry.

RT-PCR analysis/virological response

EDTA blood will be sampled at recruitment, 24 hours and then at study visits outlined in the study flow chart until the end of treatment. Blood will be processed directly to analyse viral load by qRT-PCR. Samples will be aliquoted, frozen and securely stored at ISTH until transported to BNITM at certain time points.

Assays such as ELISA and/or immunofluorescence assays will be used to determine LASV-specific IgM and IgG, as well as further IgG subclassification, and to monitor the development of LASV-specific antibodies in blood. Viral growth, isolation of LASV in cell culture, virus sequencing and unbiased metagenomic sequencing will be used to study the longitudinal impact of drug treatment (ribavirin) on LASV genomes. Sequence analysis shall be done at both ISTH and BNITM.

Laboratory analyses requiring the use of a Biosafety level 4 laboratory (virus isolation) will be performed at the BNITM. Aliquots of samples will be shipped to BNITM according to UN2814 regulations.[33]

Haematological and biochemical safety and tolerability

Blood will be sampled at baseline and then every second day until the 10th day of dosing at timepoints indicated in the study time schedule below. Full blood count and biochemistry will be performed. Biochemistry analysis will include creatinine, creatine kinase, uric acid, blood urea nitrogen, alanine aminotransferase/glutamic-pyruvic transaminase (ALT/GPT), AST/GOT, bilirubin, gamma-glutamyltransferase, amylase and serum electrolytes. Biochemical and haematological assays will be conducted by ISTH and AAU staff during the course of routine safety sampling.

Participant safety

This is a non-interventional observational study with minimal study-related risk for the participant. The biological risk in this study is limited to repeated draws of small amounts of venous blood. This risk encompasses local pain at the venepuncture site, the risk for the development of local haemorrhage due to the blood sampling and bleeding. However, even in haemorrhagic participants bleeding can be stopped by mechanical compression.

Insertion of intravenous catheters for repeat blood draws is associated with risk of local and systemic infection as in routine procedure. No further biological risks are associated with this observational study for the participants. The designated study monitor will monitor the data to ensure data reliability and patients' safety. An independent medical monitor will monitor the participants' safety data.

Before the start of the study, the personnel will receive trainings in Good Clinical Practice (GCP), research ethics, study procedures and phlebotomy. The study team will consist of personnel which is trained in supportive care of patients with LF.

Adverse events associated with phlebotomy

General definition of adverse event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Assessment of adverse events in this study

In this study, only AEs which are unfavourable and/or unintended signs, symptoms temporally associated with phlebotomy will be captured. This will be recorded in the participant case report form.

Clinical signs typical of LF will not be considered AEs unless the healthcare personnel considers these events as exceptional due to their evolution, their seriousness or another factor related to these events.

Severity

The investigator will assess the severity/intensity of the AEs using the following guidelines:

- Mild: awareness of sign or symptom, but easily tolerated.
- Moderate: enough discomfort to cause interference with usual activity.
- Severe: incapacitating with inability to work or do usual activity.
- Life-threatening.

Action taken

- Patient withdrawn from study.
- Concomitant medication required.
- Hospitalisation required or prolonged.
- Other.

Outcome

The investigator will follow-up the AE until resolution or until no further medically relevant information can be expected. AE outcome will be classified as follows:

- Resolved;
- Resolved with sequelae;
- Continuing;
- Death.

Quality control and quality assurance

Quality assurance

To ensure the quality and accuracy of the data, qualified investigators and study personnel will be selected. The protocol procedures will be reviewed with the investigators and associated personnel before the start of the study. Written instructions will be provided for collection, preparation and shipment of blood samples. The samples will be shipped following IATA (dangerous goods regulations) for the transport of category A samples (UN2814), or category B (UN3373) or exempt specimen, with dry ice (UN1845).[33] A designated Clinical Research Associate will monitor study progress to facilitate compliance with GCP which requires reported data to be accurate, complete and verifiable from source documents and that the study follows the current approved protocol and applicable regulatory and laboratory requirements. The monitoring activities will be a centralised monitoring which is both onsite and remote monitoring.

In the case of onsite monitoring, source data such as eCRF, ICF and other participant data will be reviewed for accuracy and completeness and any discrepancies will be resolved with the Principal Investigator (PI) or his/her designee, as appropriate.

Quality control of data on site

In order to ensure quality of data, several quality control (QC) measures will be put in place. Data will only be collected on validated study-specific eCRFs and logs. A stringent query process will be applied for the documentation of data. Study personnel will be trained in data acquisition and documentation.

Data management and storage

Data will be captured on study-specific password-protected eCRF on tablets located in the Lassa isolation ward. The PI will be responsible for accuracy of the data. Participants' data will only be linked to the unique identifier to ensure pseudonymity. The database will be made accessible only to dedicated staff from the institutions involved in the study. Biological samples and information will be stored for 10 years after the study results have been published. Direct access to source documentation (medical records) must be given to officials from ethics committees, regulatory authorities and from the sponsor for the purpose of verifying that the data recorded in the electronic database are consistent with the original source data. The research data will not be kept in the medical records of the patients. This would breach with confidentiality of data. Only exception: if results of the study parameters (eg, biochemical analysis) may have implication for clinical care of research participants, a copy will be provided to the responsible physician and the test result will be made available for routine care and kept in the medical records. The study identifier of this copy will be erased manually

Statistical analyses

The statistical data analyses will be performed using an appropriate software package. Details will be specified in the statistical analysis plan.

Statistical methods

PK parameters will be analysed descriptively. PK/PD analyses will be performed by employing mathematical models including potential covariates on the impact of drug exposure on viral kinetics.

Description of classical PK parameters

A non-compartmental analysis (eg, using Phoenix WinNonlin) will be performed to elucidate the classical PK parameters (Cmax, Tmax, AUC, T1/2, CL, Vd). AUC will be calculated using the linear trapezoidal method. Impairments of renal function and reductions in glomerular filtration, as assessed during routine care, will be accounted for in PK analysis, details will be specified in the statistical analysis plan.

Population PK model

A full population PK model will be developed, with the goal to characterise the typical PK parameters of ribavirin and PK variability. Linear as well as non-linear compartmental PK models will be tested using non-linear mixed-effects modelling using NONMEM. Once a suitable structural and variability model is built, all participant data will be used to find covariates significantly determining the interindividual variability in PK.

In particular, we will analyse the changes in ribavirin concentration and haemoglobin levels, ALT/GPT and uric acid. These are prognostic markers for survival, for metabolism of ribavirin and for potential side effects and toxicity (eg, haemolysis). For that purpose, a PK/tolerance model will be developed in order to relate the effect of the drugs on changes in longitudinal biological parameters as described previously for ribavirin in patients with HCV.[34]

PK-PD modelling

The PK data will be linked to the available PD data (viral kinetics). We aim to evaluate the effect (or the lack of effect) of ribavirin monotherapy on the Lassa viral kinetics. Semi-mechanistic modelling of the effect time-courses[35] will be performed using non-linear mixed-effects modelling in NONMEM and 'R'.

Protocol deviation/violation and exclusion from analysis set

Protocol deviations will be defined as non-compliance with the protocol by the investigator team that are not considered protocol violations (eg, missing one blood sample). Protocol violation will be defined as non-compliance to the protocol which reduces the quality or completeness of the data, makes the ICF inaccurate or impacts a participant's safety, rights or welfare. A protocol violation constitutes serious non-compliance and may lead to exclusion of participants from eligibility analysis and/or their discontinuation from the study.

Handling of missing data and outliers

No imputation will be applied. Missing data will be treated as such.

Exploitation of study results

All results, data, documents and inventions obtained, directly or indirectly, from the study, will be owned by the sponsor and the PI's department unless a law or local regulation states otherwise. The sponsor can use or exploit all results for their own use without any limitation of its industrial property (territory, area, duration) in consultation with the study centre. The full database will be the property of the sponsor and the PI's department and will be used for producing the final study report. The PI's department will have the right to participate with the sponsor in the publication of such results.

Data presentation

The results of the study will be made available for the sponsor and the investigator. The sponsor and the investigator will share the responsibility for the presentations and/or publications of the results. The final decision on the publication of a manuscript/summary/presentation will be taken by the sponsor and the investigator together following an internal review with the possibility of providing comments.

The participants will be informed about any information that may affect their continued participation or their health during the course of the study. The outcome of the research will be made available to ISTH, the participants on individual request and with the scientific community to improve future management of LF.

Responsibilities

Responsibilities of the study site

The study personnel shall be responsible for performing the study in accordance with this protocol and in accordance with the legislation and international guidelines under the direction of the local PI.

They are responsible for obtaining written informed consent prior to inclusion in the study, completing the study documents and recording all relevant data in relation to the study. Each study team member shall ensure that the information reported in the document is precise and accurate. The study personnel must inform the participant on all relevant aspects of the study, including the information in the participant information sheet. All this information shall be provided to the participant in layman's terms. Participant'sconfidentiality is paramount.

Prior to study inclusion, the informed consent form will have to be personally completed (first name, surname), dated and signed by the participant. The person who has conveyed the information on the study to the participant shall also sign and date the informed consent form approved by the ethics committee.

In the case where participant is unable to read and sign the participant information sheet and informed consent form, these documents will be read and explained to the participant in the local language in the presence of a witness. The participant shall put her/his fingerprint on the informed consent form and the witness shall also sign the consent form to confirm that the participant has consented willingly. A copy of the information sheet and the signed consent form shall be handed over to the participant.

Responsibility of the sponsor

The study sponsor's responsibility is towards the study team at the study site and the health authorities and shall take all reasonable measures to ensure the good conduct of the study with regard to ethics, protocol compliance, integrity and validity of the information recorded in the participant eCRF, as well as with regard to the availability of the adequate resources to ensure appropriate conduct of the study. The principal function of the study management team is to help the investigator and the sponsor to maintain a high level of ethical, scientific, technical and regulatory standards for all study-related aspects of ethics, regulations and administrative rules.

Ethical consideration

Regulations

The study shall be conducted in compliance with the Declaration of Helsinki adopted by the 18th World Medical Association Assembly in 1964, and with its amendments as well as with the Nigerian National Code for Health Research Ethics (http://nhrec.net/). This study

shall be conducted in accordance with the principles of the GCP as well as in compliance with the international and national laws and regulations in effect and in accordance with the applicable directives in Nigeria, in particular concerning the submission to the ethics committee and the protection of personal data.

Ethics approval

The study has been approved by the Health Research Ethics Committee of Irrua Specialist Teaching Hospital.

Patient and public involvement

Patients and general public were not involved in the conception and design of this study protocol. However, the concept of the study was clearly driven by the aim to close knowledge gaps and generate evidence that is currently missing during routine care of patients with LF.

Site clearance

On signature of the protocol, the PI accepts to respect the instructions and procedures described in the protocol, as well as the GCP and Good Laboratory Practices, to which he/she conforms.

Participant informed consent

The PI is responsible for ensuring that informed consent is obtained from each participant and obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures. The investigator shall explain to each study participant the nature of the study, its objective, the procedures involved, its risks and potential benefits and any discomfort it may generate.[27] This investigator is independent from the standard of care treatment of research participants. The participant will sign on the informed consent sheet after having read and voluntarily agreed to it. Where the participant is unable to read, an impartial witness should be present during the entire informed consent discussion. After inclusion, the participant may elect to withdraw from the study when he/she so wishes. The same level of attention will be dispensed to that individual. The investigator shall obtain from the participant a signed (fingerprint and signature from a witness for participants unable to read and write), written consent. If informed consent is not obtained, the patient will not be enrolled. If during the course of the research project new information become available about the treatment/the disease that is being studied, the investigator will tell the participant about it and discuss with him/her whether he/she wants to continue in the research project. If the participant decides to withdraw, the investigator will make arrangements for the regular healthcare to continue. If the participants decide to continue in the research project, he/she will be asked to sign an updated consent form.

Also, on receiving new information, the investigator might consider it will be in the participant's best interests to withdraw him/her from the research project. If this happens, the investigator will explain the reasons and arrange for the regular healthcare to continue.

Pseudonymity, confidentiality and data protection

To ensure pseudonymity of study participants, an identification number will be attributed to each participant at the time of study entry. This unique identifier will be used for the identification of study-specific documentation and labelling of samples throughout the study as well as for documentation of the electronic database.

To ensure confidentiality of the information collected eCRFs and laboratory documentation will be kept in a locked room with restricted access. The electronic database will only be accessible with a password. Only designated study personnel, the sponsor and the sponsor's delegate will have access to these documents.

Voluntariness

The participant must be informed that his/her participation is entirely voluntary, that he/she can withdraw from the study at any time and that withdrawal will not affect his/her subsequent medical treatment nor his relationship with the treating physician. If participants withdraw from the study, they will be asked whether information that has been obtained about them before they have chosen to withdraw may be used for analysis and hence may be included in reports and publications. If participants also withdraw their consent for data processing, all obtained data from them will be excluded from further analysis and processing.

Incentives

No financial incentives will be given in return of participating to the study due to the risk of manipulation and coercion. As incentive for taking part in the study, participants will receive one long-lasting impregnated mosquito net during their last appointment (approximate value US$6). Furthermore, the research participants will be provided with protein bars to balance the loss of proteins due to multiple blood drawing. In exceptional circumstances, additional material incentives such as fruit juice, candies or phone credits might be provided.

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the study protocol and will be involved in patient care, sample processing or data analysis during the study.

Funding This research is funded as part of the Global Health Protection Programme (GHPP) of the German Federal Ministry of Health, project ZMVI1-2519GHP704.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, reporting or dissemination plans of this research.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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