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La recherche opérationnelle sur les maladies infectieuses à potentiel épidémique et menaçant en Afrique Sub-saharienne

Marie Jaspard

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Marie Jaspard. La recherche opérationnelle sur les maladies infectieuses à potentiel épidémique et menaçant en Afrique Sub-saharienne. Médecine humaine et pathologie. Université de Bordeaux, 2022. Français. NNT : 2022BORD0243 . tel-03858002

HAL Id: tel-03858002

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Submitted on 17 Nov 2022

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Thèse présentée pour obtenir le grade de

DOCTEUR EN EPIDEMIOLOGIE

Université de Bordeaux

ECOLE DOCTORALE : Société, Politique et Santé Publique

SPECIALITE : Santé Publique

OPTION : Épidémiologie

Par Marie Jaspard

La recherche opérationnelle sur les Maladies Infectieuses à potentiel épidémique et menaçant en Afrique Sub-saharienne

Sous la direction de : Pr Denis Malvy

Soutenue le : 15 septembre 2022

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La recherche opérationnelle sur les maladies infectieuses émergentes à potentiel épidémique menaçant en Afrique Sub-saharienne

Résumé :

Les maladies infectieuses émergentes (MIE) représentent plus que jamais un enjeu de santé mondiale et la connaissance de leur présentation clinico-biologique est indispensable pour optimiser la prise en charge, en terme de soins de support, de traitement spécifique et de contrôle des épidémies, par exemple par la vaccination. Le risque est particulièrement important sur le continent Africain, d'où les maladies émergent régulièrement et où l'impact sur les populations fragilisées est majeur. La recherche opérationnelle représente l'un des outils pour améliorer la connaissance sur ces maladies et leur prise en charge. A titre d'exemple, 3 maladies ont tout particulièrement émergé en Afrique sub-saharienne et dans le monde ces dernières années : la fièvre de Lassa, la Maladie à Virus Ebola (MVE) et la Covid-19. La fièvre de Lassa évolue sur le mode épidémique saisonnier principalement au Nigeria mais les connaissances sur cette maladie sont encore parcellaires. De plus, le traitement recommandé, la ribavirine, ne semble pas présenter une efficacité satisfaisante pour prendre en charge efficacement les patients. Depuis la grande épidémie de 2014-2016, la MVE survient par vagues épidémiques en Afrique de l'ouest et centrale, à la faveur d'une transmission animale ou d'une rechute chez un survivant. Malgré la découverte d'un traitement spécifique, beaucoup de questions subsistent, notamment autour des soins de support et de la prévention de l'infection chez les contacts. Enfin, la pandémie de Covid-19 qui a durement touché l'ensemble de la planète depuis plus de 2 ans a des implications spécifiques sur le continent Africain qu'il convient d'explorer. Grâce à une collaboration inédite entre une ONG médicale internationale, ALIMA et une équipe de recherche clinique, l'équipe IDLIC de l'unité Inserm 1219 / PACCI, site ANRS de Côte d'Ivoire, plusieurs projets de recherche clinique sur les MIE ont été mis en place en Afrique sub-saharienne au cours des 5 dernières années.

Dans cette thèse, nous présenterons certaines des études qui ont été réalisées durant cette collaboration, sur les 3 maladies citées ci-dessus. Il s'agit d'une thèse de publications, dans laquelle seront présentés 4 publications et les perspectives qui en découlent. Les études LASCOPE (publiée dans Lancet Global Health), EVISTA (en cours de révision dans e-clinical Medicine) et COVISTA (publiée dans The International Journal of Infectious Disease) sont des cohortes prospectives décrivant la présentation clinique et biologique ainsi que le standard de soins prodigué aux patients atteints de fièvre de Lassa au Nigeria, de MVE en République Démocratique du Congo (RDC) et de Covid-19 en Guinée et Burkina Faso, respectivement. La 4^{ème} publication concerne une étude pilote menée en RDC pour évaluer la faisabilité d'une prophylaxie post exposition (PPE) par anticorps monoclonaux chez les contacts à haut risque de MVE (publiée dans The International Journal of Infectious Disease).

Les perspectives de cette thèse sont multiples. Concernant la fièvre de Lassa, le principal enjeu consiste actuellement à mettre en évidence un traitement spécifique efficace et accessible dans les pays d'Afrique de l'ouest. Pour cela, un essai thérapeutique adaptatif de phase 2-3 est en train de se mettre en place dans plusieurs pays d'Afrique de l'ouest. Pour lutter contre la diffusion de la MVE, une réflexion sur les options disponibles en terme de PPE est en cours au plan international. Enfin, concernant la Covid-19, des essais thérapeutiques et vaccinaux sont en cours en Afrique sub-saharienne.

Mots clefs : recherche opérationnelle, fièvre de Lassa, Maladie à virus Ebola, Covid-19, Afrique sub-saharienne

Operational research on emerging infectious diseases in Sub-saharan Africa

Abstract:

Emerging infectious diseases (EIDs) are more than ever a global health issue and knowledge of their clinical and biological presentation is essential to optimize management, in terms of supportive care, specific treatment and epidemic control, for example through vaccination. The challenge is particularly important on the African continent, where diseases regularly emerge and where the impact on vulnerable populations is major. Operational research is one of the tools for improving knowledge about these diseases and their management. For example, three diseases have emerged in sub-Saharan Africa and worldwide in recent years: Lassa fever, Ebola Virus Disease (EVD) and Covid-19. Lassa fever has a seasonal epidemic pattern, mainly in Nigeria, but knowledge of the disease is still incomplete. Moreover, the recommended treatment, ribavirin, does not seem to be sufficiently effective in managing patients. Since the major epidemic of 2014-2016, EVD has been occurring in epidemic waves in West and Central Africa, through animal transmission or relapse in a survivor. Despite the discovery of a specific treatment, many questions remain, particularly around supportive care and prevention of infection in contacts. Finally, the Covid-19 pandemic that has severely affected the entire planet for more than 2 years has specific implications on the African continent that need to be explored. Thanks to an unprecedented collaboration between an international medical NGO, ALIMA, and a clinical research team, the IDLIC team of the Inserm 1219 unit / PACCI, ANRS site in Côte d'Ivoire, several clinical research projects on EVDs have been set up in sub-Saharan Africa over the last 5 years.

In this thesis, we will present some of the studies that have been carried out during this collaboration, on the 3 diseases mentioned above. This is a publication thesis, in which 4 publications and their perspectives will be presented. The LASCOPE (published in *Lancet Global Health*), EVISTA (under review in *e-clinical Medicine*) and COVISTA (published in *The International Journal of Infectious Disease*) studies are prospective cohorts describing the clinical and biological presentation and standard of care of patients with Lassa fever in Nigeria, EVD in the Democratic Republic of the Congo (DRC) and Covid-19 in Guinea and Burkina Faso, respectively. The fourth publication concerns a pilot study conducted in the DRC to assess the feasibility of post-exposure prophylaxis (PEP) with monoclonal antibodies in high-risk contacts of EVD (published in *The International Journal of Infectious Disease*).

The perspectives of this thesis are multiple. Concerning Lassa fever, the main challenge is currently to identify a specific treatment that is effective and accessible in West African countries. To this end, a phase 2-3 adaptive trial is being set up in several West African countries. To combat the spread of EVD, a reflection on the available options in terms of PEP is underway at the international level. Finally, on Covid-19, therapeutic and vaccine trials are ongoing in sub-Saharan Africa.

Keywords: operational research, Lassa fever, Ebola virus disease, Covid-19, sub-Saharan Africa

Remerciements

Aux membres de mon jury pour leur disponibilité à évaluer mon travail. Je sais que votre temps est précieux et c'est un honneur pour moi de vous présenter cette thèse.

A mon directeur de thèse, Pr Denis Malvy, qui m'a soutenue et encouragée sur le plan scientifique, clinique et même personnel depuis toutes ces années. Vous avez été un véritable mentor pour moi.

A Xavier Anglaret, mon deuxième mentor. Merci pour tes conseils toujours incroyablement justes et constructifs, et de m'avoir appris ce qu'était la recherche clinique.

A Augustin Augier sans qui l'aventure CORAL n'aurait jamais vu le jour. Tu as cru en ce projet et l'as porté pendant 4 ans. Tu m'as fait confiance et je prends le relais inspiré de tes enseignements.

A Alexandre Duvignaud, pour tout ce travail accompli, y compris dans les moments difficiles où l'on se serrait les coudes, tu as été un fantastique partenaire et continuera à l'être, je l'espère.

A Beatrice, Camille, Alice, Sylvain, la grande équipe des chefs de projets CORAL, sans qui aucune étude n'aurait vu le jour. Merci pour ces années de collaboration et pour celles encore à venir.

A Moumouni Kinda, qui continue de me faire confiance, avec tant de bienveillance.

Aux équipes ALIMA de terrain, en Guinée, en RDC et au Nigeria, qui tous les jours rendent notre travail possible et construisent la recherche au quotidien. Un grand merci aux équipes médicales de l'hôpital de Owo qui ont permis de mettre en place ce grand chantier qu'est l'étude LASCOPE. Grâce à eux, un traitement actif contre la fièvre de Lassa verra le jour.

Aux équipes IDLIC et PACCI, à Valérie, Sophie, Gwenaëlle et j'en passe, qui m'ont enseigné avec une patience infinie les rouages de l'ingénierie scientifique.

Arnauld, mon mari qui m'encourage avec tout son amour, m'aide à avancer dans mes projets professionnels et dans nos projets personnels et me rappelle régulièrement que tout est possible tant qu'on est ensemble.

A ma fille Faustine et sa petite sœur à venir qui sont tout simplement ma raison de vivre.

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Épreuve de travaux dans le cadre de la thèse

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Liste des acronymes et abréviations

ALAT : Alanine Aminotransferase
ALIMA : Alliance for International Medical Action
ANRS-MIE : Agence Nationale de Recherche sur le SIDA, les hépatites virales et les Maladies Infectieuses Émergentes
ANSS : Agence Nationale de Sécurité Sanitaire
BARDA : Biomedical Advanced Research and Development Authority
BNITM : Bernhard Nocht Institut for Tropical Medicine
BSL3 : Bio Safety Level 3
CHU : Centre Hospitalo-Universitaire
CNU : Conseil National des Universités
CORAL : Clinical and Operational Research Alliance
COVID-19 : Coronavirus Disease 2019
COVISTA : Covid-19 Standard de soins
Ct : Cycle Threshold
CVS : Cardio Vascular Study
DES : Diplôme d'Étude Spécialisé
DESC : Diplôme d'Étude Spécialisé Complémentaire
DNDi : Drug for Neglected Diseases initiative
EBOV : Ebola Virus
ECG : Électrocardiogramme
EVISTA : Ebola Virus Standard de soin
FHV : Fièvres Hémorragiques Virales
GP : Glycoprotéine virale
IDLIC : Infectious Disease in Low Income Countries
IgG : Immunoglobuline G
IMC : Indice de Masse Corporelle
IMOVA : Interaction anticorps Monoclonal Vaccin anti Ebola
INRB : Institut National de la Recherche Biomédicale
Inserm : Institut National de la Santé et de la Recherche Médicale
ISPED : Institut de Santé Publique, d'Épidémiologie et de Développement
IV : Intra Veineuse
KDIGO : Kidney Disease Improving Global Outcome
LASCOPE : Lassa Fever Clinical Course and Prognostic Factor in an Epidemic Context
LASV : Lassa Virus
mAbs : Monoclonal Antibodies
MCU-PH : Maître de Conférence des Université – Praticien Hospitalier
MIE : Maladies Infectieuses Émergentes
MSF : Médecin Sans Frontière
MVE : Maladie à Virus Ebola
NICaS : Non Invasive Cardiaque System
NIH : National Institutes of Health
NP : Nucléoprotéine virale
OMS : Organisation Mondiale de la Santé
ONG : Organisation Non Gouvernementale
PK : Pharmacocinétique

PPE : Prophylaxie Post Exposition

RDC : République Démocratique du Congo

RT-PCR : Reverse Transcriptase Poly Reaction Chain

SAFARI : Pharmacokinetics, Tolerability and Safety of Favipiravir with and without Favipiravir

SARS-CoV-2 : Severe Acute Respiratory Syndrome Coronavirus 2

SMIT : Service des Maladies Infectieuses et Tropicales

UI : Unité Internationale

VIH : Virus de l'Immunodéficience Humaine

VSV : Virus de la Stomatite Vésiculaire

WALC : West Africa Lassa Consortium

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A. Introduction

Je me présente ce jour devant le jury pour soutenir ma thèse de science en épidémiologie.

Je suis médecin interniste suite à la validation de ma thèse d'exercice médical en 2011 ainsi qu'à l'obtention du DES de médecine interne en 2013 à l'université de Tours.

Au cours de mon internat des hôpitaux de Tours, j'ai eu la possibilité de participer à un certain nombre de missions humanitaires avec plusieurs ONGs, tel que Médecin Sans Frontière (MSF) et la Croix Rouge Française. En 2009, j'ai passé 6 mois à Bangui (République Centrafricaine) pour travailler comme médecin dans le centre de traitement du VIH de l'hôpital général. Cette expérience a permis la rédaction de ma thèse d'exercice sur la prévention de la transmission mère enfant du VIH à Bangui. En 2010, j'ai participé à la prise en charge des patients atteints de Cholera lors de l'épidémie qui a frappé Haïti dans les suites du tremblement de terre. En 2012 j'ai passé 10 mois au sud-est de la République Centrafricaine, à Berberati, en tant que médecin référent pour la prise en charge des enfants malnutris aigus sévères. Outre les techniques de renutrition assez codifiées, la plupart de ces enfants souffraient de maladies infectieuses non traitées, aggravant leur malnutrition. En 2014, j'ai été médecin référent sur 3 missions d'urgence avec MSF. Tout d'abord pour la gestion des blessés liés au conflit en République Centrafricaine, puis pour la prise en charge médicale des déplacés dans les camps sud soudanais, et enfin, pour la crise sanitaire liée à l'épidémie de Maladie à virus Ebola (MVE) à l'hôpital Donka en Guinée. Cette dernière expérience a été déterminante sur mon intérêt croissant pour les maladies émergentes et les épidémies.

Entre 2014 et 2016, j'ai exercé en tant que Cheffe de Clinique Assistante dans le service de Maladies Infectieuses et Tropicales à l'hôpital de la Pitié Salpêtrière, à Paris, ce qui m'a permis d'obtenir mon DESC de Maladies Infectieuses et Tropicales à Sorbonne Université Paris 6.

J'ai rencontré l'équipe Bordelaise en 2016, dans le cadre de mon Master 2 en Santé publique que j'ai réalisé à l'ISPED, Université de Bordeaux. J'ai pu réaliser mon stage de Master à PACCI, Abidjan (site ANRS-MIE de Côte d'Ivoire), où j'ai d'ailleurs vécu jusqu'en 2020.

Dès l'automne 2017, j'ai participé à la création de la plateforme CORAL en tant que coordinatrice du pôle Maladies Infectieuses Émergentes (MIE). La plateforme CORAL sera davantage décrite plus bas (paragraphe B.3), mais en quelques mots, elle est construite sur la collaboration entre l'ONG ALIMA, l'unité Inserm 1219 IDLIC et le programme PACCI, et a pour objectif d'allier les compétences des chercheurs et des humanitaires pour réaliser des projets de recherche opérationnelle dans les pays d'Afrique sub-saharienne touchés par des crises humanitaires. C'est dans ce périmètre que nous avons mis en place un certain nombre de projets qui seront détaillés ci-dessous sur la fièvre de Lassa, la Maladie à virus Ebola et la Covid-19.

Je me suis inscrite en thèse de science d'épidémiologie en 2018, à l'Université de Bordeaux sous la direction du Pr Denis Malvy pour continuer les travaux initiés dans le cadre de la

plateforme CORAL. Mon laboratoire d'accueil est l'équipe IDLIC du centre Inserm 1219 à l'université de Bordeaux, dirigée par le Dr Xavier Anglaret. Sur les conseils de mon comité de suivi de thèse, et en raison d'un congé maternité survenant en cours de thèse, j'ai prolongé ma thèse d'une année pour une durée totale de 4 ans. Pendant 4 ans, j'ai donc assuré le poste de coordinatrice du pôle MIE de CORAL en même temps que j'ai réalisé mes travaux de thèse.

La thèse sera une thèse de publications et s'articulera autour de 3 points. Tout d'abord, je présenterai le contexte du travail et les maladies investiguées, puis je présenterai les différents articles publiés ou en cours de publication dans le cadre du travail de thèse et enfin je discuterai des perspectives et suites à donner à ce travail.

B. La recherche opérationnelle : contexte du travail et maladies investiguées

1. La recherche opérationnelle et l'émergence

a) Recherche opérationnelle, recherche translationnelle

La recherche opérationnelle est historiquement une discipline liée aux mathématiques, et par extension à l'économie. Elle est définie par l'ensemble des méthodes orientées vers la recherche du meilleur choix dans la façon d'opérer en vue d'aboutir à la solution optimale (définition Larousse 1966).

L'utilisation de cette stratégie est aussi appliquée à la recherche biomédicale depuis les années 50 (1) mais avec une définition relativement différente. Nos collègues de l'ONG MSF ont défini la recherche opérationnelle comme suit : « mise en évidence des connaissances sur les interventions, les stratégies ou les outils qui peuvent améliorer la qualité, l'efficacité ou la couverture des programmes de santé dans lesquels la recherche est effectuée » (2).

En effet, en médecine, la recherche opérationnelle vise à définir les stratégies de recherche permettant d'obtenir une solution optimale pour le patient et la société. L'idée première est de baser la réflexion initiale sur les problématiques venant du patient ou de la communauté pour définir les questions de recherche et construire le projet de recherche (3).

Selon cette définition, la recherche opérationnelle comporte 3 types de méthodes : l'analyse descriptive, les analyses cas-contrôle et les cohortes prospectives ou rétrospectives. Les essais cliniques ne sont pas inclus dans cette définition, étant donné qu'ils sont réalisés dans un environnement strictement contrôlé, avec des critères d'éligibilité extrêmement précis, et donc souvent non représentatifs des conditions de terrain (en contexte humanitaire, de ressources sanitaires et économique limitées ou encore de conflit) (2). En d'autres termes, la recherche opérationnelle en médecine s'intéresse à la vraie vie et vise à avoir un impact direct sur les programmes de santé.

Par conséquent, la recherche opérationnelle implique une collaboration très étroite entre les chercheurs et les professionnels de la santé prodiguant les soins. Les responsables politiques sont aussi essentiels dans ce processus pour assurer une application des découvertes réalisées dans le cadre du projet de recherche, au sein du système de santé local. Cette composante intégrative peut parfois représenter un challenge important dans les projets de recherche opérationnelle (1,2). Ce mode de recherche très appliqué nécessite aussi un renforcement des compétences en recherche clinique pour les intervenants de terrain, avec la mise en place d'un réseau de chercheurs formés à la méthodologie de la recherche opérationnelle.

La recherche opérationnelle a été particulièrement utilisée dans la construction des connaissances sur les maladies infectieuses (VIH, tuberculose) mais aussi sur les maladies émergentes telle que la Maladie à virus Ebola (MVE). Lors de l'épidémie de MVE d'Afrique de l'ouest, en 2014-2016, un certain nombre de projets de recherche opérationnelle ont permis de mieux comprendre la maladie et donc de la combattre. La présentation clinique, biologique et les facteurs associés à la mortalité (4–9) ainsi que la transmission sexuelle de la maladie (10) sont autant d'exemples d'éléments mis en lumière par des projets de recherche opérationnelle, déterminants pour assurer la prise en charge des patients et le contrôle de la diffusion du virus (11).

Quand on parle de recherche ayant pour objectif d'impacter la prise en charge des patients sur le terrain, il n'est pas rare d'évoquer aussi la recherche translationnelle.

La recherche translationnelle est définie comme l'ensemble du processus d'investigation scientifique s'étendant « from bench to bedside », et vise à s'assurer de la « translation » efficace des découvertes depuis les scientifiques jusqu'aux patients et aux communautés (12). Elle inclut donc la recherche fondamentale, la recherche centrée sur les patients (recherche clinique) et la recherche centrée sur les populations (13). C'est une recherche interdisciplinaire car elle a l'avantage d'inclure la recherche pré-clinique. En revanche, la recherche clinique interventionnelle, tels que les essais thérapeutiques, est incluse dans la recherche translationnelle. Comme pour la recherche opérationnelle, l'objectif à long terme est d'améliorer le système de santé.

La recherche translationnelle est évidemment particulièrement adaptée à l'évaluation des stratégies d'investigation sur les maladies infectieuses émergentes (14–16). Par exemple, le séquençage du matériel génétique des virus émergents, possible grâce à une translation de la technologie depuis les laboratoires spécialisés vers les laboratoires de virologie dans les pays touchés par ces maladies, permet d'accélérer la mise en évidence des pathogènes et de tracer l'origine des épidémies. Cela a été par exemple le cas pour la résurgence de MVE en Guinée en 2021, dont les analyses par séquençage ont permis d'identifier, en un temps record, le lien avec l'épidémie de 2014-2016 (17). Cette découverte permettra peut-être de rediscuter le paradigme établi, selon lequel la transmission se fait par des sujets malades, et non par des survivants guéris.

Qu'elle soit opérationnelle et/ou translationnelle, la recherche clinique en contexte d'urgence présente des complexités propres. En effet, il est plus que jamais indispensable de prendre en compte les considérations éthiques, ainsi que les perceptions communautaires de la recherche effectuée. En effet, la gestion des rumeurs et de la défiance des populations est un volet essentiel à anticiper quand on met en place un projet de recherche en Afrique. Le travail conjoint avec les équipes de science sociale permet d'appréhender ces mécanismes.

b) L'émergence

Les Maladies Infectieuses Émergentes (MIE) sont définies par le NIH (National Institute of Health) comme des maladies infectieuses nouvellement apparues dans une population ou qui existaient déjà mais dont l'incidence ou la portée géographique augmente rapidement (18). Le principal exemple d'émergence mondiale est bien sur le VIH (19) mais la pandémie à Covid-19 a récemment rebattu les cartes. Le VIH a depuis été retiré de la liste des MIE proposée par le NIH en raison de l'évolution vers la chronicité de la maladie.

Les MIE représentent au moins 15 % de l'ensemble des agents pathogènes affectant l'humain, selon la 10e Conférence internationale sur les MIE organisée par le CDC en 2018 mais ces chiffres n'ont pas été actualisés depuis la pandémie de Covid-19 (20,21). Les zoonoses, soit les maladies infectieuses qui sont passées de l'animal à l'Homme, représentent plus de 60% des MIE (22) ce qui justifie une approche de santé globale de plus en plus plébiscitée. La santé globale utilisée ici dans le sens « une seule santé », ou « One Health » implique un effort de collaboration au niveau local et mondial pour atteindre la meilleure santé possible pour les personnes, les animaux et l'environnement (23).

Les Fièvres Hémorragiques Virales (FHV) sont un exemple de MIE de même que les viroses respiratoires. Dans la liste des pathogène prioritaires du NIH se trouvent en effet les infections par arenavirus, filovirus et coronavirus (18,21).

2. Maladies investiguées et questions de recherche clinique

a) Introduction

Du fait de la menace que les MIE font peser sur nos sociétés, en particulier au sud, nous avons décidé de nous engager dans le domaine de recherche dévolue aux maladies infectieuses émergentes. Notre travail de thèse rend compte de ce choix et de cet engagement. Grâce à notre collaboration avec l'ONG ALIMA (décrite dans le chapitre B.3), nous avons de plus accès aux sites de prise en charge existants dans les foyers Africains d'émergence. Nous avons aussi la possibilité d'ouvrir de nouveaux sites opérationnels sous réserve qu'un besoin médical eut été identifié et qu'un financement soit disponible. C'est dans ce contexte que nous avons développé un certain nombre de projets de recherche clinique, très étroitement associés aux projets opérationnels de réponse aux épidémies.

Les maladies investiguées ont été sélectionnées selon les émergences ou les réémergences déclarées dans l'agenda du travail de thèse. La grande épidémie de fièvre de Lassa survenue en 2018 au Nigéria nous a permis de nous intéresser à cette maladie clairement négligée. De même, le travail d'ALIMA et de l'équipe Inserm 1219 en Guinée, ainsi que la mission humanitaire que j'ai réalisée en 2014 dans le centre de traitement Ebola de Donka à Conakry, nous avaient particulièrement sensibilisés sur la Maladie à virus Ebola (MVE) dont les connaissances étaient encore limitées. Par conséquent, l'épidémie d'Ebola de 2019 survenue en République Démocratique du Congo (RDC) pour laquelle ALIMA avait reçu un financement opérationnel nous a semblé une bonne opportunité pour faire progresser la connaissance sur cette maladie. Enfin, la pandémie de Covid-19 survenant début 2020 a engendré un tel séisme dans le monde de la recherche qu'il nous est apparu indispensable de participer à l'effort international de recherche sur cette maladie, sa prise en charge et ses traitements spécifiques. Notre accès aux terrains africains et notre expertise en recherche opérationnelle ont largement contribué à cette implication.

Le processus de recherche que nous avons choisi est finalement assez similaire pour les 3 maladies. Tout d'abord, il s'agissait de décrire la maladie et sa prise en charge, à l'aide de cohortes prospectives observationnelles et descriptives. Il nous est apparu assez clairement que sans connaître la maladie il nous était impossible de faire évoluer sa prise en charge ou la prévention de sa propagation. Une fois la maladie bien décrite et l'expérience acquise, les questions de recherche clinique étaient formulées, en se basant sur les questions soulevées par les équipes de terrain. A titre d'exemple, l'enjeu de la fièvre de Lassa est l'accès à un traitement spécifique efficace alors qu'un traitement spécifique a déjà été identifié dans la MVE mais la question de la prophylaxie post exposition reste entière. Pour la Covid-19, les questions autour du traitement spécifique (pour les patients à risque d'aggravation) et de l'efficacité vaccinale sont majeures, spécialement en Afrique sub-saharienne. C'est de cette façon que nous avons progressivement construit les différents projets de recherche.

b) Fièvre de Lassa

La fièvre de lassa est une fièvre hémorragique virale dont le virus (LASV), de la famille des *Arenaviridae*, circule en Afrique de l'ouest au moins depuis qu'il a été découvert en 1969 dans le village de Lassa, au nord-est du Nigéria. C'est d'ailleurs au Nigéria que sont diagnostiqués la grande majorité des cas mondiaux, même si d'autres pays tels que le Benin (24), le Togo, la Guinée, le Liberia (25) et la Sierra Leone (26) déclarent aussi une petite vingtaine de cas par an (cf. tableau 1). L'incidence de la maladie en Afrique de l'ouest est certainement sous-évaluée en raison du manque d'accès au diagnostic virologique. A cet effet, une étude d'envergure de séroprévalence et d'évaluation de l'incidence de la maladie dans plusieurs pays d'Afrique de l'ouest est en cours (ENABLE) et permettra probablement d'affiner cette incidence.

C'est donc au Nigéria que la maladie sévit depuis plusieurs années avec, depuis 2018, une nette recrudescence du nombre de cas (27–29), recrudescence possiblement également liée à une amélioration du diagnostic par la généralisation du test par RT-PCR LASV (30,31). Les épidémies au Nigéria sont annuelles et saisonnières et surviennent en saison sèche, entre décembre et avril. La présentation clinique et la mortalité étaient moins bien connues avant l'étude LASCOPE (cf. chapitre C.2.a), mais la maladie était déjà décrite comme responsable de saignements extériorisés, de troubles digestifs (29) ainsi que d'atteintes rénales (32). Le traitement de référence recommandé par l'OMS et par le Nigerian Center for Disease Control (NCDC) (sans indication spécifique dans la fièvre de Lassa) est la ribavirine intraveineuse (IV) (33,34). C'est d'ailleurs le traitement utilisé depuis les années 80 chez tous les patients infectés, suite à la publication en 1976 d'une étude concluant à son efficacité mais dans laquelle les biais sont nombreux (35).

Début 2018, devant une augmentation nette des cas déclarés au Nigéria et avec notre équipe de médecins incluant des humanitaires de l'ONG ALIMA, nous avons pu réaliser une mission au sud du Nigéria pour évaluer les besoins non couverts en terme de prise en charge médicale de la fièvre de Lassa. A cette époque et comme présenté sur la figure 1, 3 hôpitaux rassemblaient la majorité des cas : Owo (Ondo state), Irrua (Edo state) et Abakaliki (Ebonyi state). Après avoir évalué ces 3 centres, le besoin principal a été identifié au « Federal Medical Center of Owo » (FMCO) où le nombre de cas était élevé et la prise en charge médicale très limitée. Les équipes d'ALIMA ont donc mis en place une intervention de soutien médical dans cet hôpital, début 2018, comportant (i) la réhabilitation du centre d'isolement pour la prise en charge de la fièvre de Lassa, (ii) l'approvisionnement en médicaments permettant d'apporter les soins de support gratuitement aux patients et (iii) la réhabilitation et l'équipement d'un laboratoire BSL 3 (bio safety level 3) permettant de réaliser des RT-PCR LASV et des analyses de biologie standard. L'approvisionnement en ribavirine et en équipement de protection individuel a été réalisé par le NCDC et l'OMS.

C'est dans ce contexte que les équipes de recherche CORAL ont pu mettre en place l'étude LASCOPE dont le protocole a été publié dans *Travel Medicine and Infectious Disease* en 2020 (36) et dont les résultats seront présentés dans le chapitre C.2.a.

Table 1 : Épidémies de fièvre de Lassa en Afrique Sub-saharienne depuis la découverte du virus (sources OMS, NCDC, revue de la littérature (24–27))

Pays	Zone géographique touchée	Année	Nombre de cas (confirmés)	Nombre de décès chez les cas	Létalité (%)
Nigéria	19/36 (Ogun, Bauchi, Plateau, Ebonyi, Ondo, Edo, Taraba, Nasarawa, Rivers, Kaduna, Gombe, Cross-River, Borno, Kano, Kogi, Enugu, Anambra, Lagos and Kwara)	2017	308	78	25
	23/36 (Edo, Ondo, Bauchi, Nasarawa, Ebonyi, Anambra, Benue, Kogi, Imo, Plateau, Lagos, Taraba, Delta, Osun, Rivers, FCT, Gombe, Ekiti, Kaduna, Abia, Adamawa, Enugu and Kano)	2018	633	171	27
	23/36 (Edo, Ondo, Bauchi, Nasarawa, Ebonyi, Plateau, Taraba, Adamawa, Gombe, Kaduna, Kwara, Benue, Rivers, Kogi, Enugu, Imo, Delta, Oyo, Kebbi, Cross River, Zamfara, Lagos and Abia)	2019	833	174	21
	27/36 (Ondo, Edo, Ebonyi, Taraba, Bauchi, Kogi, Plateau, Delta, Benue, Enugu, Nasarawa, Rivers, Gombe, Kaduna, Katsina, Sokoto, Kano, Abia, Adamawa, Borno, Kebbi, FCT, Osun, Anambra, Oyo, Ogun, Lagos)	2010	1189	244	21
	17/36 (Ondo, Edo, Ebonyi, Taraba, Bauchi, Kogi, Plateau, Delta, Benue, Enugu, Nasarawa, Kaduna, Katsina, Abia, FCT, Anambra, Borno)	2021	510	102	20
	23/36 (Edo, Ondo, Bauchi, Nasarawa, Ebonyi, Anambra, Benue, Kogi, Plateau, Taraba, Delta, Rivers, FCT, Gombe, Kaduna, Adamawa, Enugu, Kano, Katsina, Kebbi, Niger, Cross river and Oyo)	2022 (jusqu'à mai)	759	149	20
	Guinée	Guecké	2018	1	1
Kissidougou		2019	1	1	100
Beyla, Nzerekore, Faranah		2021	5	4	80
Gueckedou		2022	2	0	0
Sierra Leone	Kenema	1997	140	23	16
	Kenema	2012-2019	195	153	78
	Kenema, Kailahun, Tonkolili	2021	4	2	50
Liberia	Nimba	2006	10	7	70
		2007	13	5	38
	Bong	2013	12	8	67
	Bong, Nimba and Lofa, Margibi	2014	7	1	14
		2015	1	1	100
	Bong, Nimba, Gbarpolu, Lofa, Margibi, and Montserrado	2016	14	4	29
	Bong, Lofa, Nimba	2017	3	2	67
	Grand Bassa	2019	9	3	33
	Bong, Grand Bassa, Nimba, Margibi, Lofa, and Montserrado	2021	25	15	60
	Bong, Grand Bassa, Nimba, Margibi, Lofa, and Montserrado	2022	19	6	32
Benin		2014	13	11	85
		2016	54	28	52
		2017	2	2	100
		2018	5	5	100

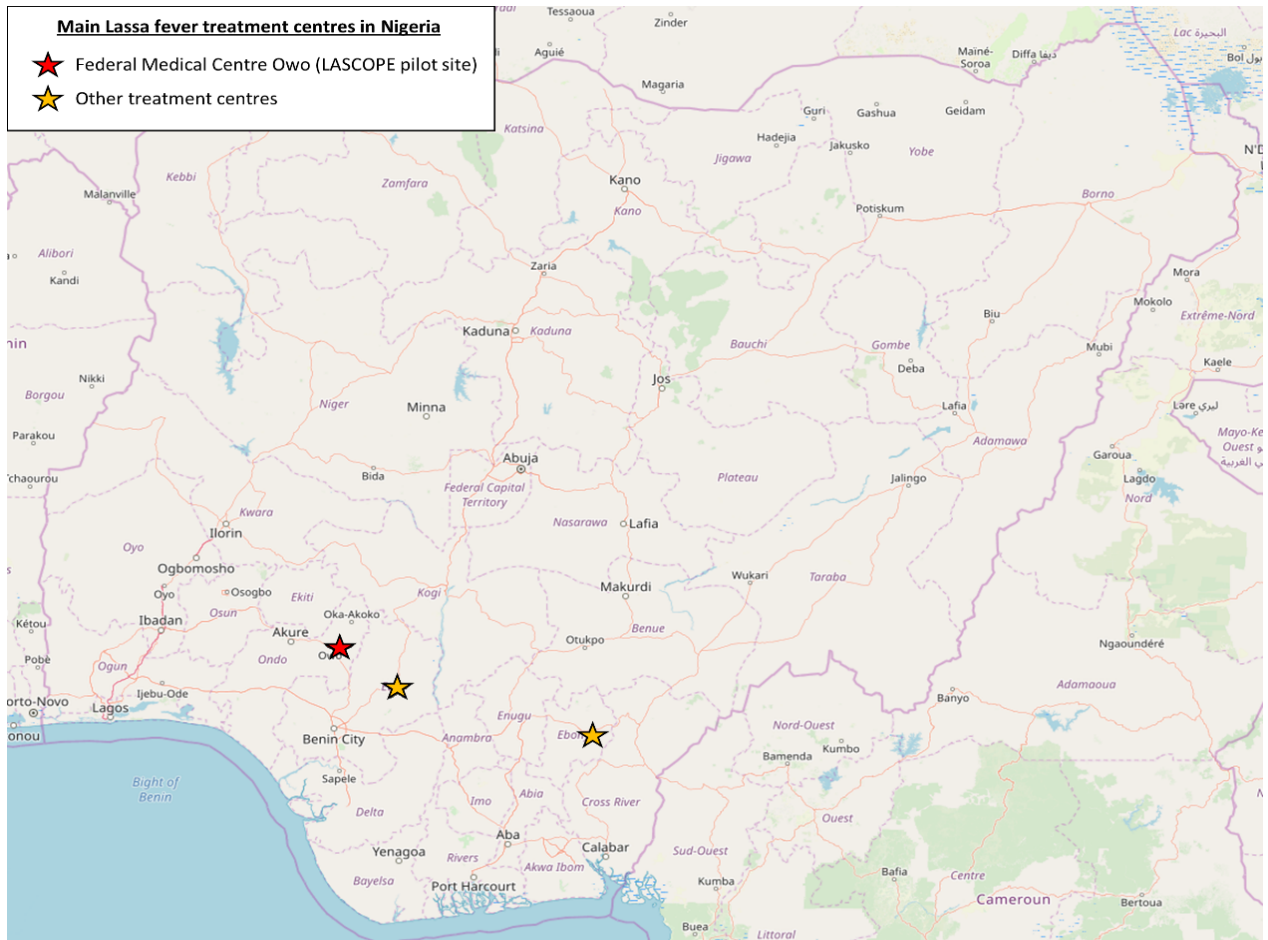


Figure 1: Principaux centres de prise en charge des patients atteints de fièvre de Lassa au Nigéria : FMCO (rouge), Irrua et Abakaliki (jaune)

c) Maladie à virus Ebola

La Maladie à virus Ebola (MVE) sévit depuis plusieurs décennies en Afrique subsaharienne, notamment en RDC où elle a été découverte en 1976 (cf. tableau 2). Mais c'est au cours de l'épidémie de grande ampleur ayant éclaté en Afrique de l'ouest (2014-2016) que les connaissances sur cette maladie négligée ont évoluée rapidement, tant en terme de présentation clinique, de pronostic et de facteurs associés à la mortalité (4–9,37). C'est au cours de cette épidémie que des projets de recherche ont été menés, permettant d'améliorer le contrôle de la diffusion de l'infection. En effet, un vaccin (r-VSV-ZEBOV-GP, ERVEBO) a montré son efficacité dans la limitation de la propagation de la maladie au sein de la communauté, grâce à une stratégie de vaccination en anneau (vaccination des contacts et contacts de contacts) (38–40). Aucun contact vacciné n'a développé la maladie au-delà de 10 jours après la vaccination.

Au cours de cette épidémie d'Afrique de l'ouest, la prise en charge clinique est restée peu invasive. L'inquiétude des soignants face à la prise en charge de patients hautement contagieux, et le manque d'accès aux techniques de réanimation de base (surveillance continue) ont limité l'évolution de la prise en charge symptomatique des patients. Elle se limitait donc à l'administration d'antibiotiques et antipaludéens, ainsi que la réhydratation parfois basée sur l'analyse biologique quand elle était disponible (9,41,42).

Du côté des traitements spécifiques, malgré plusieurs essais thérapeutiques conduits en contexte per-épidémique, aucune intervention n'a pu être identifiée comme limitant significativement la mortalité (43–45).

Ce n'est qu'en 2018-2019, au cours de la dixième épidémie survenant en RDC, que l'Institut National de la Recherche Biomédicale (INRB) a mis en place l'essai thérapeutique PALM qui a identifié 2 traitements spécifiques actifs contre la MVE. Le cocktail d'anticorps monoclonaux (mAbs) REGN-E3B et l'anticorps monoclonal MAb114 ont permis de réduire la mortalité chez les patients atteints de MVE à 33,5% et 35,1% respectivement (46).

La prise en charge symptomatique a aussi grandement évolué au cours de cette épidémie. Dans les centres de prise en charge d'ALIMA au Nord Kivu (Beni, Katwa-Butembo et Mambassa), les patients avaient accès à la biologie clinique quotidienne, à l'échographie cardio-vasculaire, au monitoring des constantes vitales, à la suppléance par oxygène, voire la transfusion sanguine. L'amélioration du niveau de standard de soin fait l'objet d'une publication qui sera exposée dans le chapitre C.2.b de la thèse.

C'est aussi au cours de cette 10^{ème} épidémie que la question de la Prophylaxie Post Exposition (PPE) dans la MVE a été identifiée comme centrale dans la lutte contre la diffusion de la maladie. En effet, selon le niveau du contact infectieux, le taux de transmission peut s'élever jusqu'à 80%. En effet, les soins prodigués à un parent malade dans un foyer au cours de l'épidémie d'Afrique de l'ouest entraîne un risque de transmission virale de 47,9% (IC 95 : 23,3-72,6) (47). Une autre étude, menée au Sierra Leone en 2014-2015 rapporte un taux de transmission intra familial secondaire de 8 à 83% selon le contact (48). Dans cette étude, 49,2% des patients ayant eu un contact rapproché et direct avec un patient sécrétant (défini par la présence de diarrhées, vomissements ou saignements extériorisés) ont développé la MVE. Ce taux de transmission s'élève à 60,8% chez les patients ayant eu un contact direct avec les fluides corporels d'un malade, et à 83,3% chez les patients ayant touché ou préparé un cadavre infecté. C'est donc en leur âme et conscience que les équipes de terrain ont administré aux contacts à haut risque, sur un mode urgent, monitoré et compassionnel, une PPE à base de mAbs. Les résultats de cette étude pilote sont présentés dans le chapitre C.2.b.

Malgré ces résultats encourageants, beaucoup de questions restent en suspens autour de l'efficacité des traitements spécifiques de la MVE et des stratégies de PPE.

Sur le plan curatif, les mAbs ont certes démontré une efficacité dans la réduction de la mortalité chez les patients atteints de MVE, mais cette diminution est nettement moins convaincante chez les patients présentant une charge virale élevée à l'admission (63,6% et 69,9% pour le REGN-E3B et le Mab114 respectivement)(46,49). De plus, ces deux médicaments n'ont pas d'action sur la constitution du réservoir viral. Les dernières épidémies survenues en Guinée (2021) et RDC (2019-2020) ont en effet pour origine la résurgence de la production virale à partir des réservoirs (organes génitaux et système nerveux central) chez des patients guéris de la maladie aiguë (17,50). Une hypothèse d'investigation est que l'association, au mAb, d'un antiviral pénétrant dans les réservoirs augmenterait l'efficacité en termes de mortalité (y compris chez les patients avec une charge virale élevée) et limiterait la constitution du réservoir et donc le risque de rechute chez les survivants. Un essai thérapeutique évaluant les bithérapies associant mAb et antiviral est en cours de mise en place par nos collègues américains du NIH en collaboration avec l'INRB.

Sur le plan de la PPE, les travaux de recherche ne font que débiter et de nombreuses questions restent à élucider avant de pouvoir conclure à la meilleure stratégie. Deux interventions sont actuellement utilisées en PPE : (i) la vaccination par ERVEBO chez les contacts et contacts de contacts, ayant une action établie sur le contrôle de la diffusion de la maladie dans la communauté, et (ii) le recours aux mAbs chez les contacts à haut risque (principalement utilisé en RDC) permettant de réduire de manière individuelle la transmission du virus. La stratégie globale de PPE doit rassembler ces deux approches. Les perspectives sur cette stratégie globale font l'objet de travaux de recherche à venir qui seront décrits dans le chapitre D.b.

Table 2 : Épidémies de Maladie à Virus Ebola en Afrique Sub-saharienne depuis la découverte du virus (sources diverses : MS, CDC, Institut Pasteur, Ministère de la santé)

Pays	# épidémie	Zone géographique	Zone(s) de santé(s)	Date de début	Date de fin	Sous-type de virus	Nombre de cas	Nombre de décès chez les cas	Létalité (%)	Traitements curatifs utilisés	Traitements préventifs utilisés
République démocratique du Congo	1	Equateur	Yambuku	août-76	août-76	Ebola Zaïre	318	280	88		
	2	Equateur	Tandala	juin-77	juin-77	Ebola Zaïre	1	1	100		
	3	Bandundu	Kikwit / Kwilu	mai-95	juin-95	Ebola Zaïre	315	250	79		
	4	Kasai-Occidental	Mweka	août-07	nov.-07	Ebola Zaïre	264	187	71		
	5	Kasai-Occidental	Mweka / Kaluamba	déc.-08	févr.-09	Ebola Zaïre	32	15	47		
	6	Province Orientale	Isiro	juin-12	nov.-12	Bundibugyo	77	36	47		
	7	Thuapa	Djera / Boende	août-14	nov.-14	Ebola Zaïre	66	49	74		
	8	Bas Uélé	Likati / Orientale	mai-15	juil.-15	Ebola Zaïre	8	4	50		
	9	Equateur	Wangata / Bikoro	mai-18	juil.-18	Ebola Zaïre	54	33	61		
	10	Nord Kivu / Ituri	Béni / Katwa / Mambassa	août-18	juin-20	Ebola Zaïre	3470	2280	66	REGENERON, MAB 114, REMDESIVIR, ZMAPP (essai PALM)	r-VSV-ZEBOV
	11	Equateur	Mbandaka	juin-20	nov.-20	Ebola Zaïre	130	55	42		
	12	Nord Kivu	Biena / Butembo	févr.-21	mai-21	Ebola Zaïre	12	6	50		
	13	Nord Kivu	Béni	oct.-21	déc.-21	Ebola Zaïre	11	9	82	REGENERON, MAB 114	r-VSV-ZEBOV
	14	Equateur	Mbandaka	avr.-22	en cours	Ebola Zaïre	4	4	100	REGENERON, MAB 114	r-VSV-ZEBOV
Guinée		Guinée forestière		déc.-13	juin-16	Ebola Zaïre	3811	2543	67		r-VSV-ZEBOV (Essai "Ebola ca suffit")
		Guinée forestière	NZK	févr.-21	juin-21	Ebola Zaïre	16	12	75	REGENERON	
Libéria				déc.-13	juin-16	Ebola Zaïre	10675	4809	45		
Sierra Leone				déc.-13	juin-16	Ebola Zaïre	14124	3956	28		
Soudan		Nzara / Maridi		1976	–	Ebola Soudan	284	151	53		
		Nzara		1979	–	Ebola Soudan	34	22	65		
				2004	–	Ebola Soudan	17	7	41		
Gabon				1994	–	Ebola Zaïre	52	31	60		
				1996	–	Ebola Zaïre	31	21	68		
				1996	–	Ebola Zaïre	60	45	75		
				2001	2002	Ebola Zaïre	65	53	82		

Côte d'Ivoire				1994	-	Forêt de Tai	1	0	0		
Ouganda				2000	-	Ebola Soudan	425	224	53		
				2007	-	Bundibugyo	149	37	25		
				2011	-	Ebola Soudan	1	1	100		
				2012	-	Ebola Soudan	24	17	71		
				2012	-	Ebola Soudan	7	4	57		
Congo (Brazaville)				2001	2002	Ebola Zaïre	59	44	75		
				2003	-	Ebola Zaïre	145	128	90		
				2003	-	Ebola Zaïre	35	29	83		
				2005	-	Ebola Zaïre	12	10	83		

d) La Covid-19 en Afrique

La maladie Covid-19 (infection par le SARS-CoV-2) est maintenant bien connue en Europe, Amérique et Asie, mais les enjeux et l'expression clinique de la pandémie sur le continent Africain sont différents. Au début de la pandémie en mars 2020, il a d'abord été nécessaire de décrire l'épidémie en Afrique et d'identifier d'éventuelles différences dans la présentation clinique et la mortalité entre le nord et le sud. Pour cela, des cohortes descriptives ont été mises en place dès le début de la pandémie, dont la cohorte COVISTA qui sera décrite dans le chapitre C.2.c. Sur le plan de la présentation clinique et des facteurs associés à la mortalité, les épidémies au nord et au sud sont extrêmement similaires. Les patients ont 50 ans en médiane, sont principalement des hommes, avec des comorbidités globalement similaires à ce que l'on retrouve en Europe, à savoir le diabète, l'obésité et l'hypertension artérielle. Les présentations cliniques sont aussi similaires à l'Europe ou aux États Unis (51–58). Le taux de mortalité estimé est plus hétérogène selon les études, selon le dénominateur utilisé pour la calculer. Certaines études évaluent la mortalité hospitalière, donc chez les patients les plus graves, entre 25 et 45% (51,52,54,55,57). D'autres prennent comme dénominateur l'ensemble des patients testés positifs pour le SARS-CoV-2, que le patient soit symptomatique ou non, avec alors une mortalité autour de 2-9% (53,58–60). Il est donc difficile de comparer cela avec les taux mis en évidence dans les pays du nord, notamment en raison du faible dépistage global et de la consultation des patients à un stade évolué de la maladie.

Cependant, un certain nombre de chercheurs estiment que la mortalité en Afrique est plus faible et que l'épidémie est moins importante que dans les pays du nord. En effet, les décès et les cas confirmés Africains représentent seulement 4,1% et 3,5% respectivement des cas mondiaux, alors que l'Afrique sub-saharienne compte pour 12,5% de la population mondiale (61,62). Cela n'est probablement pas grâce aux traitements disponibles et distribués sur le continent, car l'hydroxychloroquine et l'azithromycine n'ont démontré aucune efficacité (55,59). Les raisons pour lesquelles cette mortalité est relativement basse sont multiples. Selon les auteurs, elle peut être expliquée par (liste non exhaustive) :

- Une réponse précoce des gouvernements à la crise sanitaire, en partie liée à une expérience en terme de gestion des épidémies (61,63).
- Une pyramide des âges extrêmement différente des pays du nord, avec seulement 3% de la population africaine âgée de 65 ans ou plus (cf figure 2) (61,62,64,65).
- Des habitudes de vie des populations africaines différentes : pas de maison de retraite ou le virus circule rapidement (les personnes âgées sont maintenues à domicile avec les autres membres de la famille), davantage de zone rurale que de zone urbaine où le virus circule majoritairement, une vie quotidienne principalement à l'extérieur des (61), peu de voyage ou de déplacements internationaux (65).
- Une immunisation globale plus importante chez les Africains, avec davantage d'exposition aux pathogènes et une forte couverture de la vaccination par le BCG qui semblerait, selon une hypothèse à densifier, augmenter l'immunité contre la Covid-19 (61,65).

Cette faible mortalité (moins de 250 000 morts depuis le début de la pandémie), notamment comparée à la mortalité liée au VIH (500 000 morts par ans), à la tuberculose (500 000 morts par an) ou au paludisme (500 000 morts par an, source OMS), sont aussi potentiellement responsables du faible intérêt de la population africaine pour la Covid-19 et donc du faible taux de dépistage et de vaccination sur le continent. Quoiqu'il en soit,

l'épidémie de Covid-19 sur le continent africain ne peut pas être traitée de la même manière que l'épidémie sévissant en Europe ou aux Amériques.

Il est aussi important de considérer l'impact socio-économique que la pandémie de Covid-19 a eu sur une population déjà fragilisée par la situation sanitaire chronique (62,66). Avec la fermeture des frontières et le ralentissement voire l'arrêt des échanges internationaux, cette population vulnérable apparaît avoir plus à perdre qu'avec la maladie en elle-même. L'accès à l'alimentation, aux produits importés, aux produits de subsistances dans les familles a fortement diminué avec la crise sanitaire, laissant ces familles dans un dénuement majeur. Enfin, la diminution des aides financières internationales au profit du financement de la crise Covid a entraîné une limitation de l'accès aux soins et à l'alimentation. La Covid-19 reste une maladie du nord, qui ne doit pas faire oublier que la population africaine continue à souffrir de maux beaucoup plus préoccupants.

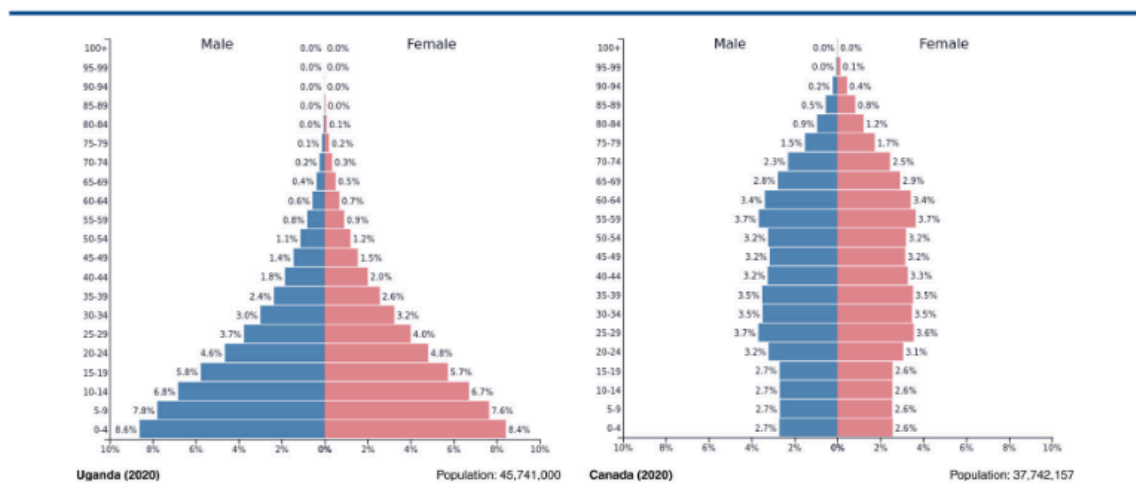


Figure 2 : Pyramides des âges au Canada et en Ouganda, Adams et al, GHSP, 2021 (62)

3. Contexte du travail : CORAL et collaborations internationales

Pour mettre en place ces projets de recherche opérationnelle, nous avons créé la structure CORAL (Clinical and Operational Research Alliance). Cette plateforme de recherche est née de la collaboration, au cours de l'épidémie de MVE en Guinée, entre ALIMA, l'équipe IDLIC du centre Inserm 1219 de l'Université de Bordeaux et le site ANRS-MIE de Côte d'Ivoire, PACCI.

ALIMA est une ONG médicale qui travaille depuis plus de 10 ans sur les maladies infectieuses et la malnutrition en Afrique sub-saharienne. Les équipes d'ALIMA sont notamment intervenues dans le cadre de l'épidémie de Choléra sévissant en Haïti en 2010, ainsi que pour l'épidémie de MVE d'Afrique de l'ouest en 2014.

L'équipe IDLIC du centre Inserm 1219 de l'Université de Bordeaux a une expertise de 15 ans de recherche clinique en Afrique sub-saharienne, notamment grâce à une collaboration particulièrement étroite avec PACCI. Les équipes de recherche travaillent principalement sur le VIH et la tuberculose, mais un élargissement des thématiques vers les MIE a été initié lors de l'épidémie de MVE d'Afrique de l'ouest.

C'est en effet autour de l'essai thérapeutique JIKI, visant à évaluer l'efficacité du favipiravir contre le virus Ebola (EBOV), que ALIMA, Inserm 1219 et PACCI se sont rencontrés et ont commencé à travailler ensemble. La plateforme CORAL est née en 2017 dans le but de formaliser cette collaboration et d'allier la prise en charge opérationnelle propre à l'action humanitaire, à la rigueur de la recherche clinique. Deux pôles de recherche ont été créés pour répondre aux questions de recherche mises en évidence par les acteurs de terrain et les chercheurs : le pôle nutrition/santé materno-infantile et le pôle MIE. J'ai été recrutée en 2017 pour coordonner le pôle MIE sous la direction du Pr Denis Malvy. Nous avons donc constitué une équipe de chercheurs, actuellement composée de 5 personnes en France et une douzaine sur le terrain.

Depuis sa création, le pôle MIE de CORAL accorde une attention particulière aux collaborations internationales. Que les acteurs internationaux soient impliqués à nos côtés sur le terrain, ou qu'ils aient une expertise particulière sur l'une ou l'autre des MIE investiguées, des liens solides ont été tissés. Au nord, nous travaillons actuellement avec le Bernhard Nocht Institut for Tropical Medicine (BNITM), Hambourg, Allemagne et l'Université d'Oxford sur la fièvre de Lassa, et avec le NIH et le BARDA (Biomedical Advance Research and Development Authorities) sur la MVE. Nos travaux sont systématiquement travaillés ou du moins argumentés en amont avec l'OMS et nous les tenons informés de leur évolution. Enfin, l'ANRS-MIE nous soutient financièrement et assure la promotion de nos essais. Au sud, les partenariats sont multiples et pas systématiquement liés à une problématique unique. En Guinée, le CHU de Donka à Conakry, ainsi que l'ANSS (Agence Nationale de Sécurité Sanitaire) sont nos partenaires privilégiés pour les essais COVICOMPARE-Guinée et COVERAGE-AFRICA, ainsi que les projets IMOVA et Integrate (cf chapitre D). Au Nigéria, les hôpitaux d'Owo, d'Irrua et de Abakaliki sont les partenaires historiques sur lesquels nous avons bâti les projets de recherche sur la fièvre de Lassa, sans oublier bien sur le Nigerian Center for Disease Control (NCDC), organisation nationale de surveillance épidémiologique sans laquelle rien de tout cela n'aurait été possible. En RDC, outre les cliniciens que nous avons formés à la recherche clinique, le partenaire principal est l'INRB (Institut National de Recherche Biomédicale) avec qui les projets PEP sont mis en place (cf chapitre C.2.b).

C. Publications

1. Format de la thèse

Cette thèse est une thèse de publications, où les différents articles publiés ou en cours de publication sont présentés par ordre thématique. Quatre articles sont donc exposés ici. D'autres publications ont vu le jour pendant la durée de la thèse mais ma participation était moins directe (publications présentées en annexes). Cependant, un grand nombre de perspectives sont nées de ces travaux, et seront détaillées dans le chapitre D de la thèse.

2. Présentation des publications

a) Fièvre de Lassa

Cohorte observationnelle descriptive au Nigéria : étude LASCOPE

En avril 2018, le protocole de la cohorte LASCOPE a été validé par le comité d'éthique et les autorités réglementaires nigérianes. Ce protocole a été publié en 2020 (36) et le manuscrit décrit l'étude comme suit : LASCOPE ("Lassa Fever Clinical Course and Prognostic Factor in an Epidemic Context") est une étude de cohorte prospective observationnelle et descriptive visant à (i) décrire les paramètres cliniques et biologiques des patients hospitalisés et présentant une fièvre de Lassa confirmée par la RT-PCR, (ii) estimer la mortalité intra hospitalière et (iii) définir les variables associées à la mortalité. C'est la première étude prospective réalisée chez les patients atteints de fièvre de Lassa et à la date du 5 juillet, près de 1000 patients Lassa confirmés ont été inclus.

Les premiers résultats de l'étude LASCOPE ont été publiés dans The Lancet Global Health en 2021 et l'article est présenté ci-dessous. Ce premier article concerne les patients inclus entre le 5 avril 2018 et le 15 mars 2020, ce qui représente un total de 510 patients analysés dont 62 décédés après une médiane de 3 jours d'hospitalisation (taux de mortalité intra hospitalière= 12%). Parmi eux, 18 avaient moins de 5 ans et 17 femmes étaient enceintes à l'admission dont une d'entre elles est décédée. La durée médiane entre l'apparition des symptômes et l'admission était de 8 jours et 15% des patients présentaient une RT-PCR < 25 Ct à l'admission. Les principaux symptômes retrouvés à l'admission étaient : fièvre (76%), douleur abdominale (31%), vomissement (38%), diarrhée aqueuse (24%) ainsi que des saignements de tout type (19%). Sur le plan biologique, à l'admission, 19% avaient des ALAT supérieures à 3N et 11% avaient une atteinte rénale (KDIGO 2 ou 3¹). Tous les patients ont reçu de la ribavirine, 18% ont reçu une suppléance en oxygène et 31% ont été transfusés. Au total, 8% des patients ont été dialysés au cours de leur prise en charge parmi lesquels 56% sont décédés. Les facteurs présents à l'admission et significativement associés à la mortalité étaient : l'âge ≥ 45 ans, le score NEWS2² ≥ 7 , les ALAT ≥ 3 N, le KDIGO ≥ 2 et la RT-PCR < 30 Ct.

Ces résultats ont permis de mieux comprendre la maladie et de préparer les projets de recherche notamment thérapeutiques à venir qui seront détaillés dans le chapitre D.a.

¹ Score KDIGO (67,68) = score d'évaluation de la fonction rénale => KDIGO 1 : augmentation de la créatinine < 2 fois la baseline ; KDIGO 2 : augmentation de la créatinine ≥ 2 et <3 fois la baseline ; KDIGO 3 : augmentation de 3 fois la baseline ou créatinine $\geq 353,6\mu\text{mol/l}$

² NEWS 2 (69) = score d'évaluation clinique basé sur la mesure de la fréquence respiratoire, saturation en oxygène, besoin en oxygène, pression artérielle systolique, fréquence cardiaque, niveau de conscience et température. Risque élevé si NEWS2 ≥ 7 , modéré si <7 et ≥ 5 , faible si <5.

Lassa fever outcomes and prognostic factors in Nigeria (LASCOPE): a prospective cohort study



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Summary

Background Lassa fever is a viral haemorrhagic fever endemic in parts of west Africa. New treatments are needed to decrease mortality, but pretrial reference data on the disease characteristics are scarce. We aimed to document baseline characteristics and outcomes for patients hospitalised with Lassa fever in Nigeria.

Methods We did a prospective cohort study (LASCOPE) at the Federal Medical Centre in Owo, Nigeria. All patients admitted with confirmed Lassa fever were invited to participate and asked to give informed consent. Patients of all ages, including newborn infants, were eligible for inclusion, as were pregnant women. All participants received standard supportive care and intravenous ribavirin according to Nigeria Centre for Disease Control guidelines and underwent systematic biological monitoring for 30 days. Patients' characteristics, care received, mortality, and associated factors were recorded using standard WHO forms. We used univariable and multivariable logistic regression models to investigate an association between baseline characteristics and mortality at day 30.

Findings Between April 5, 2018, and March 15, 2020, 534 patients with confirmed Lassa fever were admitted to hospital, of whom 510 (96%) gave consent and were included in the analysis. The cohort included 258 (51%) male patients, 252 (49%) female patients, 426 (84%) adults, and 84 (16%) children (younger than 18 years). The median time between first symptoms and hospital admission was 8 days (IQR 7–13). At baseline, 176 (38%) of 466 patients had a Lassa fever RT-PCR cycle threshold (Ct) lower than 30. From admission to end of follow-up, 120 (25%) of 484 reached a National Early Warning Score (second version; NEWS2) of 7 or higher, 67 (14%) of 495 reached a Kidney Disease–Improving Global Outcome (KDIGO) stage of 2 or higher, and 41 (8%) of 510 underwent dialysis. All patients received ribavirin for a median of 10 days (IQR 9–13). 62 (12%) patients died (57 [13%] adults and five [6%] children). The median time to death was 3 days (1–6). The baseline factors independently associated with mortality were the following: age 45 years or older (adjusted odds ratio 16·30, 95% CI 5·31–50·30), NEWS2 of 7 or higher (4·79, 1·75–13·10), KDIGO grade 2 or higher (7·52, 2·66–21·20), plasma alanine aminotransferase 3 or more times the upper limit of normal (4·96, 1·69–14·60), and Lassa fever RT-PCR Ct value lower than 30 (4·65, 1·50–14·50).

Interpretation Our findings comprehensively document clinical and biological characteristics of patients with Lassa fever and their relationship with mortality, providing prospective estimates that could be useful for designing future therapeutic trials. Such trials comparing new Lassa fever treatments to a standard of care should take no more than 15% as the reference mortality rate and consider adopting a combination of mortality and need for dialysis as the primary endpoint.

Funding Institut National de la Santé et de la Recherche Médicale, University of Oxford, EU, UK Department for International Development, Wellcome Trust, French Ministry of Foreign Affairs, Agence Nationale de Recherches sur le SIDA et les hépatites virales, French National Research Institute for Sustainable Development.

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Introduction

Lassa fever is a viral haemorrhagic fever caused by the Lassa virus. It is endemic in west Africa, where it has an estimated annual burden of 300 000 cases and 5000–10 000 deaths.^{1,2} Lassa virus is mainly transmitted from a zoonotic reservoir to humans,³ whereas human-to-human transmission is less frequent and mostly nosocomial.^{4,5} Early symptoms are not very specific,

often leading health-care workers to suspect another diagnosis and causing delays in the appropriate management. In severe cases, the course of the disease can lead to multiorgan failure and death.³ Most data on the natural history of Lassa fever are retrospective or from before the development of reliable diagnostic tools.^{6–9} Intravenous ribavirin is the recommended specific treatment despite weak evidence of its efficacy.^{6,10}

Lancet Glob Health 2021; 9: e469–78

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See Online for appendix

Research in context

Evidence before this study

Lassa fever is endemic to west Africa, with some reports of exported confirmed cases. Lassa fever is responsible for a disease burden exceeding that of all other viral haemorrhagic fevers except dengue and yellow fever. Prospective cohort evidence is needed on mortality and associated factors under the best standard of care to inform the design of future therapeutic trials. We searched PubMed up to Nov 11, 2020, for publications in English using the following search terms: ("Lassa fever" OR "Lassa virus") AND ("retrospective study" OR "prospective study" OR "cohort"). Among 19 study reports, only three reported mortality and its associated factors among patients with Lassa fever confirmed by RT-PCR. All three studies were retrospective, and only one study enrolled more than 100 patients.

Added value of this study

We monitored a cohort of 510 participants with RT-PCR-confirmed Lassa fever admitted over a 24-month period at the Federal Medical Centre of Owo, Ondo State, Nigeria. Patients received intravenous ribavirin therapy and standardised supportive care free of charge, including fluid resuscitation, antibiotics, antimalarials, blood transfusions, and renal replacement therapy where appropriate. The 30-day mortality was 13% for adults and 6% for children. The median time between symptom onset and admission to the Lassa fever ward

was 8 days. 82% of deaths occurred within 7 days of admission.

The factors associated with mortality were age, the National Early Warning Score (second version), baseline Lassa RT-PCR cycle threshold value, acute renal failure, and plasma aminotransferase level.

Implications of all the available evidence

Lassa fever displays some common characteristics with Ebola virus disease and some striking differences, all of which have implications for the design of future clinical trials. On one hand, as with Ebola, faster diagnosis and management is necessary because most patients arrive late and intra-hospital deaths occur quickly. Additionally, cycle threshold values for Lassa fever RT-PCR appear to be a useful and easy-to-use prognostic tool, for both managing clinical cases and stratifying randomisation for future trials. Furthermore, acute renal failure is a major driver of prognosis and further exploration is needed on carefully balancing recourse to fluid resuscitation, vasopressor or inotropic drugs, and dialysis in this context. On the other hand, mortality from Lassa fever is several times lower than that from Ebola. Because a very large number of participants would be required for a trial to show the efficacy of new drugs in reducing mortality, composite endpoints combining mortality with clinically significant parameters should be considered for future trials.

and concerns regarding its toxicity.¹⁰ No vaccine is available yet. For all these reasons, Lassa fever has been included in the WHO Research and Development Blueprint list of high-priority diseases.

Several antivirals and immunotherapeutics are promising candidates for treatment of Lassa fever.^{11,12} Before designing drug efficacy trials, it is essential to describe the prospective course of Lassa fever and estimate the mortality rate and associated factors under the best possible standard of care.

In 2018, in the context of an unprecedented seasonal recrudescence of Lassa fever,¹³ we established a Lassa fever ward, organised standardised care, and launched a prospective cohort of patients with Lassa fever at the Federal Medical Centre in Owo (Ondo State, Nigeria).¹⁴ In this study, we aimed to prospectively study and document the characteristics on admission, care received, and outcomes of patients registered during the first 24 months of this cohort.

Methods

Patients and setting

The protocol for the LASCOPE (Lassa fever clinical course and prognostic factors in an epidemic context) cohort has been published previously.¹⁴ Briefly, the Federal Medical Centre Owo (FMCO) is a tertiary hospital serving a large, semi-urban and rural area in Ondo State, Nigeria. From the start of the study period, all patients admitted to the

emergency room and any other department at the FMCO with confirmed Lassa fever were referred to the Lassa fever ward and invited to participate in the cohort. Patients who signed an informed consent form were included in the cohort. Patients of all ages were eligible for inclusion. Pregnant women and newborn infants were also eligible for inclusion. Lassa fever was confirmed by use of the RealStar Lassa Virus RT-PCR kit 2.0 (Altona Diagnostics, Hamburg, Germany; details presented in appendix p 4).

Informed consent was sought on admission. For underage children (younger than 18 years), the consent of at least one parent or guardian was required. The assent of the child was also sought for children older than 12 years. For adults without the capacity to give informed consent, a relative was asked to consent on behalf of the patient. When the patient regained the capacity to give informed consent, they were asked if they agreed to continue to participate. The study was approved by the Nigerian National Health Research Ethics Committee and the FMCO Research Ethics Committee. Confidentiality was guaranteed by deidentification and restricted access to study documents and databases.

Follow-up and care of patients

Following the Nigerian Centre for Disease Control guidelines, patients with suspected Lassa fever with a low index of suspicion were not started on ribavirin before RT-PCR confirmation. Patients with confirmed Lassa

fever and those without confirmation but with a high index of suspicion (defined in appendix p 3) were started on ribavirin immediately. Different intravenous ribavirin regimens were used depending on age, pregnancy status, and physician's preference (appendix p 3).¹⁵

Standard supportive care included oral or intravenous administration of fluids and analgesics, oxygen according to the patient's clinical status, antimalarials for patients with confirmed malaria, antibiotics for patients with a suspected bacterial infection, total blood transfusion for patients with severe anaemia, and intermittent haemodialysis for patients with life-threatening acute renal dysfunction. Mechanical ventilation, invasive haemodynamic monitoring, vasopressor drugs, and inotropic drugs were not available.

Patients underwent systematic biological monitoring, including testing for malaria with a rapid diagnostic test (SD BIOLINE Malaria Ag P.f, Abbott, Chicago, IL, USA) or thick blood smear on hospital admission (day 0) and the following tests on day 0, day 5, and day 10 of hospital stay: Lassa virus RT-PCR, full blood count, albuminaemia, creatininaemia, uraemia, plasma electrolytes, aspartate aminotransferase, alanine aminotransferase, and bilirubin. Women of childbearing age underwent a urine pregnancy test at day 0. Additional tests were done whenever it was deemed useful.

The criteria for hospital discharge were absence of fever or any other substantial symptoms and completion of 10 days of ribavirin therapy. Patients meeting the criteria for discharge but still having a positive RT-PCR test for Lassa virus were discharged with a prescription for oral ribavirin for an additional 7 days, as recommended in the Nigerian Centre for Disease Control guidelines (appendix p 3).¹⁵ Patients who were discharged were asked to attend an outpatient visit 30 days after admission to detect any subacute sequelae requiring specific care.

Data collection and definitions

We collected demographic characteristics, clinical measures and symptoms, the previously mentioned biological monitoring, care and treatments received, and clinical outcomes. Data were recorded with use of an expanded version of the standard WHO Lassa fever case report form.¹⁴ Clinical severity was scored with the National Early Warning Score, version 2 (NEWS2).¹⁶ Acute kidney dysfunction was classified as acute kidney injury or acute kidney failure and staged according to Kidney Disease-Improving Global Outcome (KDIGO) criteria (appendix p 2).^{17,18} Malaria was defined as presentation of signs and symptoms compatible with a malaria episode and the presence of asexual forms of *Plasmodium* sp on a thick blood smear or a positive malaria rapid diagnostic test.

Pregnancy outcomes were categorised as spontaneous miscarriage (spontaneous expulsion of the fetus before 28 weeks of amenorrhoea), intrauterine death (fetal death after 28 weeks of amenorrhoea without fetus expulsion),

maternofetal demise (concomitant death of the mother and the fetus), stillbirth (birth of neonate showing no signs of life after 28 weeks of amenorrhoea), and livebirth.¹⁹

Statistical analysis

Hospital admission was defined as day 0. We used the Kaplan-Meier method to estimate time to death between day 0 and day 30. We used univariable and multivariable logistic regression models to investigate an association between baseline characteristics and mortality at day 30. All cycle threshold (Ct) values used in the analysis were Ct values for the *GPC* gene. Sex was included a priori in all multivariable models. Age, Ct value, plasma alanine aminotransferase concentration, NEWS2, and KDIGO stage were included in all multivariable models provided they had $p < 0.05$ in univariable analysis. All other variables with less than 10% of missing values and $p < 0.05$ in univariable analysis were included in the initial multivariable model and either kept until the final model or excluded before the final model in a stepwise descending procedure. We did the main analysis with available data. We did a sensitivity analysis including all patients with missing data in a specific category for each variable to explore whether missing data for key variables could influence the final conclusion. Analyses were done with use of SAS, version 9.4.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. AD, MJ, and DG had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Between April 5, 2018, and March 15, 2020, 712 patients were admitted to the Lassa fever ward with suspected Lassa fever, of whom 178 had negative and 534 had positive Lassa RT-PCR. Of 534 patients with confirmed Lassa fever, 510 (96%) participants signed informed consents and were included in the analysis (figure 1). Of

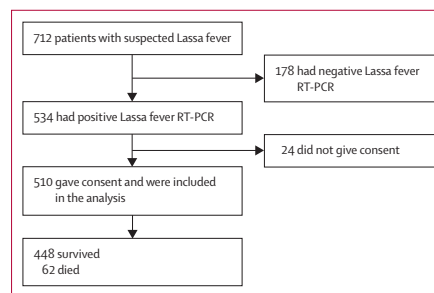


Figure 1: Flow chart of participants

	Patients with available baseline data (n=510)	Baseline	Entire follow-up
General characteristics			
Sex	510
Male	..	258 (51%)	..
Female	..	252 (49%)	..
Age, years	510
<1	..	4 (1%)	..
1-4	..	14 (3%)	..
5-17	..	66 (13%)	..
18-44	..	277 (54%)	..
45-59	..	85 (17%)	..
≥60	..	64 (13%)	..
Number of people in household	498	6 (4-8)	..
Rodents at home or in surrounding area	507	493 (97%)	..
Contact with ill or dead person within 3 weeks of admission	506	63 (12%)	..
Health workers	507	21 (4%)	..
Vital signs			
Level of consciousness	508
Alert	..	476 (94%)	437 (86%)
Confusion	..	20 (4%)	13 (3%)
Voice (reactive to)	..	2 (<1%)	5 (1%)
Pain (reactive to)	..	6 (1%)	9 (2%)
Unresponsive	..	4 (1%)	46 (9%)
Heart rate >110 per min	510	73 (14%)	167 (33%)
Systolic arterial pressure <90 mm Hg	492	26 (5%)	107 (22%)
Oxygen saturation <92%	504	34 (7%)	77 (15%)
NEWS2	484
0-2	..	199 (41%)	34 (7%)
3-4	..	130 (27%)	154 (32%)
5-6	..	90 (19%)	145 (30%)
≥7	..	65 (13%)	120 (25%)
Other clinical signs and symptoms			
Fever (temperature >38.0 °C)	509	389 (76%)	406 (80%)
Headache	508	187 (37%)	220 (43%)
Abdominal pain	509	157 (31%)	191 (38%)
Myalgia	510	101 (20%)	121 (24%)
Chest or retrosternal pain	501	59 (12%)	94 (19%)
Sore throat	508	62 (12%)	69 (14%)
Dizziness	508	127 (25%)	145 (29%)
Signs of encephalopathy			
Seizure	508	12 (2%)	27 (5%)
Delirium	510	9 (2%)	18 (4%)
Meningeal syndrome	510	4 (1%)	17 (3%)
Focal deficiency	510	3 (1%)	6 (1%)

(Table 1 continues in next column)

	Patients with available baseline data (n=510)	Baseline	Entire follow-up
(Continued from previous column)			
Aphasia or dysarthria	510	1 (<1%)	2 (<1%)
Impaired hearing or tinnitus	509	2 (<1%)	5 (1%)
Impaired vision	509	2 (<1%)	2 (<1%)
Vomiting	497	190 (38%)	241 (48%)
Watery diarrhoea	508	120 (24%)	148 (29%)
Hiccup	509	1 (<1%)	8 (2%)
Lower limbs oedema	510	10 (2%)	38 (7%)
Facial swelling	509	10 (2%)	17 (3%)
Cough	509	96 (19%)	118 (23%)
Bleeding, any type	510	98 (19%)	174 (34%)
Macroscopic haematuria	510	27 (5%)	130 (25%)
Melena	508	21 (4%)	46 (9%)
Vaginal bleeding*	228	7 (3%)	12 (5%)
Haematemesis	508	15 (3%)	15 (3%)
Gingival bleeding	510	5 (1%)	9 (2%)
Venous puncture point bleeding	509	2 (<1%)	7 (1%)
Haematochezia	508	6 (1%)	6 (1%)
Conjunctival bleeding	510	4 (1%)	4 (1%)
Epistaxis	509	5 (1%)	7 (1%)
Purpura	510	2 (<1%)	10 (2%)
Haemoptysis	510	2 (<1%)	2 (<1%)
Biological measures			
Lassa virus RT-PCR Ct value†	466	32.0 (27.6-35.2)	..
≥35.0	..	126 (27%)	..
30.0-34.9	..	164 (35%)	..
25.0-29.9	..	108 (23%)	..
<25.0	..	68 (15%)	..
Haematocrit, %	486	33 (29-38)	28 (24-33)
<25%	..	50 (10%)	153 (31%)
Platelets, 10 ⁹ per L	425	200 (118-300)	178 (104-269)
<80	..	47 (11%)	60 (14%)‡
Leucocytes, 10 ⁹ cells per L	442	5.8 (3.9-9.3)	7.4 (4.9-11.6)
>12	..	86 (19%)	105 (24%)
<4	..	116 (26%)	144 (33%)
Positive malaria diagnostic test§	315	179 (57%)	185 (59%)
Creatininaemia, µmol/L	495	86 (65-115)	92 (73-128)
Uraemia, mmol/L	500	3.5 (2.2-5.5)	3.8 (2.9-6.2)
Acute kidney dysfunction stage	495
No dysfunction	..	431 (87%)	410 (83%)
KDIGO stage 1	..	11 (2%)	18 (4%)
KDIGO stage 2 (AKI)	..	13 (3%)	10 (2%)
KDIGO stage 3 (AKF)	..	40 (8%)	57 (12%)

(Table 1 continues in next column)

	Patients with available baseline data (n=510)	Baseline	Entire follow-up
(Continued from previous column)			
Sodium, mmol/L	487	135 (131–138)	133 (129–136)
>145	..	23 (5%)	35 (7%)
<128	..	38 (8%)	84 (17%)
Potassium, mmol/L	482	3.9 (3.6–4.4)	4.2 (3.8–4.7)
>5.0	..	41 (9%)	73 (15%)
<3.5	..	99 (21%)	205 (43%)
Total carbon dioxide, mmol/L	479	23 (20–25)	22 (18–24)
<18	..	75 (16%)	107 (22%)
Albumin, g/L	405	30 (26–33)	29 (24–32)
<28	..	142 (35%)	171 (42%)
Glycaemia, mmol/L	409	4.8 (4.0–6.1)	4.2 (3.4–5.1)
<3.0	..	30 (7%)	63 (15%)
AST, U/L¶	408	80 (48–214)	93 (52–232)
>3 times the upper limit of normal range	..	154 (38%)	166 (41%)
ALT, U/L¶	421	53 (30–107)	62 (35–119)
>3 times the upper limit of normal range	..	80 (19%)	90 (21%)

Data are n, n (%), or median (IQR). Baseline values were collected on admission to hospital. For entire follow-up, values for vital signs and severity criteria are the poorest condition recorded at any time from baseline to end of follow-up; values for signs and symptoms are those reported at least once from baseline to end of follow-up; and values for biological measures are the poorest value recorded from baseline to end of follow-up. The poorest value for biological parameters was defined as the highest value for leucocytosis, creatininaemia, uraemia, kalaemia, plasma AST and ALT, and the lowest value for haematocrit, platelet count, natraemia, plasma total carbon dioxide, albuminaemia, and glycaemia. AKF=acute kidney failure. AKI=acute kidney injury. ALT=alanine aminotransferase. AST=aspartate aminotransferase. Ct=cycle threshold. KDIGO=Kidney Disease–Improving Global Outcome. NEWS2=National Early Warning Score, second version. *Among females aged 12 years or older. †The 466 (91%) available Ct values were all Ct values for the GPC gene; 44 (9%) patients had no available GPC gene Ct value, including seven patients with Ct values available for the L gene but not for GPC and 37 patients with a positive RT-PCR test result but no available Ct value; these patients with no Ct value were included during the first months of the cohort, when Lassa fever molecular diagnostic was not done on site yet and samples were sent to the Iruva Specialist Teaching Hospital laboratory (Iruva, Nigeria), and RT-PCR results were rendered as negative or positive with no Ct values. ‡Of the 60 patients, two had a platelet count lower than 20 10⁹ per L, 20 had 20–50 10⁹ per L, and 38 had 50–80 10⁹ per L. §315 participants had a malaria diagnostic test done between 7 days before and 4 days after admission; among these, 179 had at least one positive test (thick blood smear or rapid diagnostic test), of whom 40 had a positive thick blood smear test and a negative rapid diagnostic test, 119 had a positive thick blood smear test and no available rapid diagnostic test, 17 had a positive rapid diagnostic test and no available thick blood smear, and three had both tests positive; six additional participants had malaria diagnosed later during their hospital stay (all with a positive rapid diagnostic test, with thick blood smear not done). ¶The 102 (20%) AST and 89 (17%) ALT missing values were from patients included during the second half of January, 2020, because the biology device used for liver function tests was out of order.

Table 1: Baseline and follow-up characteristics

the remaining 24 patients, 15 had impaired consciousness at baseline and died with no relative available of whom consent could be asked and nine declined to take part.

	Participants
Length of hospital stay, days	
Time to death, days (n=61)	3 (1–6)
People who were discharged alive from hospital (n=449)*	11 (10–15)
Admission in the intensive care unit† of the Lassa fever ward	94 (18%)
Ribavirin therapy	
Received	510 (100%)
Time between first symptoms and first dose, days	8 (7–13)
Duration of treatment, days	10 (9–13)
Oxygen‡	
Received	92 (18%)
Maximum output, L/min	6 (5–6)
Duration, days	3 (2–7)
Total blood transfusion§	
Received	158 (31%)
Number of units (total blood pints)	2 (2–4)
Renal replacement therapy (intermittent haemodialysis)	
Received	42 (8%)
Indications¶	
Fluid overload, no response to diuretics	5 (14%)
Symptomatic hyperzotaemia	33 (94%)
Severe acid-base disorder not responding to medical treatment	1 (3%)
Number of sessions	2 (1–4)
Outcome	
Died	23 (56%)
Survived	18 (44%)
Antibacterial therapy	
Received	459 (90%)
Duration, days	8 (7–10)
Antimalarial therapy	
Received	188 (37%)

Data are n (%) for category variables and median (IQR) for continuous variables. *One patient died after discharge. †Intensive care unit refers to a specific area of the Lassa fever ward where patients were more frequently monitored for vital functions; mechanical ventilation, vasopressive drugs, and invasive haemodynamic monitoring were not available. ‡Missing data for one patient. §Missing data for two patients. ¶Missing data for six patients; four participants had more than one indication for renal replacement therapy: symptomatic hyperzotaemia plus fluid overload not responding to diuretics (n=3) and symptomatic hyperzotaemia plus severe acid-base disorder not responding to medical treatment (n=1). ||Antimalarial therapy administered within the Lassa fever ward (treatments before admission are not included).

Table 2: Care and treatments

Of the 510 participants, 258 (51%) were male and 252 (49%) female. The median age was 32 years (IQR 21–47), with 84 (16%) participants younger than 18 years and 426 (84%) adults (table 1). 269 (53%) participants came directly from their homes and 241 (47%) were referred from other facilities (82 [16%] from private clinics, 34 [7%] from primary public facilities, and 125 [25%] from secondary or tertiary public facilities). The median time between first symptoms and admission to the Lassa fever ward was 8 days (IQR 7–13). Before admission, 376 (74%)

	Available data in the univariable analysis	Participants who died	Univariable		Multivariable	
			Crude odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Sex						
Female	252	28 (11%)	1 (ref)	..	1 (ref)	..
Male	258	34 (13%)	1.21 (0.71-2.07)	0.48	1.19 (0.45-3.16)	0.72
Age, years						
<45	361	25 (7%)	1 (ref)	..	1 (ref)	..
≥45	149	37 (25%)	4.44 (2.56-7.70)	<0.0001	16.30 (5.31-50.30)	<0.0001
NEWS2						
<7	419	30 (7%)	1 (ref)	..	1 (ref)	..
≥7	65	30 (46%)	11.10 (6.02-20.50)	<0.0001	4.79 (1.75-13.10)	0.0023
Plasma ALT						
<3 U/LN	341	21 (6%)	1 (ref)	..	1 (ref)	..
≥3 U/LN	80	23 (29%)	6.15 (3.19-11.80)	<0.0001	4.96 (1.69-14.60)	0.0036
KDIGO stage						
<2	442	26 (6%)	1 (ref)	..	1 (ref)	..
≥2	53	28 (53%)	17.90 (9.18-35.00)	<0.0001	7.52 (2.66-21.20)	<0.0001
Lassa RT-PCR Ct						
≥30	290	8 (3%)	1 (ref)	..	1 (ref)	..
<30	176	46 (26%)	12.50 (5.72-27.20)	<0.0001	4.65 (1.50-14.50)	0.0078

Data are n (%) unless otherwise specified. ALT=alanine aminotransferase. Ct=cycle threshold (GPC gene as a target). KDIGO=Kidney Disease-Improving Global Outcome. NEWS2=National Early Warning Score, second version. U/LN=upper limit of normal range.

Table 3: Association between mortality and baseline characteristics, multivariable analysis (n=377)

patients had received antimalarials and 14 (3%) ribavirin therapy.

Clinical and biological features of patients on admission and during follow-up are presented in table 1 and the appendix (pp 6–9). The median Ct value for GPC in Lassa virus RT-PCR tests at baseline was 32 (IQR 27.6–35.2; appendix p 18). The most common clinical characteristics were fever, pain (headache, myalgia, chest, or abdominal pain), digestive disorders (vomiting or diarrhoea), neurological disorders (dizziness or impaired consciousness) and haemorrhagic signs, of which macroscopic haematuria and melena were the most frequent.

Overall, between baseline and end of follow-up, 120 (25%) of 484 patients reached a NEWS2 of 7 or higher; 67 (14%) of 495 reached a KDIGO stage of 2 or higher; 174 (34%) of 510 had at least one haemorrhagic sign, 73 (14%) of 508 had a consciousness disorder of any grade; 77 (15%) of 504 had oxygen saturation lower than 92%; 153 (31%) of 486 had a haematocrit level lower than 25%; 90 (21%) of 421 had plasma alanine aminotransferase over three times the upper limit of normal; and 171 (42%) of 405 had albuminaemia lower than 28 g/L. Male patients had more frequent acute kidney injury and acute kidney failure and elevated alanine aminotransferase at baseline than did female patients (appendix p 10).

All participants received intravenous ribavirin therapy for a median of 10 days (IQR 9–13; table 2). Ribavirin was started a median of 8 days (7–13) from the onset of symptoms. 92 (18%) participants received oxygen therapy, 158 (31%) had at least one blood transfusion, and 42 (8%) underwent dialysis. Of the 510 participants, 130 still had a positive Lassa virus RT-PCR at day 10, 53 at day 15, 30 at day 20, and 12 at day 25 (appendix p 19). The median time to hospital discharge in patients who survived was 11 days (IQR 10–15).

62 (12%) participants died, including five (6%) of 84 children (four girls and one boy, aged 2 weeks, 4 months, 1 year, 12 years, and 15 years) and 57 (13%) of 426 adults. The median time to death was 3 days (IQR 1–6). Death occurred within 18 days of the onset of symptoms for 48 (81%) of 59 participants with a known date of first symptoms (appendix p 20). Of the 62 deaths, 51 occurred within 7 days of hospital admission, seven between 8 and 19 days, and four more than 20 days after admission (appendix p 21). The four patients who died after 20 days were a 33-year-old man and two women (22 and 45 years) who developed acute kidney failure and underwent dialysis, and a 76-year-old woman with persisting hyponatraemia and severe hepatitis after completing her treatment for Lassa fever (her post-treatment RT-PCR was negative). All but one death occurred in hospital. The participant who died after hospital discharge was a 2-week-old girl at the time of admission. She was discharged at day 12 and died at home 1 week later. Her cause of death was unknown. In univariable analysis, the risk of death increased significantly with increasing age and with the following baseline characteristics: high body-mass index, impaired consciousness, at least one other sign of encephalopathy, low oxygen saturation, accelerated heart rate, watery diarrhoea, at least one sign of bleeding, low platelet count, high leucocyte count, high neutrophil count, high uraemia, high creatinaemia, hypernatraemia, hyperkalaemia, low total carbon dioxide, low albuminaemia, high plasma aspartate aminotransferase or alanine aminotransferase, high plasma bilirubin, high KDIGO grade, high NEWS2 score, low Lassa fever RT-PCR Ct value, and dipstick haematuria (appendix pp 11–15).

We observed no association between mortality and sex, time from first symptoms to hospital admission, time from first symptoms to ribavirin treatment start, a positive malaria test, pregnancy, or any other symptoms or test results (appendix pp 11–15).

In multivariable analysis, age, NEWS2, KDIGO stage, plasma alanine aminotransferase, and RT-PCR Ct value were independently associated with the risk of death (final model, table 3; findings from a sensitivity analysis including patients with missing values are presented in the appendix [p 16]). The probability of death at day 30 was 7.2% for participants younger than 45 years and 24.8% for those aged 45 years or older; 3.5% for participants with baseline NEWS2 of 0–2, 7.7% for those

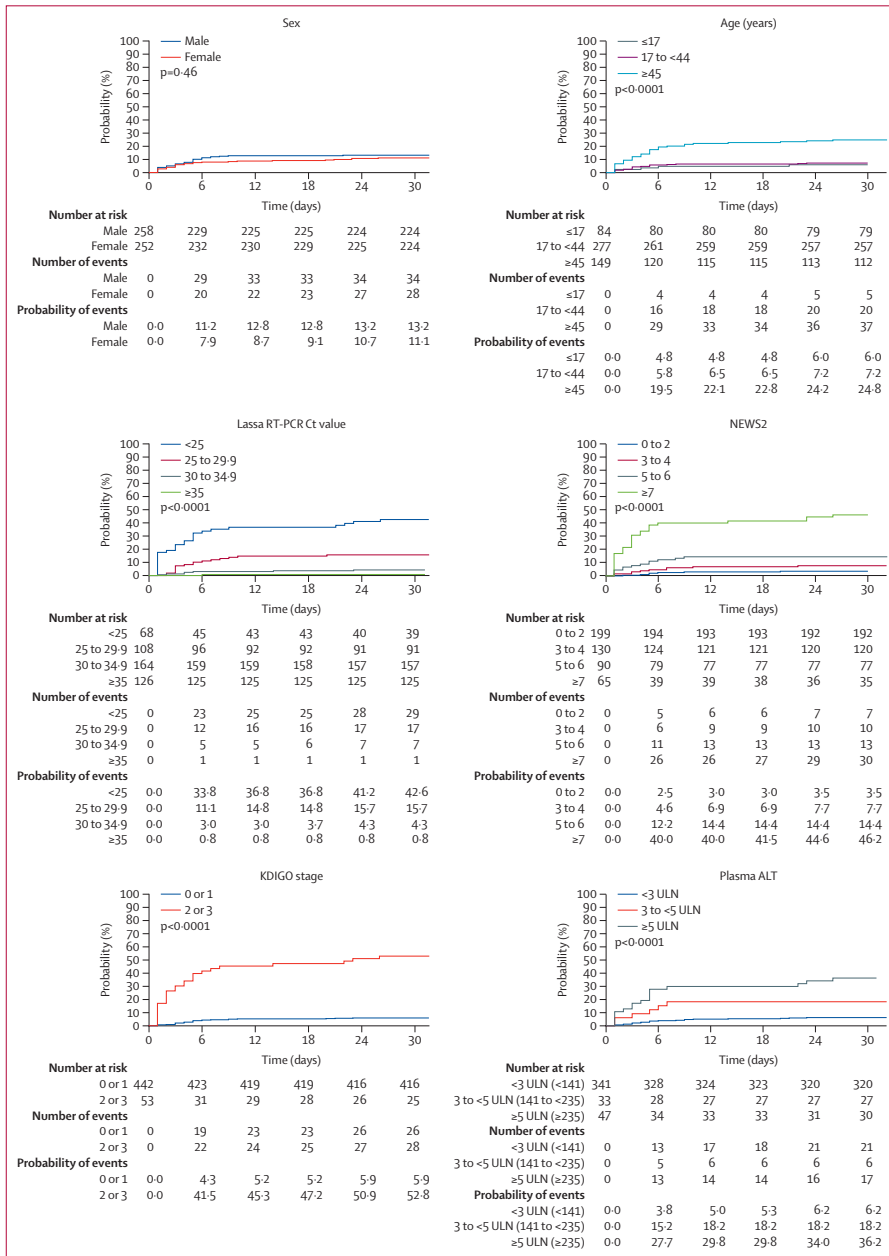


Figure 2: Kaplan-Meier probability of death, according to baseline characteristics
 ALT=alanine aminotransferase. Ct=cycle threshold. KDIGO=Kidney Disease-Improving Global Outcome. NEWS2=National Early Warning Score, second version. ULN=upper limit of normal range.

with NEWS2 3–4, 14.4% for those with NEWS2 5–6, and 46.2% for those with NEWS2 of 7 or higher; 5.9% for participants with a baseline KDIGO stage of 0–1 and 52.8% for those with KDIGO stage of 2–3; 6.2% for participants with baseline plasma alanine aminotransferase values lower than 3.0 times the upper limit of normal, 18.2% for those with values 3.0–4.9 times the upper limit of normal, and 36.2% for those with values of 5.0 or more times the upper limit of normal; and 42.6% for participants with a baseline Ct value lower than 25.0, 15.7% for those with Ct 25.0–29.9, 4.3% for those with Ct 30.0–34.9, and 0.8% for those with Ct 35.0 or higher (figure 2). The distribution of baseline Ct values for Lassa fever RT-PCR by age and baseline NEWS2, KDIGO grade, and plasma alanine aminotransferase value is detailed in the appendix (p 17).

Of the 172 women of childbearing age, seven (4%) reported a recent miscarriage between the onset of symptoms and hospital admission and 17 (10%) were pregnant on admission. Of these 17 ongoing pregnancies, 12 ended while the participant was in hospital and two ended after hospital discharge (5 days and 5 weeks after discharge). The three remaining women were discharged while still pregnant and their pregnancy outcome was still unknown at the time of the analysis. The 14 documented pregnancy outcomes were six spontaneous miscarriages, one intrauterine death, one maternofetal demise, and six livebirths. The maternofetal demise occurred on day 0. All six live newborn babies tested negative for Lassa fever.

Discussion

To our knowledge, this is the first prospective study of patients with acute symptomatic Lassa fever confirmed by RT-PCR. Patients received appropriate care in a specific Lassa fever ward, including the possibility of oxygen therapy, blood transfusion, and dialysis. All received intravenous ribavirin in accordance with national and international guidelines. Our findings provide a comprehensive description of clinical and biological characteristics of Lassa fever and their relationship with mortality. More importantly, they provide prospective estimates from a large population for key parameters that will be useful for designing future therapeutic trials.

In this study, the frequency of haemorrhagic signs was higher than that reported at a national level in Nigeria.¹³ The disease displayed a lower mucosal haemorrhaging pattern, in which macroscopic haematuria, melena, and vaginal bleeding were the most frequent manifestations. Thrombopenia was common and moderate.^{20,21} These findings suggest that haemorrhaging might be driven by platelet dysfunction,^{20,22} endothelial dysfunction,²² and coagulopathy²³ rather than thrombocytopenia. We observed a high frequency of hypoalbuminaemia, which might be due to inflammation, digestive loss, renal loss, or vascular leakage.²³ Kidney dysfunction and its

complications (acidosis, hyperkalaemia, and hyperazotaemia) was a dominant feature and a major risk factor for death. However, mortality in patients with severe kidney dysfunction was lower than previously reported,⁸ possibly because of the early access to free-of-charge dialysis. High plasma aminotransferase levels were frequent.^{6,8,24} This was truer for aspartate aminotransferase than for alanine aminotransferase, suggesting that, beyond hepatic tissular damage,^{21,25} some degree of haemolysis or myolysis might occur. Finally, our data show that Lassa fever greatly influences pregnancy outcomes. Seven (4%) women of childbearing age reported miscarriages between the onset of symptoms and hospitalisation. 17 (10%) women were pregnant on hospital admission and eight (57%) of 14 ongoing pregnancies ended with the death of the baby during the hospital stay.^{19,26}

All participants received ribavirin therapy. The time between first symptoms and administration of ribavirin was not associated with mortality, contrary to a previous report.⁶ The proportion of patients with a haematocrit level below 25% increased from 10% at baseline to 31% over the entire study follow-up. Ribavirin-induced haemolysis might have occurred even where multiple potential causes of anaemia existed in this context, such as inflammation, massive fluid administration, or haemorrhaging. Most deaths occurred within the first few days. This, together with the 9-day delay between first symptoms and hospital admission, suggests that special efforts should be made to promote earlier diagnosis and faster hospitalisation.

When designing future comparative trials, the expected mortality rate in the reference group will be key for calculating the number of participants needed. In this study, 62 (12%) patients with Lassa fever died, including 57 (13%) of 426 adults and five (6%) of 84 children. These numbers are conservative estimates because some patients who died shortly after admission to hospital could not be asked for informed consent and thus were not included in the analysis. However, we suggest that trials comparing new Lassa fever treatments to a standard of care should take no more than 15% as the reference mortality rate. Given such a mortality rate in the reference group of any trial, large numbers of participants would be required to show that a new treatment can decrease mortality. Therefore, future trial investigators might consider using a composite endpoint, combining mortality with a clinically significant indicator of adverse outcome. We suggest that the worsening of the KDIGO stage and the need for dialysis could be a valuable candidate, because acute kidney failure is a cause of death but renal-associated death can be avoided if the standard of care includes dialysis. Because of the strong association between mortality and baseline Ct value, alanine aminotransferase value, NEWS2, and KDIGO stage, future trial investigators might also consider stratifying the randomisation by one or more of these variables.

Although no prespecified universal cutoff exists for Ct values for Lassa fever RT-PCR assays, we believe that all participants in our study were confirmed cases of Lassa fever. The bell-shaped distribution of the observed Ct values is consistent with a cutoff value at 42 in our setting (appendix p 18). Patients with high Ct values had much lower mortality rates than those with low Ct values, in the same order of magnitude as previously reported in patients with Ebola. This suggests that Ct values are a suitable surrogate for viral load and a useful tool in Lassa fever as well as in Ebola, two diseases where viral load is strongly associated with mortality.²⁷

Our study had several limitations. First, the study was not designed to provide new evidence on the benefit–risk ratio of ribavirin, which is still needed. Second, leucocytosis and high neutrophil counts were more frequent in patients who died. However, bacterial cultures were not routinely available. Even if leucocytosis could be due to a stress response in patients with multiorgan failure, it would have been interesting to document the frequency of bacterial morbidity and its potential prognostic value. Third, several other biological variables—such as clotting parameters, inflammatory markers, platelet function, and stigmas of haemolysis or myolysis—were not available, which limits our ability to discuss the pathogenesis underlying some disease features. Finally, we used multivariable logistical regression to analyse the association between mortality and several key characteristics previously shown to be associated with mortality in Ebola virus disease. Our findings are consistent with the hypothesis that there are common clinical and biological features between Ebola and Lassa fever, even if mortality is much higher with Ebola virus disease than with Lassa fever. However, we should point out that odds ratios are not relative risks, and thus the magnitude of the odds ratios we present here does not reflect the respective clinical importance of one variable relative to the others.²⁸ Additionally, we did not find any significant association between mortality and other characteristics such as sex or the time from first symptoms to ribavirin treatment start, but the absence of association in a sample of patients does not prove the absence of association in the population.²⁹

In conclusion, this study provides prospective evidence on Lassa fever characteristics under the best possible standard of care in Nigeria. Future studies should explore whether survival could be improved by upgrading the standard of care and whether ribavirin should continue to be part of this standard. Whether the standard of care includes ribavirin or not, future comparative therapeutic trials should actively promote early diagnosis and consider using a composite outcome and stratifying randomisation by baseline Ct value and a clinical or biological severity score.

Contributors

AD, MJ, APS, PH, XA, OOA, LAA, and DM designed the study. AD, MJ, ICE, BSe, CA, ClG, ATA, MD, SO, BSé, JKV, AA, LAA, and OOA

set up the Lassa ward, enrolled the patients, followed up the patients, and acquired clinical data. MB-C, JS, ND, EO-E, LAA, SD, SG, and ANA set up the Lassa laboratory, did the tests, and acquired biological data. AD, MJ, and DG had access to the raw data and verified and analysed the data. AD, MJ, OOA, XA, and DM interpreted data and drafted the manuscript. All authors revised the manuscript critically for important intellectual content and approved the final version before submission.

Declaration of interests

DM reports grants from the Institut National de la Santé Et de la Recherche Médicale (INSERM)/Research and Action Targeting Emerging Infectious Diseases (REACTing) during the conduct of the study. PH reports grants from the Wellcome Trust and UK Department for International Development during the conduct of the study. SG reports grants from the German Research Foundation and German Government during the conduct of the study. AD reports grants from the French National Research Institute for Sustainable Development during the conduct of the study. All other authors declare no competing interests.

Data sharing

The anonymised individual data and the data dictionary of the LASCOPE study will be made available to other researchers by the coordinating investigator Prof Denis Malvy (denis.malvy@chu-bordeaux.fr) after approval of a methodologically sound proposal and the signing of a data access agreement.

Acknowledgments

We thank all the participants in the LASCOPE cohort study, their relatives, and their caregivers as well as Dr Chikwe Ihekweazu (Nigerian Centre for Disease Control) and Prof Adebola Olayinka (Nigerian Centre for Disease Control & WHO Country Office in Nigeria). This study was funded by INSERM/REACTing, the African Coalition for Epidemic Research, Response and Training consortium (European and Developing Countries Clinical Trials Partnership grant agreement RIA2016E-1612), the University of Oxford, the UK Department for International Development, the Wellcome Trust, the French Ministry of Foreign Affairs, the Agence Nationale de Recherches sur le SIDA et les hépatites virales, and the French National Research Institute for Sustainable Development. This study was done within the framework of CORAL (Clinical and Operational Research Alliance), a research consortium including the INSERM 1219 Bordeaux Public Health Centre (Bordeaux, France), the ANRS-funded PACCI programme (Abidjan, Côte d'Ivoire) and the humanitarian organisation ALIMA (Dakar, Senegal). The board of directors of CORAL are Renaud Becquet (INSERM 1219, methodological co-chair), Susan Shepherd (ALIMA, medical co-chair), Raoul Moh (PACCI), Augustin Augier (ALIMA), Moumouni Kinda (ALIMA), Marie Jaspard (ALIMA and INSERM 1219), Benjamin Séri (PACCI), Claire Levy-Marchal (ALIMA) and Xavier Anglaret (INSERM 1219 and PACCI).

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b) Maladie à virus Ebola

Cohorte observationnelle descriptive en République Démocratique du Congo : étude EVISTA

Au cours de la dixième épidémie de MVE, les équipes de l'ONG ALIMA ont été immédiatement mobilisées au Nord Kivu et en Ituri (Est de la RDC, Région des grands Lacs) pour assurer la prise en charge des patients infectés. Près de 3500 personnes ont été infectées par EBOV au cours de cette épidémie qui représente la plus importante survenant en RDC. C'est donc dans ce contexte purement opérationnel que les médecins intervenant dans la prise en charge des patients à Beni, Katwa, Butembo et Mambassa se sont employés à améliorer la prise en charge clinique avancée, notamment en terme de soins de support, afin de limiter la mortalité des patients (70,71). De plus, c'est au cours de cette épidémie que l'essai thérapeutique PALM a été mis en place et a permis d'évaluer l'efficacité de 3 médicaments spécifiques de la MVE, le REGN-EB3, le MAb 114 et le Remdesivir (46). En parallèle avec cette double prise en charge clinique (symptomatique et spécifique), les équipes de recherche CORAL ont déployé un projet de recherche nommé EVISTA (Ebola Virus STandard de soin), cohorte observationnelle prospective visant à décrire le standard de soin prodigué au cours de cette épidémie et de proposer un score de risque de mortalité à 28 jours basé sur des variables recueillies à l'admission.

Au total, 711 patients ont été inclus dans cette cohorte, parmi lesquels sont 323 sont décédés (45%). 13% avaient 5 ans ou moins, 4,5% (32 femmes) étaient enceintes et 33% rapportaient avoir été vaccinés avant l'hospitalisation. Plus de la moitié des patients inclus (54%) présentaient une RT-PCR NP inférieure ou égale à 22 Ct. Les soins de support reçus étaient : antibiothérapie probabiliste (100%), traitement par inotropes (16%), oxygénothérapie (38%), transfusion de sang total (14%), pose de sonde urinaire (32%) ou gastrique (7%) et pose de cathéter intra-osseux (1%). De plus, au cours de cette épidémie, l'accès au diagnostic de biologie standard (électrolytes, numération sanguine, bilan hépatique et rénal) a permis un suivi plus rapproché des patients et un traitement symptomatique orienté sur les défaillances d'organe identifiées.

Le score de risque PREDS a été construit à l'aide des données recueillies dans la cohorte EVISTA et comporte les variables suivantes (chacune pondérées selon leur association avec la mortalité) : ALAT > 5N (0-3 points), âge ≥ 50 ans (0-1 point), créatinine > 1,3mg/dl (0-3 points), délais entre les premiers symptômes et l'admission < 4 jours (0-1 point), NEWS2 > 4 (0-2 points) et RT-PCR NP ≤ 22Ct (0-3 points). Un score supérieur à 6 à l'admission est associé à une mortalité supérieure à 50%. Le score doit maintenant être testé au cours d'une épidémie de MVE pour validation externe.

En tout état de cause, l'amélioration du niveau des soins de support fournis et l'utilisation de ce genre de score pour effectuer un triage précoce des patients selon leurs caractéristiques à l'admission représentent des outils majeurs pour améliorer la survie des patients atteints de MVE, en parallèle avec l'implémentation des traitements spécifiques.

Ce travail a été soumis à Lancet e-Clinical Medicine (journal du groupe Lancet). Les derniers échanges avant publication sont en cours. Le manuscrit est présenté ci-dessous et les « supplementary data » en annexe 3.

Development of the PREDS score to predict in-hospital mortality of patients with Ebola virus disease under advanced supportive care: results from the EVISTA cohort in the Democratic Republic of the Congo

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Background:

As in-hospital mortality remains high for patients with Ebola virus disease (EVD) despite new treatment options, the role of supportive care is paramount. The aim of the EVISTA cohort was to describe advanced supportive care and to search for determinants of EVD mortality in the Democratic Republic of the Congo (DRC) through the development of PREDS, a simple risk score for predicting in-hospital death.

Methods:

EVISTA is a multicenter prospective cohort recruited during the 10th EVD outbreak in DRC; all consecutive patients admitted across three Ebola Treatment Centers (ETCs) were included in the study. Demographic, clinical, biological, virological, and supportive care data were collected. To develop PREDS, the patients were randomly allocated to either a training set or a validation sample, and a multivariable Cox proportional hazards model was developed to predict EVD 28-day mortality.

Findings:

Between August 1st, 2018 and December 31st, 2019, 711 patients were enrolled in the study. 623 (88%) received investigational treatment, 111 received a vasopressor drug, 101 received a blood transfusion and 250 received oxygen therapy; 15 were managed by cardio-pulmonary ultrasound. Overall, 323 (45%) patients died. The PREDS score, based on ALT, creatinine, NEWS2, RT-PCR, age and symptom duration, ranged from 0 (risk estimate of death = 7.3%) to 13 (risk estimate of death = 99.8%).

Interpretation:

The implementation of advanced supportive care is possible for EVD in emergency settings. PREDS is a simple, accurate tool that could help in orienting early advanced care for at-risk patients.

The Ebola outbreak in DRC that occurred between August 2018 and June 2020 involved 3,470 cases and resulted in 2,287 deaths. It affected three provinces in the east of the country (Ituri, North Kivu and South Kivu).¹

In 2014-2016, the West African outbreak witnessed the development and implementation of a specific Ebola Virus (EBOV) vaccine (r-VSV-ZEBOV, ERVEBO, Merck) for ring vaccination of contacts and contacts of contacts.^{2,3} In addition, there were several interventions in Eastern DRC in 2018-2020 to improve the management of patients infected with EBOV. The MEURI (Monitored Emergency Used for Unregistered Intervention) and PALM trials each evaluated four investigational drugs: Remdesivir (an antiviral); REGN-EB3, Zmapp and MAb114 (monoclonal antibody products).⁴

For most of these treatment strategies, the mortality remains high for patients with high viral load on admission (> 60%).⁴ For severe cases, supportive care still remains an important therapeutic strategy.⁵⁻¹⁰ However, the precise spectrum of supportive care is wide and there is no clear indication as to its application, as it depends on a combination of several complicated actions.¹¹ The spectrum of supportive care therefore requires definition in order to optimize its feasibility in the field.

Early triage of patients also remains of prime importance in reducing mortality rates; understanding which patients are more at risk of death and have the highest chance of being saved is a crucial issue to address. To date, no study has specifically evaluated the individual risk of death from EVD in the field using a large set of contextual data.

Thus, the present study set out to describe the supportive care provided to patients during the 10th Ebola outbreak in the DRC, and to develop an individual death risk score model, herein referred to as PREDS (PRedicting Ebola Death risk Score).

Methods

Design

This work is designed as a care-focused, descriptive, prospective, systematic and longitudinal study.

Participants and settings

Within the context of the operational response to Ebola outbreaks in the DRC, the national Ministry of Health, with the support of the French NGO ALIMA (Alliance for International Medical Action), has established three Ebola Treatment Centers (ETC) in the North Kivu and Ituri provinces, at the Beni, Katwa and Mambassa urban centers. The EVISTA (Ebola Virus STandard of care) cohort was set up for the duration of the outbreak, with the aim of describing the standard of care provided to Ebola patients and the clinical and biological course of the disease. All patients with a positive EBOV RT-PCR test admitted to an ETC during the inclusion period were included in the study.

Care and follow-up

All EVD patients received a standard of care in accordance with the DRC Ministry of Health recommendations and WHO guidelines.¹¹ Clinical evaluation was carried out using a modified version of the NEWS2 (mNEWS2) score without the respiratory rate, for practical reasons.¹²

Advanced supportive care tools were used to improve clinical management. Another innovative measure, the Biosecure Emergency Room (BER), was developed for treating ebola cases in individual spaces within the units.^{5,13,14}

Biological analysis

The diagnostic status for EVD was confirmed by the INRB laboratory (Institut National de Recherche Biomedicale, Kinshasa, DRC) deployed at each ETC using an Altona Gene Expert RT-PCR, with quantitative results for nucleoprotein (NP) cycle threshold (Ct) and glycoprotein (GP) Ct of EBOV. A metabolic analysis was carried out at admission and then based on physician demand using a point-of-care Piccolo blood chemistry analyzer with AmLyte 13 disk (Sysmex), in accordance with the manufacturer's recommendations. We were thus able to measure albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, blood urea nitrogen, calcium, creatine kinase, creatinine, C reactive protein (CRP), glucose, potassium, sodium and total bilirubin for each patient. A Poch-100i blood cell analyzer (Sysmex) was used to measure full blood cell count,

specifically for white blood cells, red blood cells, haematocrit, platelets, lymphocytes, monocytes and neutrophils.

Data collection

Data regarding the demographic and clinical situations, biological parameters and medication received were collected for all patients included, using a standardized case report form (CRF available on request) and entered in a secure database. Data regarding vaccination status on admission (with r-VSV-ZEBOV) and interval between vaccination and admission to ETC were collected based on patient declaration. The source documents used to complete the CRFs were the patient's medical file and the laboratory register.

Statistical analysis

Baseline characteristics, follow-up supportive care, treatment, and observed outcomes were summarized using median and interquartile range (IQR) for continuous variables, and count and percentage for categorical variables. Statistical comparisons were carried out using the Wilcoxon rank-sum test for continuous variables and chi-squared test for categorical variables.

Time-to-event was estimated as the period from date of admission to date of death for the patients who died. Patients who survived the whole study period ceased to be monitored at the end of their follow-up period, i.e. day 28. Univariate Cox proportional hazard regression models were used to assess the association of key baseline characteristics with risk of death for all patients. Hazard ratio (HR) estimates and their corresponding 95% confidence intervals (CI) and p value associated with each baseline characteristic were reported.

A multivariable Cox proportional hazard model was developed to search for determinants of death before day 28 after admission. To develop and validate the PREDS score, patients were randomly allocated to either a training sample (2/3 of the data), used to develop and parametrize the prediction model, or a validation sample (1/3 of the data), used to test out-of-sample prediction model performance. The selection of candidate predictors included in the model was based on the literature, the results from the univariate Cox models, and input from clinical experts. Alternative models based on the least absolute shrinkage and selection operator (LASSO) regression method were also explored. Since prediction performance was similar for the full model (i.e. including all candidate predictors) and the LASSO-selected models, the results reported here are those based on the full model.

The HR estimates, 95% CI and p values associated with each risk factor are reported. Predictive performance was assessed in the validation set by 1) model concordance, measured by the C statistics, and 2) model calibration for risk of death at 28 days, quantified by the Hosmer–Lemeshow goodness-of-fit test. The Breslow estimator of the baseline hazard was combined with the HRs to obtain the predicted risk of death for each patient at 28 days from admission date.

The PREDS was generated based on this multivariate Cox model. PREDS was developed as a simple points-based system to characterize patient risk scores using the methods of Sullivan and D'Agostino.¹⁵ Creatinine was categorized according to a clinically relevant cut-off, and a reference value operationalized as the midpoint was calculated using the 1st and 99th percentile values to minimize the influence of extreme values. A base risk profile was set to correspond to the lowest risk category for each variable. The constant for the points system (number of regression units corresponding to one point) was defined as the increase in risk associated with a 2-unit (mg/dL) increase in creatinine. Factor states associated with a higher risk of death were assigned more points, so a higher points total represented greater risk. The points for each predictor were totaled and a table indicating risk (defined as the probability of dying within 28 days of admission) for each points total was established. To evaluate the robustness of the risk-scoring algorithm, the points system based on the training sample was used to generate risk scores for patients in the validation sample, and the correlation between the points-based risk scores and the Cox model-based risk scores was assessed.

Finally, to evaluate the impact of missing data, a sensitivity analysis was carried out to assess the model's predictive ability in multiple imputed held-out (i.e. validation) datasets. This approach allowed the robustness of the prediction model to be evaluated across a range of imputed datasets to assess how well the model would perform in other datasets with no missing data. Multiple imputation was carried out using the multivariate imputation by chained equations (MICE) approach,¹⁶ which has been the preferred approach for handling missing data based on published simulation studies.¹⁷ We generated 200 validation imputations. Model concordance C

statistics, p-value for the Hosmer–Lemeshow goodness-of-fit test, and correlation coefficient between points-predicted risk and model-predicted risk were obtained for each imputed validation set. The mean values across all 200 validation sets were then calculated and compared with the respective values obtained in the non-imputed (empirical) validation set.

All statistical analyses were performed with the R statistical package version 4.0.3 or later (R Foundation for Statistical Computing, <https://www.R-project.org/>).

Ethical consideration

The consent of patients included in the cohort was (i) extrapolated from their signed consent to participation in the RCT or MEURI studies (the data collected were part of the medical support we had provided for these patients) or (ii) collected through specific consent forms for EVISTA.

The EVISTA protocol was approved by the DRC National Ethics Committee (151/CNES/BN/PMMFI2019) and registered with clinicaltrials.gov (NCT04815175).

Results

Baseline characteristics (table 1 and supplementary table 1)

Between August 1st, 2018 and December 31st, 2019, 711 patients in total were eligible for analysis (figure 1). The mean (\pm SD) age was 28 (\pm 17) years. Patients were admitted at a median of 4 days from onset of symptoms. 14% of patients presented with bleeding and 17% with a neurological disorder on admission.

In terms of vaccination status, 175 (33%) declared they had been vaccinated with the rVSV-ZEBOV-GP vaccine. Of the 134 patients who reported their vaccination date, 93 had been vaccinated 10 days or less before admission (median 7 days, IQR 5-8), and 41 more than 10 days before admission (median 15 days, IQR 12-41).

Biological parameters at baseline showed a low platelet count in 158 patients (54%), a high white blood cell count in 138 patients (46%), and an increased level of creatinine in 271 patients (49%). The CRP value was above 5mg/l for 512 patients (99%). Serum sodium was low ($<$ 130 mmol/l) for 183 patients (33%) and 166 (35%) had an abnormal level of serum potassium. Values for AST and ALT were more than 5 times the upper limit for the normal range in 224 patients (59%) and 262 patients (48%) respectively. The albumin level was low (\leq 35g/l) in 490 patients (88%). In terms of viral load measurement, the EBOV RT-PCR NP Ct value was \leq 22 for 356 patients (54%), while the GP Ct value was \leq 22 cycles for only 83 patients (13%).

Follow-up (supplementary table S1)

Almost all patients (94%) experienced viral-illness-like symptoms such as fatigue (91%), anorexia (70%) and headache (57%). Half of them showed respiratory symptoms and a critical oxygen saturation below 92%. In addition, 86% of patients had digestive symptoms, of which 75% diarrhea and 34% dysphagia. A total of 238 patients (35%) experienced an impaired level of consciousness (CVPU on the ACVPU scale) and 264 (37%) had no neurological symptoms. The most common neurological symptoms were agitation and coma (in 25% and 29% of patients respectively). 103 patients (15%) experienced seizure during follow-up. Overall, 275 patients (39%) had bleeding symptoms of some type, the most common being venous puncture point bleeding, melena, gingival bleeding and hematemesis (20%, 14%, 12% and 11% respectively).

Investigational treatment and patient care (table 2, supplementary tables S6, S7, S8, and supplementary figure S1)

Overall, 623 patients (88%) were given an investigational treatment, 453 (73%) in the context of RCT and 170 (27%) MEURI.

Of the patients who received an investigational therapeutic regimen, their mNEWS2 scores, ALT, creatinine and EBOV viral loads on admission were lower than those for patients who received no investigational drugs (supplementary table S6).

Most patients were managed in the BER from the time of admission, mainly for biosafety reasons. Other supportive interventions are detailed in table 2.

Outcomes

Of the 711 patients involved, 323 (45%) died after a median of 2 days (IQR 1-5) from admission and 9 days (IQR 6-12) from onset of symptoms (table 2). In addition, lethality dropped to 36% and 29% in patients hospitalized for more than 24 hours and 48 hours respectively.

Of the patients who received investigational treatment, the case fatality rate was 39% (supplementary table S6). 79 (91%) of the 87 patients who received no specific treatment died, and of these 79, 64 (81%) died within the first 24 hours of admission (supplementary figure S1).

A total of 32 pregnant women and 85 children aged 5 or under were included in the study. Details of the outcomes for these specific groups are given in figure 2 and supplementary table S4 respectively.

A total of 175 patients who declared they were already vaccinated with the rVSV-ZEBOV vaccine had less severe symptoms on admission and a lower mortality rate (30% vs 49%) (supplementary table S3).

PREDS: risk score for death at 28 days (table 3, 4, 5, annex 2)

The training sample to develop the prediction model comprised 279 patients randomly selected. Six variables were included in the prediction model as described in the Methods section: ALT, creatinine, mNEWS2, EBOV RT-PCR, age, and time from 1st symptom to admission. Table 3 shows the HR for the candidate risk factors corresponding to the univariable and multivariable Cox models in the training sample. The crude and adjusted HRs are qualitatively similar. The adjusted HRs in the multivariable model indicate that all the candidate risk factors were associated with an increase in the risk of death, particularly ALT > 5N (HR=2.48), mNEWS2 score > 4 (HR=2.31) and EBOV RT-PCR NP Ct value ≤ 22 (HR=2.56). A smaller but significant association was also found for increases in creatinine (HR = 1.19). The predictive performance of the multivariable Cox model showed good concordance (C = 86.24%) and calibration with the Hosmer- Lemeshow test (p = 0.12) for risk of death at 28 days in the validation sample (supplementary figure S4).

The PREDS score was developed by attaching points according to the HR of the multivariable Cox model (table 4 and annex 2). The total risk score for death at 28 days varied from 0 (risk estimate = 1.9%) to 13 (risk estimate = 80.8%) (table 5).

The correlation between points-based risk scores and the Cox model-based risk scores in the validation sample (N = 140) was 93.8%.

The sensitivity analyses indicated that the model had a similar predictive value across the 200 multiple imputed held-out datasets. The mean C statistic was 82.2%, compared to 86.2% in the empirical validation set. The mean p-value for the Hosmer–Lemeshow goodness-of-fit test was 0.09, while the corresponding value in the empirical dataset was 0.12. Finally, the mean correlation coefficient between points-predicted risk and model-predicted risk was 92.7%, compared to 93.8% in the empirical dataset.

Discussion

This study, characterizing more than 700 consecutive EVD patients represents to our knowledge the largest prospective observational cohort for EVD.

The initial clinical presentation of these patients was similar to that described during the 2014-2016 Ebola outbreak in West Africa.^{14,18–22} Likewise, hyponatremia, renal dysfunction, thrombocytopenia, dyskaliemia and hepatic failure are factors previously described in EVD.^{21,23,24} We also recognized that acute renal failure, high viral load, and a high ALT level were indicators of a fatal outcome.^{14,23,24}

The overall mortality rate for our cohort was 45% - higher than that reported in the PALM trial in which patients with severe symptoms on admission were not included.⁴

Symptomatic treatment has improved since the West African epidemic.^{9,25} Innovative options such as admission and management in BER, cardiopulmonary ultrasound to guide vascular filling, monitoring of biological parameters allowing the adaptation of electrolyte compensation and oxygen therapy are now common procedures in the field.^{5,13} Some patients have even benefited from the placement of a central venous or bone catheter, or treatment with a vasopressive drug to manage cardio-circulatory shock. There have been strong calls for improvements in care standard of care by caregivers,^{6–10} including the development of specific treatments. These latter treatments only have a partial impact on fatality rates in severe patients on admission,^{4,26} which is

largely supported by the experience of EVD management in northern countries with high levels of supportive care and showing mortality rates of less than 20%, even without specific treatments.²⁷ The optimization of supportive care should therefore always involve three elements: (i) very close monitoring of the patient's hydration level (vital constant, electrolytes and possibly cardiopulmonary ultrasound), (ii) early access to oxygen therapy, antibiotics and blood transfusion and (iii) the possibility of advanced resuscitation care such as central catheter placement, dialysis or mechanical ventilation, which could lower fatality rates. The availability of innovative measures such as BERs for severe patients could also help with close monitoring while maintaining bio-medical safety for caregivers.^{5,13}

However, recourse to these advanced supportive care measures, even where they are available, remains limited in poor-resource settings. In this context, patient triage is of prime importance. The NEWS2 score on admission and for clinical follow-up appears to be a useful marker of severity that is compatible with operational constraints; however, it omits important risk factor variables that have been included in the PREDS scoring model developed here, so the PREDS model represents an important element of this study.

One previously-published study attempted to generate a scoring system for evaluation of death, nonetheless it did not include central biological and virological parameters.²⁸ PREDS, on the other hand, does include those biological and clinical data, which might help early distinction of patients requiring immediate advanced supportive care from those at a lower risk of negative outcome. In the framework of our study, we propose with the aim to improve survival rates, that patients who score 6 or more (a 45% risk or higher of 28-day mortality) should benefit immediately from innovative advanced care. This aggressive strategy should be tested with further cohorts to confirm its general applicability and accuracy.

This study has a few limitations. First, due to its observational nature, it did not allow the effects of advanced supportive care to be distinguished from those of specific investigational treatments on mortality rates. Second, PREDS was validated in a specific setting and requires external validation in future epidemics in the field. To address missing data, we conducted a sensitivity analysis using multiple imputed validation datasets to test the external validity of the prediction model and subsequent risk-scoring system, which indicated that the model performed well on these multiple imputed held-out datasets. Finally, our data relate not to the last outbreak of all but to the last major outbreak, but the vaccine, treatments and standards of care remain unchanged.

In conclusion, despite the development of specific treatments for EVD, mortality remains high, especially in patients who present with critical symptoms on admission to the ETC. Improving the availability of advanced supportive care is therefore essential as a first step in strategic management of the disease. In addition, early triage after admission at the ETC, aimed at identifying patients at risk of death, is key to managing strategies. Considering the trends in EVD cases in recent outbreaks, it is likely that interventions will be carried out at multiple small ETCs quickly set up near to where cases of the disease develop, and that PREDS will be useful in this regard for identifying severe cases at these small intervention units.

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Figure

Figure 1: inclusion flowchart

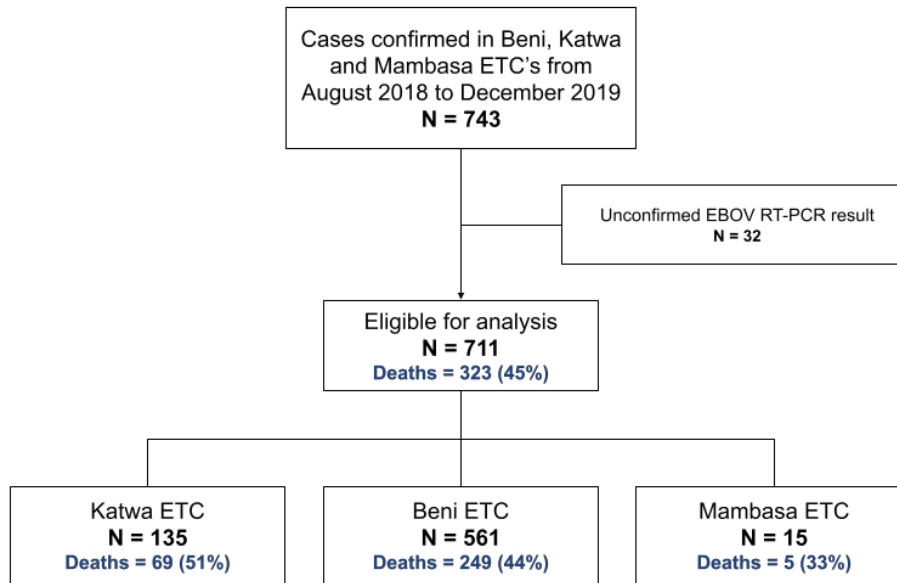
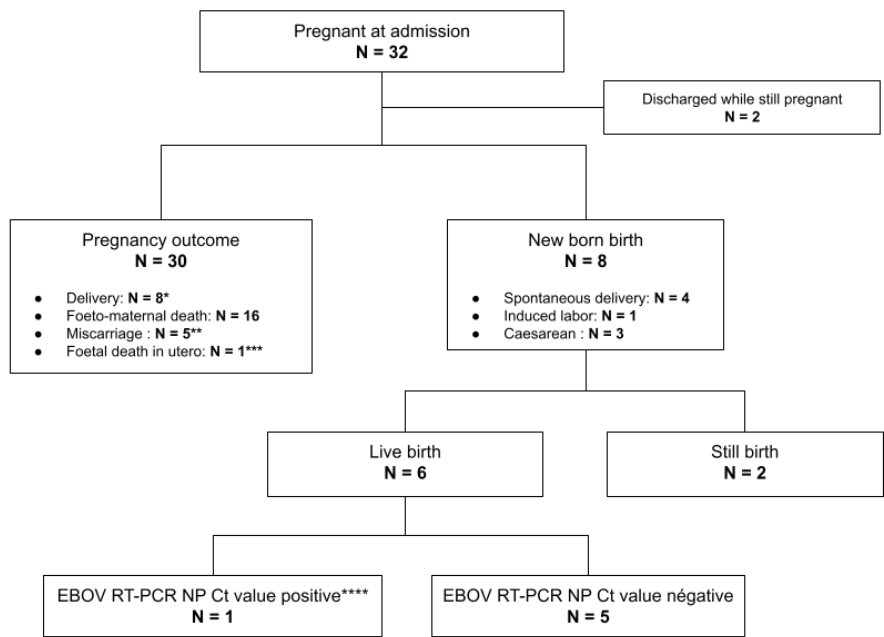


Figure 2: pregnancy flowchart



* Two mother died 24 and 48h after
 ** One mother died 5 days after
 *** One mother died 24h after
 **** Recovered

EVISTA – Tables

Table 1. Baseline characteristics

	Number (N=711)	n
Age, n (%)		676
≤ 5 yr	85 (13%)	
≤ 28 days	6 (1%)	
6 to 18 yr	101 (15%)	
19 to 49 yr	406 (60%)	
≥ 50 yr	84 (12%)	
Sex, n (%)		711
Male	302 (42%)	
Female	409 (58%)	
Positive result on pregnancy test, n (%)	32 (8%)	
Vaccination		
Patient-reported vaccination with rVSV, n (%)	175 (33%)	533
Time from vaccination to admission (days), median [IQR]	8 [5;11]	134
> 10 days before admission, n (%)	41 (31%)	
≤ 10 days before admission, n (%)	93 (69%)	
Time from 1st symptom to admission (days), median [IQR]	4 [2;7]	697
Vital signs, n (%)		
Respiratory rate ≥ 24 breaths per minute	328 (51%)	641
Heart rate ≥ 110 per minute	207 (31%)	666
Diastolic Arterial Pressure < 60 mmHg	134 (23%)	579
Systolic Arterial Pressure < 90 mmHg	89 (15%)	579
SpO ₂ < 92%	63 (10%)	602
Mean Arterial Pressure < 65 mmHg ⁽¹⁾	49 (8%)	579
Symptoms, n (%)		
Any type of bleeding symptom ⁽²⁾	123 (17%)	711
Any type of neurologic symptom ⁽³⁾	99 (14%)	711
Level of consciousness, n (%)		
ACPVU classification: ⁽⁴⁾		638
Alert	577 (90%)	
Impaired	61 (10%)	
mNEWS2 score, n (%) ⁽⁵⁾		496
Total score ≤ 4	357 (72%)	
Total score > 4	139 (28%)	
Hematology, median [IQR]		
Hemoglobin — g/dl	14 [12;15]	301
Hematocrit — %	40 [36;45]	298

Platelets — G/l	145 [97;222]	294
Leucocytes — G/l	10 [5;21]	300
Lymphocytes — G/l	3 [1;6]	232
Neutrophils — G/l	5 [3;12]	224
Monocytes — G/l	0.4 [0.2;1.0]	222
Renal function, median [IQR]		
Urea — mg/dl	18 [9;48]	553
Creatinine — mg/dl	1.1 [0.7;3.6]	548
Electrolytes and Biochemistry, median [IQR]		
CRP — mg/l	39 [11;109]	517
CPK — U/liter	674 [267;2116]	519
Sodium — mmol/l	131 [128;134]	551
Potassium — mmol/l	4.2 [3.7;4.8]	469
Amylase — U/liter	106 [72;177]	534
Corrected calcemia— mmol/l ⁽⁶⁾	2.3 [2.2;2.5]	554
Albumine — g/l	28 [23;33]	556
Glycemia — mg/dl	97 [79;121]	556
Liver function, median [IQR]		
Bilirubin — mg/dl	0.6 [0.5;1.1]	511
ALT — U/liter	212 [60;589]	544
AST — U/liter	292 [93;1018]	378
Virology, n (%)		
EBOV RT-PCR NP Ct value ≤ 22	356 (54%)	655
EBOV RT-PCR GP Ct value ≤ 22	83 (13%)	644

Footnotes

⁽¹⁾ Mean Arterial pressure = 2/3 Diastolic Arterial Pressure + 1/3 Systolic Arterial Pressure

⁽²⁾ Epistaxis, hematemesis, hematuria, hemoptysis, melena, purpura, conjunctival bleeding, gingival bleeding, venous puncture point bleeding

⁽³⁾ Headache, focal neurological deficit, disorientation/confusion, agitation, convulsions, coma/consciousness disorder, photophobia.

⁽⁴⁾ ACVPU classification: A: Alert; C: new confusion; V: responsive to voice; P: responsive to pain, U: Unresponsive

⁽⁵⁾ NEWS2 score: National Early Warning Score 2nd version; mNEWS2: modified NEWS2 score without respiratory rate

⁽⁶⁾ Corrected calcemia = measured calcemia – 0.025*(albuminemia – 40); measured calcemia in mmol/L, albuminemia in g/L

Table 2. Care, treatment and outcome

	Number (N=711)	n
Supportive care		
Antibiotics, n (%) ⁽¹⁾	636 (100%)	638
Duration (days), median [IQR]	8 [4;14]	
Inotropes, n (%) ⁽²⁾	111 (16%)	711
Received 1 dose	69 (62%)	
Received 2 doses or more	42 (38%)	
Oxygen therapy, n (%)	250 (38%)	661
Duration (days), median [IQR]	2 [1;3]	188
Highest flow, n (%)		218
< 10 l/min	154 (71%)	
≥ 10 l/min	64 (29%)	
Transfusion, n (%)	101 (14%)	711
Volume (ml), median [IQR]	450 [250;525]	99
Urinary catheter, n (%)	199 (32%)	628
Feeding tubes, n (%)	45 (7%)	654
UltraSound, n (%)	15 (2%)	711
Intraosseus catheter, n (%)	7 (1%)	659
Hospitalized in cube, n (%)	560 (85%)	656
Investigational treatment		
Investigational treatment received, n (%)	623 (88%)	710
Program, n (%)		
MEURI ⁽³⁾	170 (27%)	
PALM RCT ⁽⁴⁾	453 (73%)	
Drugs, n (%)		
MAB114	197 (32%)	
Regeneron	191 (31%)	
Remdesivir	122 (20%)	
Zmapp	107 (17%)	
Outcome		
Dead, n (%)	323 (45%)	711
Time from admission to death (days), median [IQR]	2 [1;5]	
Alive, n (%)	388 (55%)	711
Time from admission to discharge (days), median [IQR]	17 [14;22]	

Footnotes⁽¹⁾ Ceftriaxone, cefixim, metronidazole, gentamycin, amoxicillin, ciprofloxacin, cloxacillin⁽²⁾ Adrenaline, noradrenaline⁽³⁾ Consultation on Monitored Emergency Use of. Unregistered and Investigational Interventions⁽⁴⁾ Mulangu et al, NEJM, 2019

Table 3. Hazard ratios for the candidate risk factors for the univariable and multivariable Cox models

	Univariable			Multivariable		
	cHR	95% CI	P value	aHR	95% CI	P value
ALT > 5N (U/L)	6.12	(3.95, 9.46)	0.0000 *	2.48	(1.46, 4.23)	0.0008 *
Creatinine (mg/dL)	1.27	(1.20, 1.34)	0.0000 *	1.19	(1.12, 1.26)	0.0000 *
mNEWS2 score > 4	3.75	(2.54, 5.54)	0.0000 *	2.31	(1.54, 3.48)	0.0001 *
EBOV RT-PCR NP Ct value ≤ 22	4.78	(3.17, 7.21)	0.0000 *	2.56	(1.56, 4.19)	0.0002 *
Age ≥ 50 years	1.20	(0.71, 2.01)	0.4946	1.5	(0.89, 2.54)	0.1281
Time from 1st symptom to admission (days) ≥ 4	1.90	(1.29, 2.79)	0.0012 *	1.44	(0.97, 2.16)	0.0733

Footnotes

CI: confidence interval

cHR: crude Hazard Ratio

aHR: adjusted Hazard Ratio: Hazard Ratios for each variable shown in the table is adjusted on the other variables shown in the table

Table 4. Points associated with each of the categories of the PREDS score

	Categories	Points
ALT > 5N (U/L)	No	0
	Yes	3
Age (years)	< 50	0
	≥ 50	1
Creatinine (mg/dL)	≤ 1.3	0
	> 1.3	3
Time from 1st symptom to admission (days)	< 4	0
	≥ 4	1
mNEWS2 score > 4	No	0
	Yes	2
EBOV RT-PCR NP Ct value ≤ 22	No	0
	Yes	3

Table 5. Risks associated with point totals of the PREDS score

Point total	Risk estimate
0	0.073224
1	0.101521
2	0.139898
3	0.191161
4	0.258192
5	0.343248
6	0.446721
7	0.565364
8	0.690567
9	0.808205
10	0.902185
11	0.962092
12	0.990019
13	0.998475

Comme exposé dans le chapitre précédent, nous avons pu mettre en place une étude de cohorte descriptive en parallèle avec l'essai thérapeutique PALM, visant à évaluer l'efficacité des traitements spécifiques chez les patients infectés par EBOV. La mise en place de cette cohorte a justifié une mission de supervision en RDC (Nord Kivu, a Béni, Katwa et Butembo) en août 2019. Au cours de cette mission, une nouvelle question de recherche a été mise en évidence, au fil des discussions avec les équipes de terrain. En effet, avec l'administration des traitements spécifiques de la MVE aux patients infectés dans le cadre de l'essai PALM, les soignants ont rapidement eu la volonté d'administrer les mêmes traitements pour les contacts à haut risque d'infection : les personnels soignants ayant eu un accident d'exposition, les nouveau-nés de mères infectées, les proches prodiguant des soins rapprochés aux malades. L'institut National de Recherche Biomédicale (INRB) a alors rendu possible l'administration du REGN-EB3 ou du MAb114 pour les contacts à haut risque, et les équipes ont pu administrer ces médicaments dans ce contexte. Cet usage compassionnel a fait l'objet d'une publication conjointe avec l'INRB, présentée ci-dessous.

Ainsi, pendant la 10^{ème} épidémie de MVE au Nord Kivu (RDC), les équipes opérationnelles d'ALIMA, conjointement avec les équipes de l'INRB ont donc administré l'un ou l'autre des mAbs validés comme candidats dans le cadre de l'essai thérapeutique PALM (Mab114 ou REGN-EB3) à 23 contacts à risque élevé ou intermédiaire de MVE définis selon la classification du risque établi par l'INRB. Ces participants n'avaient jamais reçu de vaccination contre la MVE. Parmi ces individus, aucun n'a développé de MVE ou n'a présenté de RT-PCR positive au cours du suivi qui était de 14 jours.

Cette étude pilote suggère donc que l'administration de mAbs chez les contacts à haut risque pourrait être utilisée en prophylaxie post exposition, et permettre de limiter la propagation de l'épidémie.



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Short Communication

Post-exposure prophylaxis following high-risk contact with Ebola virus, using immunotherapies with monoclonal antibodies, in the eastern Democratic Republic of the Congo: an emergency use program



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ARTICLE INFO

Article history:

Received 23 August 2021

Revised 20 September 2021

Accepted 22 September 2021

Keywords:

Ebola virus disease
post-exposure prophylaxis
monoclonal antibodies

ABSTRACT

Introduction: With the development of therapeutics and vaccine against Ebola virus disease (EVD), the question of post-exposure prophylaxis for high-risk contact has emerged. Immunotherapies (monoclonal antibodies [mAbs]) recently validated for treating infected patients appear to be a good candidate for protecting contacts.

Design: During the tenth EVD outbreak in the Democratic Republic of the Congo, we have administrated mAbs (Mab114 or REGN-EB3) to high and intermediate-risk contacts of EVD patients.

Results: Overall, 23 non-vaccinated contacts received mAbs after a median delay between contact and post-exposure prophylaxis of 1 day (interquartile range 1–2). All contacts were free of symptoms, and all had negative reverse transcriptase-polymerase chain reaction 14 days after the contact.

Conclusion: Immunotherapies appear to be promising candidates to protect EVD contacts. Interaction with vaccine needs to be analyzed and a larger study on efficacy conducted.

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Introduction

Over the past decade, there have been significant advances in the management of Ebola virus disease (EVD), including the development of a vaccine (VSV-ZEBOV-GP, ERVEBO, Merck, USA) indicated for ring vaccination of contacts (Henao-Restrepo et al., 2017), and two immunotherapies (MAB114 and REGN-EB3) effective against mortality in patients infected with Ebola virus (EBOV) (Mulangu et al., 2019). Consequently, new strategic and operational

issues have also emerged, including protection of high-risk contacts, defined as post-exposure prophylaxis (PEP).

Vaccination with ERVEBO is beneficial for breaking the transmission chains for EVD but has no place in PEP because antibody production occurs after 10 days (Fischer et al., 2018; Iversen et al., 2020). Monoclonal antibodies (mAbs) represent a good therapeutic option to be evaluated in PEP, as they effectively reduce mortality when administered early in the course of the disease and are easy to administer as they require only one intravenous injection.

Design

During the tenth EVD outbreak in the Democratic Republic of the Congo, national response teams and caregivers of the non-governmental organization The Alliance for International Medical Action administered MAB114 or REGN-E3B on an emergency-use basis for high-risk contact (direct contact with skin barrier breach with a confirmed EVD patient) and intermediate-risk contact (di-

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<https://doi.org/10.1016/j.ijid.2021.09.053>

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rect contact without skin barrier breach with an EVD patient), according to the WHO classification.

Results

Between 1 July 2019 and 31 January 2020, teams at the Ebola treatment centers in Beni, Katwa and Mambassa administered MAb114 (N = 21) or REGN-E3B (N = 2) to 23 individuals. These individuals had never received any Ebola vaccine. The median age of the participants was 30 years (interquartile range (IQR) 20–43) and 14 (61%) were female. Among them, 8 (35%) were children <10 years old, 2 of whom were treated at birth (in utero exposure from EBOV-infected mothers). Only 4 participants were health care workers. The contact was high-risk for 18 (78%) individuals and intermediate-risk for 5 (see table). The median time from contact to the administration of mAb was 1 day (IQR 1–2). The participants were monitored until day 14 by reverse transcriptase-polymerase chain reaction (RT-PCR) and on-demand clinical follow-up. No participants developed symptoms of EVD and all RT-PCR tests were negative.

Discussion

The secondary attack rate of EVD in the literature ranges from 37% for direct contact to 80% for handling dead bodies (Bower et al., 2016). The use of mAbs in PEP has been shown to be effective in mice and non-human primates (Brannan et al., 2019; Froude et al., 2018). In addition, 4 health care workers have previously been treated with a combination of mAbs and the antiviral favipiravir after intermediate or high-risk exposure; none of them developed EVD (Jacobs et al., 2015).

However, certain points need to be discussed. First, the World Health Organization's definition of contact cases does not mention the source patient's clinical status. It seems likely that contact with a patient with 'wet' Ebola (secretions, vomiting/diarrhea or external bleeding) is more likely to result in contamination than contact with a 'dry' Ebola case. In addition, the notion of skin barrier breach, which classifies contact as high-risk, is not always easy to identify in the field, particularly when it occurs despite personal protective equipment. Therefore, we propose that PEP be offered to individuals who have had direct contact (with or without skin barrier breach) with a confirmed case of 'wet' Ebola. Second, mAbs do not provide sustained immunity and vaccination is subsequently mandatory. However, ERVEBO and mAbs share the same viral target, the envelope glycoprotein (GP) (Cagigi et al., 2018). Therefore, it is likely that the vaccine will be inhibited by mAbs, especially when administered concomitantly (Fischer et al., 2018). Third, the overall protection strategy (PEP and vaccination) needs to be assessed in terms of feasibility. If the PEP alternative is more effective when administered soon after contact, as in our study, any strategy that leads to a delay, e.g., a few days after vaccination as proposed by Cross et al. (Cross et al., 2020), might decrease the efficacy and therefore render it an unsatisfactory operational option. On the other hand, both the minimum time between mAb and vaccine and the maximum time between contact and PEP administration remain to be defined.

Conclusion

In conclusion, the use of mAbs in PEP represents an attractive option in the protection of those who have had contact with an EVD patient; however, phase 2 and phase 3 studies to validate the effectiveness of this strategy are required, and the issues of feasibility and mAb-vaccine interaction need to be addressed.

Author contribution statement

Marie Jaspard (MJ), Sylvain Juchet (SJ), Beatrice Serra (BS), Denis Malvy (DM) designed the study. MJ, SJ, BS, Baweve Mayoum (BM), Issa Malam Kanta (IMK), Seto Camara (SC), Placide Mbala (PM) and Richard Kojan (RK) set up the study, enrolled and followed the patients and recorded clinical data.

MJ, SJ, DM had access to the raw data and performed the analysis.

MJ, SJ, DM drafted the manuscript.

All authors revised the manuscript critically for important intellectual content and approved the final version before submission.

Conflict of interest

All authors declare no conflict of interest

Role of the funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical considerations

The use of mAbs for Ebola virus disease patients was approved by the National Ethics Committee in the Democratic Republic of Congo within the PALM trial (University of Kinshasa). Each administration of mAbs in this pilot post-exposure prophylaxis emergency use was approved by the Ebola response committee led INRB (institute de recherche biomédicale), Kinshasa.

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c) Covid-19

Avec l'arrivée de la pandémie de Covid-19 au début de l'année 2020, il a été évident que la documentation de la maladie spécifiquement sur le continent africain devenait une priorité de recherche pour les équipes de CORAL. De plus, les équipes d'ALIMA ont décidé d'intervenir dans les pays où elles étaient déjà présentes, pour assister les équipes locales dans la prise en charge des patients infectés par le SARS-CoV-2. C'est ainsi que le projet de cohorte prospective descriptive des patients infectés par le SARS-CoV-2 a été mis en place, greffé sur la prise en charge opérationnelle des patients par ALIMA. Le projet COVISTA (COVID-19 STANDARD de soins) a été déployé au Burkina Faso et en Guinée et a permis d'inclure les patients Covid symptomatiques admis dans 3 centres de prise en charge Covid entre mars et novembre 2020.

Au total, 1805 patients symptomatiques et hospitalisés ont été inclus dans cette étude. Parmi eux, 63% étaient des hommes et 22% avaient 60 ans ou plus. 12% étaient diabétiques, 21% hypertendus et 18% avaient un IMC (indice de masse corporelle) supérieur ou égal à 30. Les signes d'atteinte respiratoire haute étaient présents chez 24% des patients, 61% présentaient une atteinte respiratoire basse et 15% avaient une saturation en oxygène inférieure à 94% à l'admission. Selon la classification de l'OMS, 48% étaient non sévères, 42% avaient une sévérité modérée et 10% une présentation clinique sévère à leur arrivée à l'hôpital. La quasi-totalité (95%) des patients ont reçu un traitement spécifique comme recommandé à cette date par les autorités sanitaires du pays, à savoir l'hydroxychloroquine et/ou l'azithromycine et/ou le lopinavir. 86 patients (5%) sont finalement décédés après une médiane de 5 jours d'hospitalisation. Les facteurs significativement associés à la mortalité et à l'aggravation clinique étaient l'âge supérieur ou égal à 60 ans, le sexe masculin et l'hypertension artérielle. Le diabète était uniquement associé à l'aggravation clinique.

Cette étude a permis de démontrer la similarité de la présentation clinique et des facteurs de risques associés à la mortalité et à l'aggravation clinique avec les patients des pays du nord. L'interprétation du taux de mortalité et sa comparaison avec celle des autres pays est cependant difficile, car le dépistage est encore peu accessible dans ces pays, et l'estimation du nombre de cas totaux est probablement sous-évaluée.



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Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijidINTERNATIONAL
SOCIETY
FOR INFECTIOUS
DISEASES

Clinical presentation, outcomes and factors associated with mortality: A prospective study from three COVID-19 referral care centres in West Africa



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ARTICLE INFO

Article history:

Received 30 March 2021

Received in revised form 7 May 2021

Accepted 11 May 2021

Keywords:

SARS-CoV-2

COVID-19

Sub-Saharan Africa

Mortality

Comorbidities

ABSTRACT

Objectives: The overall death toll from COVID-19 in Africa is reported to be low but there is little individual-level evidence on the severity of the disease. This study examined the clinical spectrum and outcome of patients monitored in COVID-19 care centres (CCCs) in two West-African countries.

Methods: Burkina Faso and Guinea set up referral CCCs to hospitalise all symptomatic SARS-CoV-2 carriers, regardless of the severity of their symptoms. Data collected from hospitalised patients by November 2020 are presented.

Result: A total of 1,805 patients (64% men, median age 41 years) were admitted with COVID-19. Symptoms lasted for a median of 7 days (IQR 4–11). During hospitalisation, 443 (25%) had a SpO₂ < 94% at least once, 237 (13%) received oxygen and 266 (15%) took corticosteroids. Mortality was 5% overall, and 1%, 5% and 14% in patients aged <40, 40–59 and ≥60 years, respectively. In multivariable analysis, the risk of death was higher in men (aOR 2.0, 95% CI 1.1; 3.6), people aged ≥60 years (aOR 2.9, 95% CI 1.7; 4.8) and those with chronic hypertension (aOR 2.1, 95% CI 1.2; 3.4).

Conclusion: COVID-19 is as severe in Africa as elsewhere, and there must be more vigilance for common risk factors such as older age and hypertension.

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<https://doi.org/10.1016/j.ijid.2021.05.024>

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Introduction

As of 05 May 2021, it was estimated that 153 million people had been infected with SARS-CoV-2 and that COVID-19 had killed 3.2 million people worldwide. Africa accounted for 3% of deaths from COVID-19, although 17% of the world's population lives there. Europe (10% of world population, 34% of deaths from COVID-19) and North America (4% of world population, 19% of deaths from COVID-19) have been impacted comparatively much higher ([WHO Coronavirus \(COVID-19\) Dashboard, n.d.](#)). Therefore, the death toll from COVID-19 seemed lower in Africa than in Europe and North America, although some predictions indicated the opposite ([Martinez-Alvarez et al., 2020](#)). This may have been due to the heterogeneity of systems for reporting cases and causes of death, the level of preparedness in a continent that has faced threatening epidemics in the recent past, or differences in environmental characteristics, circulating strains or population susceptibility ([Galloway et al., 2020](#); [Nagai et al., 2020](#); [Price-Haywood et al., 2020](#); [Williamson et al., 2020](#)). To study this latter point, it would be useful to verify whether the clinical presentation, mortality and factors associated with COVID-19 are similar in Africa to those described in other continents. There have been a few retrospective population or hospital-based cohorts reporting mortality rates from COVID-19 in Africa so far, but no cohorts that provide individual-level prospective evidence on the severity of the disease ([Abdela et al., 2020](#); [Bouille et al., 2020](#); [El Aidaoui et al., 2020](#); [Elimian et al., 2020](#); [Kirenga et al., 2020](#); [Mekolo et al., 2021](#); [Nachega et al., 2020](#)). This study reports prospectively collected clinical data on patients hospitalised in the COVID-19 referral care centres of two West African countries between March and November 2020.

Methods

Participants, settings and follow-up

Burkina Faso and Guinea, two West African countries, decided at the start of the COVID-19 pandemic to accommodate everyone detected as carriers of SARS-CoV-2 in specific centres, whether asymptomatic or symptomatic, and in the latter case regardless of the intensity of their symptoms. The Ministries of Health in the two countries set up the COVID-19 referral care centres (CCCs) in collaboration with the non-governmental association ALIMA (The Alliance for International Medical Action). In Guinea, the CCC was opened at the Donka hospital in Conakry. In Burkina Faso, two CCCs were opened at the Tengandogo and Clinique Princesse Sarah hospitals, in Ouagadougou. This study describes the data collected from all people hospitalised with symptomatic COVID-19 and a positive RT-PCR SARS-CoV-2 test in each of these three centres. Those placed in isolation at the centres and asymptomatic were excluded from this study. The study period was between 01 April and 04 November 2020 in Guinea; and between 01 March and 12 November 2020 in Burkina Faso.

Care and follow-up

All people received basic care for COVID-19, as defined in the recommendations of the ministries of health of the two countries. No additional medical action or treatment was introduced for participants in this study. Basic care was similar at the three centres, including: (i) anti-infective treatment considered to have an anti-SARS-CoV-2 effect and recommended by the national authorities; (ii) oxygen therapy via nasal probe or mask, in the event of an SpO₂ of <90%; and (iii) the possibility of corticosteroid therapy (methylprednisolone 120 mg/d or dexamethasone 6 mg/d

for 3 days then decreased over a total of 10 days), anticoagulant treatment (enoxaparin 100 IU/kg twice a day) and broad-spectrum antibiotic therapy, of variable indication depending on the period and treating physician's decision.

Throughout the study, the anti-SARS-CoV-2 treatment recommended in the two countries consisted of dual therapy combining 5 days of azithromycin (500 mg at day 1 and then 250 mg/day) with 10 days of hydroxychloroquine (200 mg x tds). In Guinea, physicians had the choice of alternately using either hydroxychloroquine as monotherapy, lopinavir/ritonavir (200/50 mg bd for 10 days) as monotherapy or lopinavir/ritonavir in combination with hydroxychloroquine and azithromycin.

Each of the three centres had an intensive care unit, allowing patients on oxygen to be closely monitored and non-invasive or mechanical ventilation to be used if required.

Data

Data were recorded using the WHO COVID-19 rapid core case report form (CRF) ([Global COVID-19 clinical platform: rapid core case report form \(CRF\), n.d.](#)). In the context of the three urgently-established CCCs, the data were first collected on hard copy CRF and subsequently entered into an electronic database. The variables considered essential for the analysis were then monitored and the source data verified. Only these variables are presented in this article. The variables were: patient verification (exhaustivity of the patient list); sex; age; comorbidities; date of first symptoms; signs, symptoms, measurement of pulse oximetry (SpO₂) at baseline and during follow-up; oxygen therapy, anti-infectious treatments, corticosteroid therapy; and vital status at the end of follow-up. Radiological data, and biological data other than the result of the initial SARS-CoV-2 test were not monitored.

Statistical analysis

The follow-up time was the time between admission to hospital and death or discharge alive from hospital. There was no post-discharge follow-up. The primary endpoint was in-hospital death. The secondary endpoint was COVID-19 clinical worsening, defined as a combination of an SpO₂ < 94% at least once during follow-up (including at baseline), use of oxygen therapy at any time during follow-up, or death. The analysis describes the baseline characteristics, care received during follow-up, outcomes, and factors associated with COVID-19 clinical worsening or death.

The Kaplan Meier method was used to estimate the probability of occurrence of the primary and secondary endpoints over time, overall and by sex, age category, and presence or absence of the main comorbidities. Univariable then multivariable logistic regression were used to analyse the association between the primary and secondary endpoints and the following characteristics: sex, age (<60 vs. ≥60 years), time between first symptom and hospitalisation (≤7 vs. >7 days), chronic hypertension declared by the patient (yes vs. no), diabetes declared by the patient (yes vs. no), and initial severity (in three grades, mild, moderate, severe, according to the May 2020 WHO definitions). Variables associated with the endpoint with a $p < 0.20$ in univariable analysis were included in the multivariable model. The analyses were carried out using the software R, version 4.0.3

Ethical considerations

The study was approved in Burkina Faso by the Comité d'Éthique pour la Recherche en Santé (ID number 2020-6-116) and in Guinea by the Comité National d'Éthique pour la Recherche en Santé (ID number 069/CNERS/20). The committees of the two

Table 1
Baseline characteristics (n = 1,805).

Country, n (%)		
Burkina Faso	212	12%
Guinea	1,593	88%
Sex, n (%) ^a		
Male	1,151	64%
Female	651	36%
Age, years, n (%) ^a		
<20	132	7%
20–39	691	39%
40–59	579	32%
≥60	388	22%
Comorbidities, n (%)		
Diabetes	219	12%
Chronic hypertension	386	21%
Any other ^b	147	8%
Time from first symptom to admission (days), median [IQR] ^a	7	[4; 11]
Signs and symptoms, n (%)		
Fever ≥38.0 °C	297	16%
Fatigue	748	41%
Loss of smell or taste	490	27%
Headache	673	37%
Arthralgia or myalgia	294	16%
Higher respiratory tract symptoms ^c	425	24%
Any lower respiratory tract symptom	1,110	61%
Cough	833	46%
Dyspnoea	505	28%
Chest pain	294	16%
Any digestive tract symptom	311	17%
Vomiting	156	9%
Diarrhoea	95	5%
Abdominal pain	125	7%
Glasgow <15	21	1%
Other signs or symptoms ^d	36	2%
SpO2 under ambient air		
≥94%	1,545	86%
90–93%	83	5%
<90%	177	10%
Severity, n (%) ^e		
Mild	867	48%
Moderate	761	42%
Severe	177	10%

N, number; %, percentage; IQR, interquartile range.

• Mild: Symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia.

• Moderate:

- Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia, including SpO2 ≥ 90% on room air.
- Child with clinical signs of non-severe pneumonia (cough or difficulty breathing + fast breathing and/or chest indrawing) and no signs of severe pneumonia.

• Severe:

- Adolescent or adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: respiratory rate >30 breaths/min; severe respiratory distress; or SpO2 < 90% on room air.
- Child with clinical signs of pneumonia (cough or difficulty in breathing) + at least one of the following: Central cyanosis or SpO2 < 90%; severe respiratory distress (e.g., fast breathing, grunting, very severe chest indrawing); general danger sign: inability to breastfeed or drink, lethargy or unconsciousness, or convulsions (55, 56). Fast breathing (breaths/min): <2 months: ≥60; 2–11 months: ≥50; 1–5 years: ≥40.

^a Missing values: sex, n = 3; age, n = 15; time since first symptoms, n = 517.

^b Chronic cardiac disease (n = 25, 1%), current smoking (n = 12, 1%), chronic pulmonary disease (n = 17, 1%), active or previous tuberculosis (n = 23, 1%), asthma (n = 39, 2%), asplenia (n = 0, 0%), chronic kidney disease (n = 13, 1%), malignant neoplasm (n = 4, 0%), chronic hepatic disease (n = 19, 1%), chronic neurological disorder (n = 8, 0%), HIV infection (n = 5, 0%).

^c Runny nose, nasal congestion, sneezing.

^d Other signs or symptoms: confusion (n = 18, 1%), seizure (n = 6, 0%), conjunctivitis (n = 7, 0%), skin rash (n = 3, 0%), skin ulcer (n = 1, 0%), lymphadenopathy (n = 3, 0%), bleeding (n = 1, 0%).

^e Adapted from WHO May 2020 interim guidance (WHO, 2020a).

countries authorised the use of medical data collection in accordance with WHO methodology to describe the course of the COVID-19 disease during routine care, without requesting signatures for specific consent.

Role of the funding source

The funding sources took no part in designing the study, collecting, analysing and interpreting data, writing the report or making the decision to submit the article for publication. MJ, BS and SJ had access to the raw data. The corresponding author had full access to all data and the final responsibility for submitting it for publication.

Results

During the study period, 1,938 people (Guinea: 1,593; Burkina Faso: 345) were admitted to one of the three CCCs with a positive SARS-CoV-2 test on nasopharyngeal swab. Of these, 133 were completely asymptomatic and not included in the analysis. The remaining 1,805 were included in the analysis (Guinea: 1,593; Burkina Faso: 212). Their baseline characteristics are detailed in Table 1; 64% were men and 36% women, and the median age was 41 years (IQR 30–57, range 1–99 years). The two most common comorbidities were chronic hypertension (21%) and diabetes (12%). The median weight was 73 kg (IQR 60–85). Height, and therefore body mass index (BMI), could only be measured in 645 people. In this subgroup, the median BMI was 25 kg/m² (IQR 22–29), and 119 (18%) had a BMI of ≥30 kg/m². At the time of admission, symptoms lasted for a median of 7 days (IQR 4–11). The clinical picture was dominated by signs of lower respiratory tract involvement, present in 1,110 (61%) patients. Digestive signs were present in 311 (17%) patients. A total of 177 (10%) patients had an initially measured SpO2 of 90%, and 177 (10%) were considered severe from the start on the basis of this SpO2 or other signs of respiratory severity.

The follow-up characteristics are detailed in Table 2. During hospitalization, 1,697 people (95%) received at least one specific treatment recommended by national authorities, and 97 (5%) received none. The most common treatment was the hydroxychloroquine plus azithromycin combination, received by 1,244 (73%) participants. Between admission and the end of follow-up, 231 people (13%) had a measured SpO2 of <90% at least once, 237 (13%) received oxygen and 266 (15%) received corticosteroids.

The rate of clinical worsening was 31% overall; 24% in women and 34% in men; 23%, in patients aged <60 years and 57% in those ≥60 years; 59% in diabetics and 27% in non-diabetics; 51% in patients with self-declared chronic hypertension and 25% in those without hypertension.

Overall, 86 (5%) patients died within a median of 5 days (IQR 2–10) of hospital admission. The final percentage of deaths by group was: 3% in women and 6% in men; 0%, 1%, 5% and 14% in patients aged <20, 20–39 years, 40–59 years and ≥60, years, respectively; 13% in diabetics and 4% in non-diabetics; 12% in patients with chronic hypertension and 3% in those without hypertension; and 26%, 3% and 2% in patients initially considered to be at a severe, moderate and mild stage, respectively. Patients aged <60 years at admission accounted for 38% of death (33/86). For these patients, median age was 50 years (IQR 41–56), 60% were at a severe stage at admission and 32% and 36% had diabetes and chronic hypertension, respectively. In the primary analysis, the risk of death was significantly higher in men, those aged ≥60 years, those with chronic hypertension, and those initially considered to be at a severe stage. The duration of symptoms prior to admission was not significantly associated with the risk of death (Table 3A, Figure 1 and 2). In the secondary analysis, male patients, those aged ≥60 years, those with chronic hypertension, and those with diabetes had a higher risk of clinical worsening (Table 3B, Figure 1 and 2). Patients who recovered were discharged after a median of 11 days (IQR 8–16).

Table 2
Follow-up characteristics (n = 1,805).

Vital signs during follow-up, n (%)		
Heart rate >110 beats/min at least once	338	19%
Glasgow <15 at least once	37	2%
SpO2 under ambient air, worse value		
<90%	231	13%
90–93%	212	12%
≥94%	1,362	75%
Care received, n (%)		
Specific drugs: ^a		
Hydroxychloroquine + azithromycin	1,697	95%
Hydroxychloroquine	1,244	73%
Hydroxychloroquine + azithromycin + lopinavir/ritonavir	227	13%
Hydroxychloroquine + lopinavir/ritonavir	212	12%
Lopinavir/ritonavir	7	0.4%
No specific drugs	7	0.4%
Other drugs	97	5%
Other antibiotics		
Corticosteroids	1,491	83%
Systemic anticoagulation	266	15%
Received oxygen therapy	315	17%
237	13%	
WHO ordinal scale (higher grade) ^b		
Stage 4: Oxygen by mask or nasal prongs	151	64%
Stage 5: Non-invasive ventilation or high-flow oxygen	83	35%
Stage 6: Intubation and mechanical ventilation	3	1%
Admitted to intensive care unit	275	15%
Final vital status		
Death, n (%)	86	5%
Time from admission to death (days), median [IQR]	5	[2; 10]
Alive, n (%)	1,719	95%
Time from admission to discharge (days), median [IQR] ^a	11	[8; 16]
Combined secondary outcome		
Death or oxygen therapy or oxygen saturation <94%, n (%)	552	31%

N, number; %, percentage; IQR, interquartile range.
^a Missing values: Specific drugs, n = 11; time from admission to discharge, n = 1.
^b See reference (WHO, 2020b).

Discussion

It is believed that this is the first African study describing the clinical presentation and outcomes of COVID-19 among symptomatic patients prospectively monitored in specialist hospital care centres. This study has strengths in that it provides partial answers to some of the questions surrounding COVID-19 in Africa, and weaknesses in that it leaves other questions unanswered.

In two West African countries where the policy was to hospitalise everyone with a positive SARS-Cov-2 RT-PCR test, regardless of the severity of the clinical picture, 5% of symptomatic people who were hospitalised died. Mortality was 14% in patients

aged ≥60 years and 26% in people with severe respiratory signs on admission. Although no comparisons can be made between hospitals in different countries with different hospitalisation practices and levels of care, these figures are consistent with those described elsewhere (Lv et al., 2020; Olumade and Uzairue, 2021; Singer et al., 2020) and strongly suggest that COVID-19 is at least as serious in Africa as it is in other continents (Boulle et al., 2020; Elimian et al., 2020; Kirenga et al., 2020; Nachega et al., 2020). If the reported death toll from COVID-19 is lower in Africa than in Europe or the USA (Chilimuri et al., 2020; Galloway et al., 2020; Rossi et al., 2020; Zhou et al., 2020), it is probably not because the disease is less lethal in Africa. This reasoning is also compatible with the 2.8%, 9.2% and 13.2% mortality rates reported from retrospective population or hospital-based cohorts in South Africa, Nigeria and the Democratic Republic of the Congo (Boulle et al., 2020; Elimian et al., 2020; Nachega et al., 2020) as well as the 5.6% of an African meta-analysis (Olumade and Uzairue, 2021).

In the present study, the predominant clinical presentation was respiratory. Digestive signs and general signs, headaches, myalgia and asthenia were nevertheless quite frequent, as was a lack of taste and smell. Mortality was twice as high in people with hypertension, 2 times higher in men, and 3 times higher in people aged ≥60 years. These clinical spectrum and factors associated with mortality are also consistent with what has been described elsewhere (Boulle et al., 2020; El Aidaoui et al., 2020; Elimian et al., 2020; Guan et al., 2020; Huang et al., 2020; Kirenga et al., 2020; Nachega et al., 2020; Singer et al., 2020; Zhou et al., 2020). They emphasise the particular severity of the disease in groups at risk, in Africa as elsewhere, and the need to set up appropriate care to try to reduce the high mortality rate in these groups.

The first type of appropriate care is oxygen therapy. In the present study, participants received oxygen therapy when their SpO2 dropped below 90%, but few people with an SpO2 of between 90% and 93% did. Very few patients also benefited from non-invasive or invasive ventilation. It can be hypothesised that expanding the indications for oxygen therapy and increasing options for ventilation could have reduced mortality (Baker et al., 2020).

Regarding other ways to improve care, the data is of very limited value. The anti-infective treatments recommended in both countries and widely prescribed to patients in this study have not been proven effective so far (Kaptein et al., 2020; Maisonnasse et al., 2020; RECOVERY Collaborative Group et al., 2020; Skipper et al., 2020). The data do not provide any new insights in this regard. In the present study, a large percentage of patients received

Table 3A
Factors associated with the risk of death.

		Univariable					Multivariable				
		n/N	%	cOR	95% CI	p	n/N	%	aOR	95% CI	p
Sex	Female	18/651	3%	-	-	-	18/643	3%	-	-	-
	Male	68/1,151	6%	2.2	[1.3; 3.8]	0.002	68/1,144	6%	2.0	[1.1; 3.6]	0.019
Age	<60 years	33/1,402	2%	-	-	-	33/1,400	2%	-	-	-
	≥60 years	53/388	14%	6.5	[4.2; 10.4]	<0.001	53/387	14%	2.9	[1.7; 4.8]	<0.001
Diabetes	No	58/1,586	4%	-	-	-	58/1,570	4%	-	-	-
	Yes	28/219	13%	3.9	[2.4; 6.2]	<0.001	28/217	13%	1.3	[0.8; 2.3]	0.32
Chronic hypertension	No	41/1,419	3%	-	-	-	41/1,403	3%	-	-	-
	Yes	45/386	12%	4.4	[2.9; 6.9]	<0.001	45/384	12%	2.1	[1.2; 3.4]	0.006
Initial severity grade ^a	Mild	18/867	2%	-	-	-	18/857	2%	-	-	-
	Moderate	22/761	3%	1.4	[0.7; 2.7]	0.30	22/753	3%	1.3	[0.7; 2.6]	0.37
	Severe	46/177	26%	16.4	[9.4; 30.0]	<0.001	46/177	26%	9.0	[5.0; 16.8]	<0.001
Time since first symptom	≤7 days	31/778	4%	-	-	-	-	-	-	-	-
	>7 days	27/510	5%	1.4	[0.8; 2.3]	0.28	-	-	-	-	-

n = total number of patients in the category; n = number of patients in the category who died; CI: confidence interval; cOR: crude odds ratio; aOR: adjusted odds ratio; the OR for each variable shown in the table was adjusted on the other variables shown in the table.

^a See reference (WHO, 2020b).

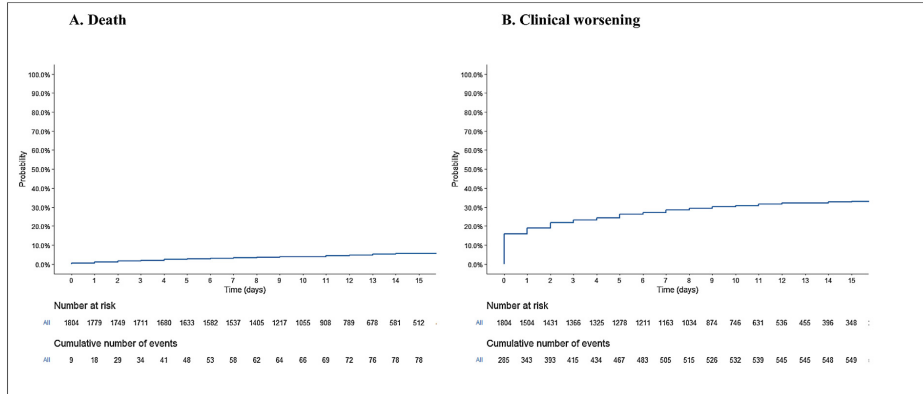


Figure 1. Kaplan Meier estimate of the probability of clinical worsening and death in the overall population.

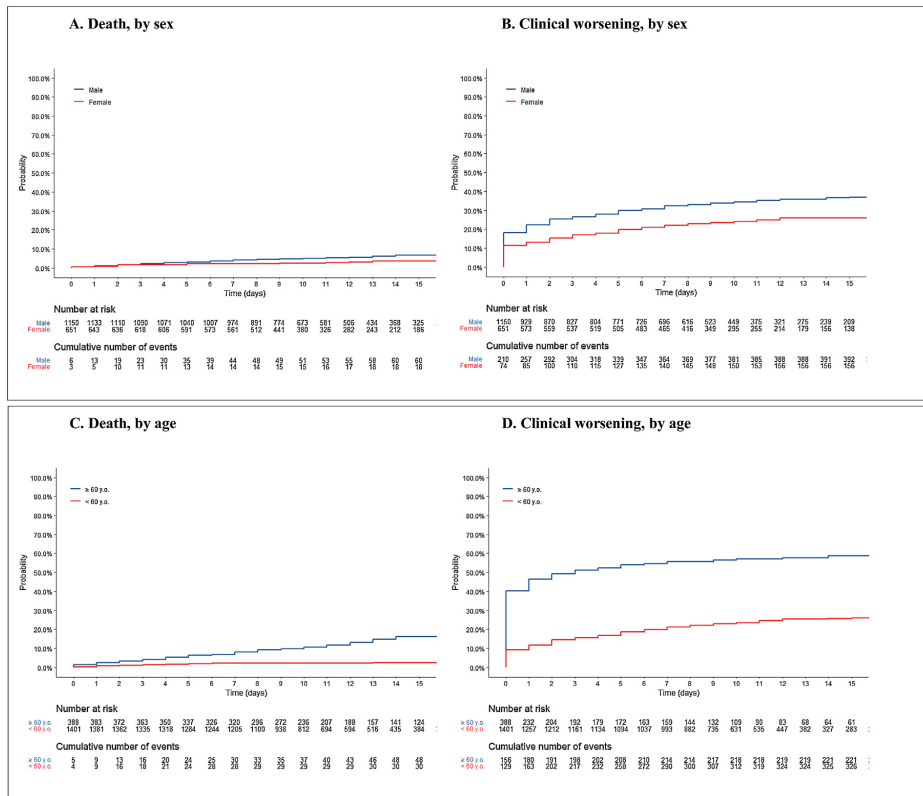


Figure 2. Kaplan Meier estimate of the probability of clinical worsening and death, according to sex, age, and comorbidities.

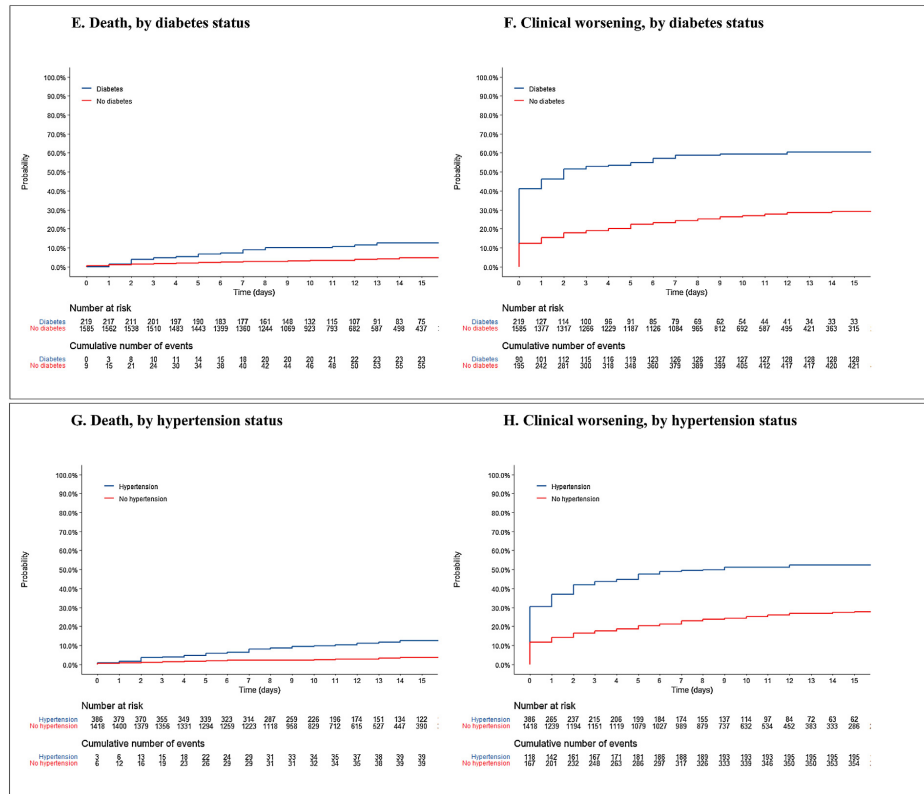


Figure 2. (Continued)

Table 3B
Factors associated with the risk of clinical worsening.

		Univariable					Multivariable				
		n/N	%	cOR	95% CI	p	n/N	%	aOR	95% CI	p
Sex	Female	157/651	24%	–	–	–	157/643	24%	–	–	–
	Male	394/1,151	34%	1.6	[1.3; 2.0]	<0.001	392/1,144	34%	1.6	[1.3; 2.1]	<0.001
Age	<60 years	327/1,402	23%	–	–	–	326/1,400	23%	–	–	–
	≥60 years	223/388	57%	4.4	[3.5; 5.6]	<0.001	223/387	58%	3.1	[2.4; 4.0]	<0.001
Diabetes	No	423/1,586	27%	–	–	–	420/1,570	27%	–	–	–
	Yes	129/219	59%	4.0	[2.9; 5.3]	<0.001	129/217	59%	2.3	[1.6; 3.1]	<0.001
Chronic hypertension	No	356/1,419	25%	–	–	–	354/1,403	25%	–	–	–
	Yes	196/386	51%	3.1	[2.4; 3.9]	<0.001	195/384	51%	1.7	[1.3; 2.2]	<0.001
Time since first symptom	≤7 days	223/778	29%	–	–	–	–	–	–	–	–
	>7 days	188/510	37%	1.5	[1.1; 1.8]	0.002	–	–	–	–	–

n = total number of patients in the category; n = number of patients in the category who had an SpO2 < 94%, received oxygen, or died; CI: confidence interval; cOR: crude odds ratio; aOR: adjusted odds ratio; the OR for each variable shown in the table was adjusted on the other variables shown in the table.

corticosteroids; although this percentage is consistent with what could be expected based on SpO2 figures, there are no more details on its duration or precise indications. In addition, the data do not allow comment on anything concerning elaborate management of the complications of the disease in its inflammatory phase, but since substantial resuscitation capacities are limited, there is little hope for improving survival this way. Hopes for therapy lean more toward earlier intervention. Treatments of this nature are still

experimental, and it is important for African centres to participate in trials of new curative treatments.

Finally, this study was also limited as follows. Because it was carried out at the height of an emergency, the monitoring of collected data was limited to a small number of key variables to ensure the analysis provided solid descriptive elements. Since the number of variables was limited, the analysis of factors associated with outcomes probably suffered from confusion bias.

Nevertheless, the analyses were consistent with what was known elsewhere, and this external consistency has the advantage of increasing confidence that the data are robust. It is therefore believed that the main results, which are the overall and group mortality rates, are credible.

In conclusion, COVID-19 is a serious disease in Africa, as it is elsewhere. Men are more likely to experience complications than women, and certain common risk factors such as age, diabetes and hypertension should make us particularly vigilant in the event of COVID-19 in these countries. Further studies should now focus on finding ways to reduce mortality from COVID-19 in Africa, overall and in these at-risk groups.

Data sharing statement

The anonymised individual data and the data dictionary of the study will be made available to other researchers by Professor Denis Malvy (denis.malvy@chu-bordeaux.fr) after approval of a methodologically sound proposal and the signature of a data access agreement.

Author contribution statement

MJ, SJ, BS, RK, ED, AP, MSS, Eudoxie Koumbem (EK), Halidou Tinto (HT), Adama Sanou (ASa), Apoline Sondo (ASo), Billy Sivahera (BSH), Caroline Martin (CM), Moumouni Kinda (MK), Joseph Donamou (JD), Jean-Paul-Yassa Guilavogui (JPG), Fode Bangaly Sako (FBS), Fode Amara Traore (FAT), Flavien Kabore (FK), Brice Bicaba (BB), Hans-Joerg Lang (HJL), Sani Sayadi (SS) and Augustin Augier (AA) set up the study in Burkina Faso and Guinea, enrolled and followed the patients and recorded clinical data.

MJ, SJ, MSS, ED and BS had access to the raw data.

MJ, SJ, BS and XA performed the analysis. MJ, SJ, BS, ED, MSS, RK, Olivier Marcy (OM), XA and DM drafted the manuscript.

All authors revised the manuscript critically for important intellectual content and approved the final version before submission.

Conflict of interest

All authors declare not conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Ethical approval

The study was approved in Burkina Faso by the Comité d'Éthique pour la Recherche en Santé (ID number 2020-6-116) and in Guinea by the Comité National d'Éthique pour la Recherche en Santé (ID number 069/CNERS/20).

The committees of the two countries authorised the use of medical data collection in accordance with WHO methodology to describe the course of the COVID-19 disease during routine care, without requesting signatures for specific consent.

Acknowledgments

We thank all the patients involved in the current study, as well as their caregivers and the investigators and research staff at the participating care centres. We thank the World Health Organization for technical advice.

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D. Discussion et Perspectives

a) Fièvre de Lassa

En parallèle et dans les suites de la cohorte LASCOPE, plusieurs études ont été mises en place à l'hôpital de Owo, Etat d'Ondo, Nigéria. Dans l'ensemble, 2 grands volets de recherche ont été développés :

- L'extension de LASCOPE et les études ancillaires
- Les projets de recherche clinique visant à mettre en évidence des nouveaux traitements contre la fièvre de Lassa. Pour cela, 3 projets sont en cours ou en préparation :
 - o Une étude de phase 2 visant à évaluer l'activité du favipiravir dans le traitement de la fièvre de Lassa (étude SAFARI)
 - o Une analyse des données des patients LASCOPE pour déterminer le meilleur critère de jugement principal possible à utiliser dans les essais thérapeutiques de phase 3
 - o Un essai thérapeutique de type plateforme adaptative pour évaluer l'efficacité des différents traitements disponibles contre la fièvre de Lassa.

Études ancillaires à la cohorte LASCOPE (étude PK et CVS) et extension de LASCOPE

En collaboration avec nos collègues de l'université d'Oxford, deux études ancillaires de LASCOPE ont été mise en place en 2019-2020.

La première étude vise à évaluer la pharmacocinétique (PK) de la ribavirine IV chez les patients traités pour fièvre de Lassa et inclus dans l'étude LASCOPE. En effet, le régime administré suite à l'étude de Mc Cormick en 1976 (35) est le suivant : dose de charge de 33mg/kg puis 16mg/kg/6 heures de J1 à J4 puis 8mg/kg/8 heures de J5 à J10. Ce régime thérapeutique, baptisé « régime Mc Cormick », a été recommandé par le NCDC pour tous les patients Lassa confirmés jusqu'en novembre 2018 (33). Au cours de la grande épidémie de 2018, nos collègues cliniciens du site d'Irrua ont spontanément modifié l'administration de la ribavirine. Le régime dit « régime Irrua » est plus simple d'administration, car requiert moins d'injection et donc moins d'entrée dans la zone à haut risque. Il comporte une dose de charge de 100mg/kg administrée en 2 fois à 8h d'intervalle, puis une dose unique quotidienne de 25mg/kg de J2 à J7 puis une dose unique quotidienne de 12,5mg/kg de J8 à J10. Les recommandations du NCDC ont donc changé à la fin de l'années 2018 et ce « régime Irrua » est maintenant recommandé pour les adultes non enceintes (34).

L'étude PK/LASCOPE vise donc à décrire la pharmacocinétique de la ribavirine quand elle est administrée en utilisant le « régime Irrua ». Pour cela, 51 patients ont été inclus et des prélèvements sanguins ont été réalisés avant l'administration de ribavirine à J1, J3, J5, J7, J10 et à 30 min, 1,5h, 4h, 6h, 8h, 10h après l'administration de la ribavirine à J1, J5 et J10. Le sang a été recueilli sur des papiers buvards pour être conservé à -40° sur site en attendant d'être transporté en Angleterre. Les données cliniques et biologiques recueillies sont celles de LASCOPE. A ce jour, les papiers buvard ont été acheminés en Angleterre et sont en train d'être analysés. Nous espérons pouvoir travailler sur ces données en fin d'année 2022, dans le but d'évaluer la PK du « régime Irrua ».

La deuxième étude vise à décrire la tolérance cardiovasculaire et la survenue du choc hypovolémique chez les patients inclus dans la cohorte LASCOPE. Pour cela, 107 adultes ont été inclus dans l'étude CVS (Cardio Vascular Study)/LASCOPE. Les patients étaient soumis à plusieurs examens fonctionnels et morphologiques pour évaluer la fréquence du choc, la fuite vasculaire, la fonction cardiaque et pulmonaire, la résistance vasculaire périphérique ... Les examens étaient les suivants :

- Le NICaS (Non Invasive Cardiac System) est une intervention non invasive utilisant la bio-impédance permettant d'évaluer la fonction cardiaque, la résistance vasculaire périphérique et le volume total d'eau corporelle. Le NICaS était réalisé tous les jours entre l'admission et la sortie d'hospitalisation.
- L'échographie thoracique et abdominale permettait d'évaluer la présence d'épanchement pleural, de péricardite, d'ascite et de mesurer l'index de collapsibilité de la veine cave inférieure. Elle était réalisée tous les jours de J0 à J5 puis à J7, J10 et à la sortie d'hospitalisation
- L'échographie cardiaque évaluait la fonction cardiaque et était réalisée à la même fréquence que l'échographie thoracique et abdominale.
- L'ENDO-PAT 2000 est une intervention non invasive évaluant la fonction endothéliale. Elle était réalisée à l'admission et à la sortie d'hospitalisation.
- L'électrocardiogramme (ECG) était réalisé à J0, J5, J10 et à la sortie d'hospitalisation.

A ce jour les inclusions sont finalisées et les données fonctionnelles et morphologiques sont en cours d'analyse. Nous espérons pouvoir les publier en début d'année 2023.

Enfin, l'étude LASCOPE continue ses inclusions sur le site historique de Owo, où elle n'ont jamais été interrompues depuis avril 2018. Mais un deuxième site a été ouvert en mai 2022 à l'hôpital d'Abakaliki (Ebonyi state) en collaboration avec MSF. Pour l'instant, un seul patient Lassa confirmé provenant de ce site a été inclus mais l'ouverture a eu lieu après la saison épidémique au Nigéria qui se déroule, pour rappel, chaque année de décembre à mars.

La recherche clinique visant à mettre en évidence de nouveaux traitements actifs contre la fièvre de Lassa

- Traitement de référence : la ribavirine

Le traitement de référence pour traiter la fièvre de Lassa est en 2022 la ribavirine, suite à une publication datant des années 70 (35). Cependant, de plus en plus de cliniciens et chercheurs émettent des réserves quant à l'efficacité de ce traitement et mettent en garde contre la toxicité notamment hématologique. Nos collègues allemands ont réalisé une revue de la littérature pour évaluer l'efficacité de la ribavirine dans la fièvre de Lassa (72). D'après les auteurs, seule l'étude de Mc Cormick a permis d'évaluer, de manière prospective, l'efficacité de la ribavirine chez les patients Lassa, et la conclusion n'est pas très convaincante : la ribavirine serait efficace chez les patients avec des ALAT > 150 UI, mais sans efficacité chez ceux présentant des ALAT < 150 UI. De plus, à la lecture approfondie de l'étude de Mc Cormick, plusieurs biais ont été mis en évidence : un certain nombre de déviations non expliquées au protocole, l'utilisation de données contrôle historiques, l'allocation aux différents bras d'intervention (ribavirine orale, ribavirine IV ou plasma de convalescent) non randomisée ... De la même façon, nos collègues anglais ont demandé à avoir accès aux données complètes

de l'étude de Mc Cormick pour pouvoir refaire les analyses (73). Ils aboutissent aux mêmes conclusions, à savoir que la ribavirine IV présente peut-être une discrète efficacité chez les patients avec un bilan hépatique perturbé, mais est plutôt davantage toxique que le contrôle chez les patients avec un bilan biologique hépatique normal (cf. figure 3).

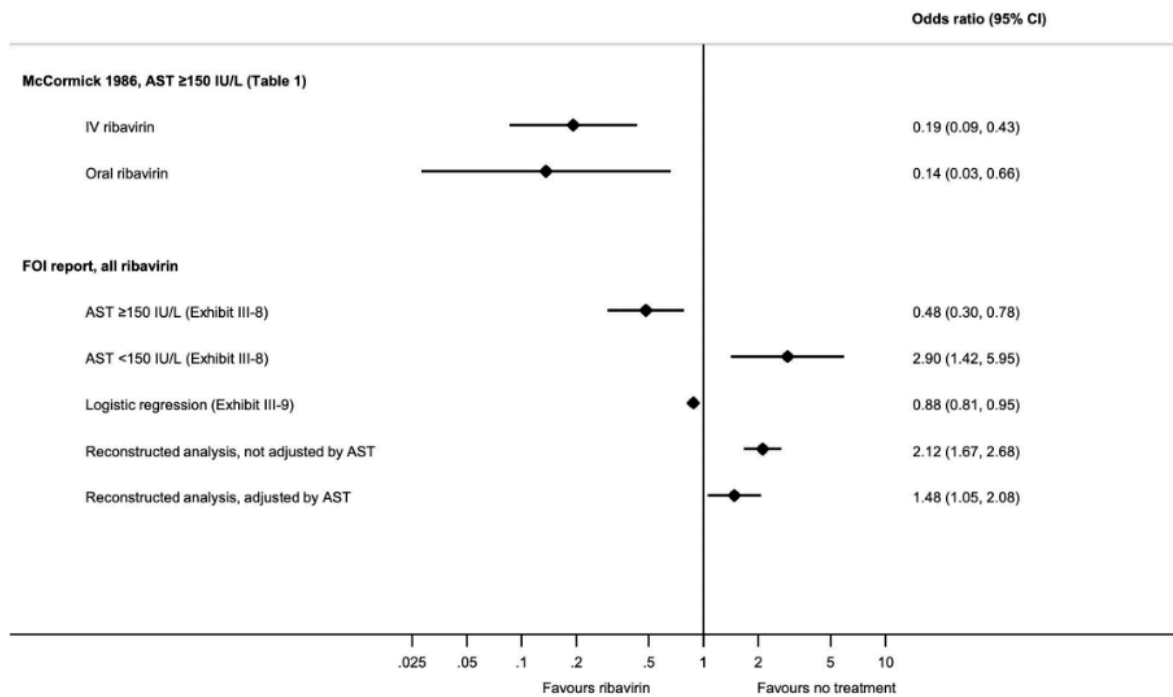


Figure 3 : Odds Ratio du risque de décès dans les sous-groupes de traitement après analyse des données de Mc Cormick, Salam et al, plus NTD, 2021

- Autres traitements antiviraux

Dans ce contexte, il apparaît urgent de trouver des alternatives thérapeutiques à la ribavirine. Pour cela, deux grandes questions doivent être adressées : (i) quels médicaments sont prometteurs et disponibles pour être évalués au cours d'un essai clinique ? et (ii) quel schéma d'étude est acceptable et faisable dans ce contexte épidémiologique ?

Pour répondre à la première question, nous avons réalisé un mapping des médicaments disponibles ainsi que leur niveau de préparation en phase pré clinique et en phase 1. Ils sont listés dans le tableau 3 ci-dessous.

Table 3: médicaments en cours d'évaluation en phase pré clinique en phase clinique 1 et 2 ayant démontré une efficacité contre le virus Lassa

Molécule	Classe	Cible	In vitro (EC50)	Efficacité préclinique LASV	Phase I	Phase II	Commentaires
Favipiravir	Petites molécules antivirale	ARN-pol	18 µM	Cochon d'inde Souris Macaque	Dose grippe OK Doses élevées en cours (FAVIDOSE)	SAFARI (en cours)	Problème de dose efficace sur LASV
ARN-75039	Petites molécules anti virales	Inhibiteur de fusion tardif	<0,3 nM	Cochon d'inde	Fin 2022 (USA)	-	Coût de production faible
LHF-535	Petites molécules anti virales	Inhibiteur de fusion précoce	0,3 nM	Cochon d'inde	OK	-	↘ sensibilité du lignage I Coût production élevé
Arevirumab-3	Immunothérapie (cocktail d'anticorps monoclonaux)	Anti-GP (inh. fusion)	<1 nM	Cochon d'inde Macaque	?	-	Ø mutant d'échappement Problème d'accessibilité

L'antiviral favipiravir est actuellement évalué en phase 2 sur les sites d'Owo et d'Irrua au Nigéria, sous la promotion et la coordination du Bernhard Nocht Institut for Tropical Medicine (BNITM), Hambourg, Allemagne. En effet l'essai SAFARI vise à évaluer la sécurité, la tolérance et la pharmacocinétique du favipiravir administré à la dose de 1200mg/12h pendant 10 jours (après une dose de charge de 6000mg réparti en 3 doses) en comparaison avec la ribavirine (« régime Irrua »). Les patients inclus ne présentent pas de signe de sévérité et 20 patients par bras sont nécessaires pour réaliser les analyses. A ce jour, 30 patients ont été inclus sur les 40 attendus. Cependant, un doute persiste sur l'efficacité du favipiravir à cette dose relativement faible de 1200mg/12h, notamment en raison de la faible efficacité à cette dose chez le macaque infecté par LASV (74) mais aussi chez les patients Ebola dans l'essai thérapeutique JIKI (44,75). Il est donc nécessaire d'évaluer, chez les patients Lassa, des doses plus fortes en phase 2 avant de pouvoir sélectionner la dose qui sera évaluée en phase 3. A noter qu'un essai chez les volontaires sains est en cours en France pour évaluer la sécurité et la tolérance de doses plus élevées de favipiravir (1600mg/12h, 2000mg/12h et 2400mg/12h). Cet essai FAVIDOSE pourra permettre de justifier de l'utilisation à venir de dose élevées, si elles s'avèrent être bien tolérées.

Pour répondre à la deuxième question, à savoir quel schéma d'étude permettrait d'évaluer l'efficacité de nouveaux traitements dans la fièvre de Lassa, plusieurs démarches ont été entreprises. Il est admis par la communauté scientifique que le critère de jugement principal ne peut résider en la seule analyse de mortalité (76). En effet, un taux de mortalité de 12% induirait d'inclure un effectif très important de patients, ce qui est donc difficilement atteignable considérant l'épidémiologie de la fièvre de Lassa dans son aire d'extension actuelle en Afrique de l'ouest. Le critère de jugement principal doit donc être plus fréquent. La définition d'un critère composite, donc constitué de la mortalité mais aussi d'autres variables corrélées avec l'aggravation clinique ou biologique des patients, semble être une solution intéressante pour prouver l'efficacité d'un traitement en maintenant un effectif d'inclusion raisonnable.

Pour établir ce critère de jugement principal composite, une analyse des données de LASCOPE est en cours. L'objectif est de tester différents critères composites et leur association avec l'évolution défavorable. Il conviendra aussi d'établir un critère composite faisable sur le terrain et facilement reproductible d'un site à l'autre. La publication devrait être prête pour soumission en fin 2022.

En parallèle, à l'initiative de nos collègues de l'Université d'Oxford, le consortium WALC (West Africa Lassa Consortium) a été mis en place en janvier 2021 pour établir, de manière consensuelle un protocole générique pour l'évaluation des nouveaux médicaments disponibles pour lutter contre la fièvre de Lassa. Ce consortium comporte des membres (Université d'Oxford, MSF West And Central Africa, NCDC, ALIMA et Kineta (= firme développant le LHF 535, cf. tableau 3)) et des experts scientifiques de différentes organisations (BNITM, OMS, ministère de la santé et autorités réglementaires des pays touchés ...). Les questions autour du critère de jugement principal ont été aussi largement discutées et le critère composite qui semblait rassembler l'opinion générale était le suivant :

- ⇒ décès ou hypotension artérielle (définie par une pression artérielle moyenne <65mmHg sur 2 mesures consécutives à 4h d'intervalle) ou insuffisance rénale (définie par le score KDIGO à 3) ou insuffisance respiratoire (définie par SpO₂/FiO₂ ≤ 315 sur 2 mesures consécutives à 4h d'intervalle) entre J0 et J28.

De plus le schéma d'étude choisi par le consortium était celui d'un essai plateforme adaptatif phase 2-3 de supériorité, randomisé, avec la ribavirine comme bras contrôle. Le protocole générique devrait être publié d'ici à la fin de l'année 2022. Il doit être accessible à toute équipe de recherche souhaitant réaliser cet essai thérapeutique sur la fièvre de Lassa.

- Essai thérapeutique de phase 3 : projet Integrate

Suite à tout ce qui a été décrit ci-dessus, et au travail réalisé dans LASCOPE, SAFARI et au sein du consortium WALC, la mise en place de l'essai thérapeutique est par conséquent l'étape suivante. Compte tenu du nombre de cas par an, un seul essai plateforme peut être conduit, dans lequel seront progressivement inclus l'ensemble des médicaments disponibles selon leur niveau de préparation. Nous avons donc établi le consortium Integrate, qui rassemble à ce jour BNITM, CORAL, l'ANRS-MIE, l'hôpital de Owo et l'hôpital de Irrua pour travailler sur la mise en place de cet essai. L'objectif est de débiter au plus vite l'essai en s'inspirant du protocole générique produit par le WALC mais aussi des expériences de terrain sur Owo et Irrua en terme de prise en charge et de recherche clinique. Cet essai débutera en premier lieu à Owo et Irrua, le temps de renforcer les capacités médicales, de laboratoire et de recherche clinique d'autres sites potentiels tels qu'Abakaliki ou Bauchi au Nigéria, Phebe au Liberia, Kenema au Sierra Leone et Nzérékoré en Guinée. Le premier traitement à être évalué sera le favipiravir, avec évaluation de 2 doses en phase 2 puis sélection d'une dose pour la phase 3. Dès lors que les autres médicaments seront prêts pour entrer en phase 2, à savoir principalement le LHF-535 et l'ARN-75039, un comité indépendant évaluera leur niveau de préparation et validera ou non leur intégration dans l'essai plateforme.

A ce jour, le synopsis de l'essai est bien établi, de même que le consortium et ses différents partenaires. La recherche de financement est en cours, mais plusieurs pistes ont déjà été identifiées (EDCTP, ANRS-MIE, ministère de la santé allemand ...). L'objectif serait d'initier le projet début 2023 et de démarrer les inclusions fin 2023 - début 2024.

b) Maladie à virus Ebola

Groupe de travail international - Prophylaxie Post Exposition dans la Maladie à virus Ebola

Les perspectives de recherche concernant la MVE concernent principalement l'évaluation des stratégies de PPE chez les contacts à haut risque. En effet, pour pouvoir avancer sur cette perspective, un certain nombre de questions doivent être adressées :

- I. Quelle est l'efficacité réelle de la vaccination par ERVEBO quand elle est administrée en PPE chez les contacts à haut risque ?
- II. Quelle définition du contact à haut risque à utiliser pour classifier les contacts ?
- III. Quels sont les candidats, autre que la vaccination, dont l'efficacité en PPE doit être évaluée ?
- IV. Quel est le design idéal pour une étude évaluant l'efficacité de la PPE ?
- V. Faut-il craindre une interaction entre les mAbs et le vaccin si ces deux interventions sont administrées conjointement ?

Pour tenter de répondre à ces questions, un groupe de travail international a été mis en place par l'ANRS-MIE. Ce groupe de travail rassemble autour d'une même table l'ensemble des acteurs qui s'intéressent à cette question : INRB (RDC), ANSS (Guinée), Université de Bordeaux, Inserm 1219, ANRS-MIE, MSF, ALIMA, NIH, CDC, BARDA, OMS ainsi que divers experts internationaux dont l'expertise sur la MVE est précieuse. Ce groupe de travail est dirigé par Dr Xavier Anglaret (Université de Bordeaux, Inserm 1219), Lori Dodds (NIH) et Pr Placide Mbala (INRB). Je suis responsable de l'animation scientifique de ce groupe.

L'objectif de ce groupe est de répondre aux questions ci-dessus pour préparer le terrain d'un essai thérapeutique évaluant l'efficacité de la PPE dans la MVE. Le travail scientifique consiste donc à :

- I. Rassembler les données de la littérature sur l'efficacité du vaccin en PPE chez les contacts à haut risque, notamment parmi les données non publiées qu'il conviendra de demander aux investigateurs des différentes études publiées sur le vaccin ERVEBO.
- II. Réfléchir collectivement à la meilleure définition des cas contacts, en repensant la définition « historique » de l'OMS en 2015 (77) à la lumière des expériences terrain passées et des nouvelles définitions du niveau de risque élaborées par l'INRB depuis.
- III. Mettre en évidence, à la lumière des données disponibles, les candidats qui pourront être évalués en PPE. Cela implique d'évaluer leur efficacité pré clinique et éventuellement clinique, leur tolérance et l'accès de ces molécules pour un déploiement sur le terrain.
- IV. En collaboration avec les équipes de biostatistique et méthodologie, identifier le meilleur schéma d'essai thérapeutique pour cette évaluation. L'essai randomisé et contrôlé permettrait d'obtenir le niveau de preuve le plus élevé, mais l'effectif représentera un enjeu important au vu du faible nombre de cas confirmés lors des dernières épidémies (RDC 11^{ème} = 130 cas, 12^{ème} = 12 cas, 13^{ème} = 11 cas, 14^{ème} = 4 cas, Guinée 2021 = 16 cas). La question du bras contrôle est évidemment essentielle, car le placebo serait très mal accepté par les populations. La vaccination devrait donc servir de bras contrôle. Le taux d'attaque chez les contacts à haut risque est aussi assez discuté, pourtant cette donnée est indispensable au calcul de l'effectif. Enfin, la

population de l'étude doit être définie, avec notamment une question sur l'inclusion ou non des personnes déjà vaccinées ou survivantes d'épidémies antérieures.

- V. Identifier le risque d'interaction entre les différentes interventions quand elles sont administrées conjointement dans le cadre de la PPE (cf. ci-dessous).

Le premier « délivrable » de ce groupe de travail sera la rédaction d'une publication conjointe dont les contours doivent se dessiner plus clairement lors de la prochaine réunion qui aura lieu mi-septembre 2022.

Étude de l'interaction entre le vaccin ERVEBO et les anticorps monoclonaux : essai IMOVA

Comme évoqué ci-dessus, il va être nécessaire d'évaluer l'efficacité de l'utilisation des mAbs en PPE chez les contacts à haut risque. Cependant, l'administration d'un mAb après un contact à haut risque pour éviter la contamination immédiate ne doit pas empêcher la vaccination par ERVEBO qui limite le risque d'infection à long terme. Par conséquent, la solution idéale serait d'administrer en même temps le mAb pour sécuriser le contact à risque et le vaccin pour conférer une immunité prolongée dans le temps. Or, il est probable que le mAb, dont la cible est la glycoprotéine (GP) virale, inhibe la réplication vaccinale (le vaccin étant constitué d'un vecteur répliquant, le virus VSV dont la GP a été intégralement remplacée par une GP de virus Ebola) et en limite donc l'efficacité.

Dans ce contexte, l'hypothèse principale de l'essai IMOVA (Interaction entre les anticorps Monoclonaux et le Vaccin contre la MVE) est que l'administration conjointe du vaccin ERVEBO et d'un mAb entraînera la diminution de l'activité vaccinale. L'étude a aussi pour objectif d'évaluer le délai minimum à respecter entre l'administration du mAb et celle du vaccin pour préserver l'efficacité vaccinale.

L'essai IMOVA est coordonné par le pôle MIE de CORAL. Le promoteur et financeur est l'ANRS-MIE. Il se déroulera en Guinée en collaboration avec l'ANSS et inclura 135 volontaires sains. Les inclusions devraient pouvoir commencer en 2023, selon l'avancement des discussions avec les firmes (MSD-Merck pour ERVEBO et REGENERON pour le REGN-E3B). Le schéma de l'étude est présenté dans la figure 4.

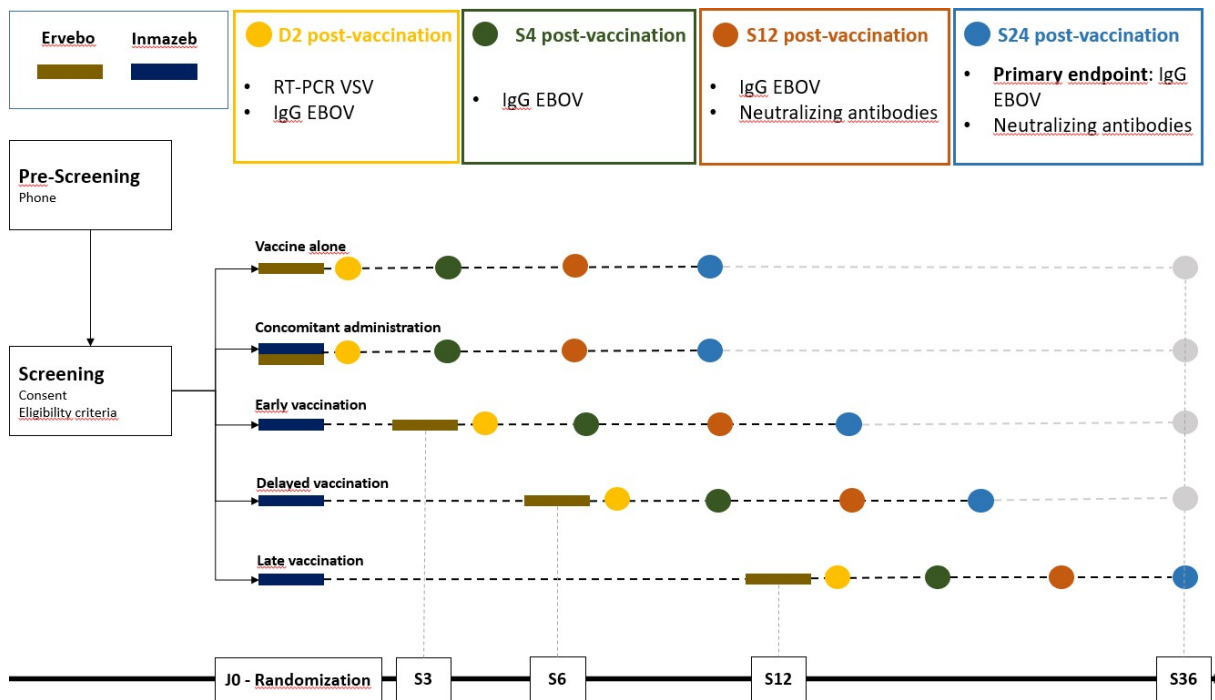


Figure 4 : Schéma d'étude de l'essai IMOVA

* Inmazed = REGN-E3B

A noter que nos collègues Cross et al, dans une publication de Nature Communication en juillet 2020 intitulée « Prior vaccination with rVSV-ZEBOV does not interfere with but improves efficacy of post exposure antibody treatment », concluent à l'absence d'interaction entre les mAbs et le vaccin ERVEBO chez les primates (78). Cependant, les auteurs ont évalué l'efficacité de l'association mAb + vaccin pour limiter l'infection par le virus Ebola chez les singes, et n'ont nullement analysé la qualité de la réponse vaccinale en terme de production d'IgG anti GP virale en cas d'administration conjointe. La question reste donc entière.

c) COVID-19

Pour contribuer à l'effort scientifique international relatif à la réponse à l'épidémie de Covid-19, et du fait de notre expertise en terme de recherche en Afrique sub-saharienne, le pôle MIE de CORAL coordonne actuellement 2 études sur la Covid-19 : COVERAGE-AFRICA et COVICOMPARE-Guinée.

- COVERAGE-AFRICA

L'étude COVERAGE-AFRICA est un essai adaptatif multi bras multi étapes de phase 2-3 visant à évaluer des médicaments spécifiques contre la Covid-19 chez les patients à risque de forme grave et présentant une infection à SARS-CoV-2 précoce sans signe de sévérité ou avec une sévérité modérée. Les patients à risque, donc éligibles, sont définis comme suit : patients âgés de 40 ans ou plus ou adultes avec au moins une comorbidité parmi l'obésité (IMC≥30 kg/m²), le diabète traité et l'hypertension artérielle traitée. Les patients éligibles présentent une infection symptomatique précoce sans sévérité ou avec une sévérité modérée définie par la présence de symptômes depuis moins de 7 jours, une saturation en oxygène <94% et

l'absence de signe de gravité clinique (fréquence respiratoire < 25 par minute, tension artérielle > 90/60 mmHg ou < 160/100 mmHg, absence de diarrhée ou vomissement). Les sites retenus pour cette étude sont le Burkina Faso (sites à Ouagadougou et Bobo Dioulasso) et la Guinée (site à Conakry) et 169 participants par bras doivent être inclus pour mettre en évidence une supériorité (avec 3 analyses intermédiaires). Le critère de jugement principal est la survenue d'une désaturation $\leq 93\%$ ou le décès entre J0 et J14. Les inclusions ont débuté le 12 mars 2021 et sont toujours en cours. Les médicaments ayant été évalués jusque-là sont, le telmisartan (arrêté en octobre 2021 devant un défaut de preuve d'efficacité pré clinique et clinique), l'association ciclesonide-nitazoxanide (arrêté pour futilité) et l'association budesonide-fluoxétine (inclusions en cours).

Compte tenu de la courbe épidémique en Guinée, les inclusions ont été interrompues depuis janvier 2022 mais les équipes sont maintenues en veille en cas de nouvelle vague épidémique.

A noter que le projet COVERAGE-AFRICA est adossé à un consortium international, nommé ANTICOV, coordonné par DNDi (Drugs for Neglected Diseases initiative) et qui rassemble 14 sites de recherche clinique (en Afrique sub-saharienne + Brésil) menant l'étude décrite ci-dessus. Les résultats de COVERAGE-AFRICA seront agrégés avec les résultats des autres sites ANTICOV pour en augmenter la pertinence.

- COVICOMPARE-Guinée

L'étude COVICOMPARE a été mise en place en France pour évaluer l'immunogénicité des vaccins contre le SARS-CoV-2 chez les sujets âgés en comparaison avec les sujets jeunes. En effet, en raison de l'immunosénescence attendue chez les sujets âgés, l'hypothèse est que la réponse vaccinale chez ces sujets est moindre et raccourcie. Les vaccins Moderna, Pfizer et Jansen ont été évalués en France et les analyses sont en cours.

Le pôle MIE de CORAL a été approché par les coordinateurs de l'essai en France pour mettre en place COVICOMPARE en Guinée, dans le but d'évaluer les vaccins disponibles sur le continent africain. Après discussion avec les autorités guinéennes, le choix s'est porté sur le vaccin développé par la firme Sinopharm, vaccin largement utilisé en Guinée. Les inclusions ont débuté en avril 2022 et se poursuivront pour 6 mois. Les participants sont suivis 24 mois au total avec des analyses immunologiques régulières. L'ajout d'un deuxième vaccin à l'essai COVICOMPARE-Guinée est en discussion actuellement.

d) Perspectives générales

Après la soutenance de la thèse, je vais donc poursuivre les projets de recherche présentés dans les paragraphes précédents, probablement sous la forme d'un post doctorat en 2022-2023. Cependant j'aimerais ensuite reprendre mes activités hospitalières. Le Pr Karine Lacombe m'a proposé un poste universitaire dans son service de maladies infectieuses et tropicales à l'hôpital Saint Antoine à Paris, que j'ai accepté avec honneur. L'objectif de ce poste est de développer la santé internationale dans le service, tant en terme de recherche, que d'enseignement et de prise en charge clinique. En effet, l'expertise assez particulière que j'ai pu développer au cours de ma thèse, notamment sur cette approche de recherche opérationnelle à l'international, de même que les nombreux partenariats mis en place, représentent des volets de ma spécialité médicale (maladies infectieuses et tropicales) qu'il

me semble important de développer et d'enseigner aux plus jeunes. Il faudra mettre en place des modules de formations, sous forme d'enseignements dirigés, de masters ou de diplômes universitaires pour que cette approche soit enseignée et donc cette activité pérennisée. La collaboration avec l'Université de Bordeaux et l'équipe du Pr Malvy sera maintenue et renforcée. Pour cette prise de poste, je suis passée en pré CNU (Conseil National des Universités) dans la discipline de maladies infectieuses et tropicales et j'ai reçu un avis favorable. Les perspectives sont donc de prendre un poste de praticien hospitalier contractuel en septembre 2023 au SMIT de saint Antoine, de passer en commission d'effectif et en CNU au printemps 2024 pour être éventuellement nommée maître de conférence des universités – praticien hospitalier (MCU-PH) en octobre 2024 si j'ai l'accord de Sorbonne Université.

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Annexes

Annexe 1 : Autres publications sur la fièvre de Lassa, revue de la littérature sur l'efficacité de la ribavirine en préclinique

PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Ribavirin for treating Lassa fever: A systematic review of pre-clinical studies and implications for human dosing

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OPEN ACCESS

Citation: Salam AP, Duvignaud A, Jaspard M, Malvy D, Carroll M, Tarning J, et al. (2022) Ribavirin for treating Lassa fever: A systematic review of pre-clinical studies and implications for human dosing. *PLoS Negl Trop Dis* 16(3): e0010289. <https://doi.org/10.1371/journal.pntd.0010289>

Editor: Andrea Marzi, National Institute of Allergy and Infectious Diseases Division of Intramural Research, UNITED STATES

Received: November 11, 2021

Accepted: February 28, 2022

Published: March 30, 2022

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Data Availability Statement: All relevant data are within the manuscript.

Funding: The UK Public Health Rapid Support Team is funded by UK Aid from the Department of Health and Social Care and is jointly run by UK Health Security Agency and the London School of Hygiene & Tropical Medicine. The views expressed in this publication are those of the authors and not

Abstract

Ribavirin is currently the standard of care for treating Lassa fever. However, the human clinical trial data supporting its use suffer from several serious flaws that render the results and conclusions unreliable. We performed a systematic review of available pre-clinical data and human pharmacokinetic data on ribavirin in Lassa. In in-vitro studies, the EC50 of ribavirin ranged from 0.6 µg/ml to 21.72 µg/ml and the EC90 ranged from 1.5 µg/ml to 29 µg/ml. The mean EC50 was 7 µg/ml and the mean EC90 was 15 µg/ml. Human PK data in patients with Lassa fever was sparse and did not allow for estimation of concentration profiles or pharmacokinetic parameters. Pharmacokinetic modelling based on healthy human data suggests that the concentration profiles of current ribavirin regimes only exceed the mean EC50 for less than 20% of the time and the mean EC90 for less than 10% of the time, raising the possibility that the current ribavirin regimens in clinical use are unlikely to reliably achieve serum concentrations required to inhibit Lassa virus replication. The results of this review highlight serious issues with the evidence, which, by today standards, would be unlikely to support the transition of ribavirin from pre-clinical studies to human clinical trials. Additional pre-clinical studies are needed before embarking on expensive and challenging clinical trials of ribavirin in Lassa fever.

Author summary

Lassa fever is a viral disease endemic to West Africa that has been highlighted by the World Health Organisation as one of the top ten epidemic threats to global health. Ribavirin has been used to treat Lassa fever for more than 30 years. However, the clinical

necessarily those of the Department of Health and Social Care.

Competing interests: The authors have declared that no competing interests exist.

evidence for ribavirin is poor. We conducted a systematic review of the pre-clinical and human pharmacokinetic data in Lassa. We found that the minimum concentrations of ribavirin that inhibited Lassa virus replication varied hugely according to the experimental design and the type of cell and Lassa virus strain used. There was no comprehensive data on the concentrations of ribavirin in the blood of Lassa fever patients. Modelling of ribavirin concentration data from healthy humans suggest that the doses of ribavirin currently used to treat Lassa fever patients do not reliably achieve the concentrations required to inhibit viral replication.

Introduction

Lassa fever is an acute viral disease caused by Lassa virus (LASV) that can lead to a viral haemorrhagic fever syndrome in some patients. It is endemic in several West African countries, including Nigeria, Sierra Leone, Guinea and Liberia [1]. As a result of its mortality, paucity of therapeutic and preventative interventions, and epidemic potential, Lassa fever has been highlighted by the World Health Organisation (WHO) as one of the top ten global epidemic threats [2,3]. Intravenous ribavirin is the therapeutic standard of care for Lassa fever. Ribavirin is part of national treatment guidelines in endemic countries [4], is recommended by the WHO, is on the WHO list of essential medicines for viral haemorrhagic fevers, and has been in use for over 30 years [5]. It is the only drug therapy currently used to treat Lassa fever.

The evidence base for ribavirin in Lassa fever primarily relies on one clinical trial conducted in the early 1980s in Sierra Leone [6]. This trial concluded that ribavirin reduced mortality in Lassa fever and should be used in all patients [6]. However, this trial suffered from several serious flaws that render the results and conclusions of the trial unreliable [7]. More recent analysis of previously unavailable data from a larger cohort of Lassa fever patients in Sierra Leone suggests that ribavirin may actually increase mortality in some patients [7]. Two recent systematic reviews both came to the same conclusion, that there is insufficient evidence to support the routine use of ribavirin in Lassa fever patients and that there is an urgent need to re-evaluate the use of ribavirin in Lassa fever [8,9]. However, before embarking on expensive and logistically complex trials of ribavirin in Lassa fever, it is worth reviewing the pre-clinical data supporting the use of ribavirin in Lassa fever in order to help design and inform such trials. Here, we present and discuss the results of a systematic review of the pre-clinical data for ribavirin in Lassa fever as well as human pharmacokinetic (PK) data. The results of our review highlight that the data suffer from several serious limitations, which need to be addressed prior to any clinical trials of ribavirin or before trials of new anti-viral therapies in which ribavirin is used as control therapy.

Methods

Search terms

Pubmed and Embase were searched using the search term: Lassa AND (ribavirin OR virazole), up until the 27th of July 2021. There were no language restrictions. Non-English language articles were translated using a professional translation service. Two authors independently screened article titles and abstracts, reviewed full text articles, and extracted data. A third author resolved any disagreements. Articles were assessed according to specific domains. Here we define "pre-clinical" data as in-vitro data and animal efficacy and PK data. We also investigated human PK studies as the PK and pharmacodynamics (PD) of ribavirin in Lassa fever

patients are directly relevant to questions of dosing and target concentrations. The following inclusion criteria were applied for each domain:

- In-vitro data: studies presenting original data on the effect of ribavirin on LASV replication in-vitro. We excluded studies that used minigenome-based assays, though we did include studies that used recombinant LASV expressing fluorescent protein.
- Animal efficacy data: studies presenting original data on the effect of ribavirin on survival in animals experimentally infected with LASV in comparison to placebo or no treatment. We restricted articles to only those that investigated ribavirin monotherapy.
- Mechanism of action data: studies presenting original in-vitro or in-vivo data on the mechanism of action by which ribavirin acts in Lassa fever.
- Animal PK data: studies presenting original data on ribavirin concentrations in animals experimentally infected with LASV.
- Human PK data: studies presenting original data on ribavirin concentrations in humans with laboratory confirmed Lassa fever.

Data extraction

For each article, the following data were extracted: first author, year of publication, and PMID number. In addition, the following data were extracted for each specific domain:

- In-vitro studies: LASV strain, cell type, multiplicity of infection (MOI), timing and duration of ribavirin exposure, timepoint at which viral replication was assessed, EC50 (concentration of ribavirin resulting in a 50% reduction in viral replication), EC90 (concentration of ribavirin resulting in a 90% reduction in viral replication), CC50 (concentration of ribavirin resulting in a 50% reduction in cell viability), SI50 (selectivity index, a value that indicates the relationship between the compound's effective and toxic concentrations, calculated as CC50/EC50). Where EC50/90 were expressed as μM , they were converted into $\mu\text{g/ml}$.
- Animal studies: LASV strain, animal model, inoculation dose and route, sample size in ribavirin group, sample size in control group, dose and route and duration of ribavirin, time from inoculation to starting ribavirin (days), prophylaxis versus treatment (for the purpose of this review we considered treatment as initiation of ribavirin after symptom onset and prophylaxis as initiation of ribavirin before symptom onset since initiating drug therapy after symptom onset is more clinically relevant in humans as "treatment"), number of deaths in each group, whether ribavirin extended time to death, reduced peak viral titers, and reduced aspartate aminotransferase (AST), and any toxicity issues. We only extracted data on groups that received ribavirin monotherapy. Data were not extracted on groups that received ribavirin in combination with another antiviral.
- Mechanism of action data: LASV strain, human or animal data, study type (if human), sample size, experimental method, summary of findings.
- Animal PK data: LASV strain, animal model, experimental groups (i.e. infected and non-infected) dose, frequency, route and duration of ribavirin, matrix for concentration measurement, PK findings.
- Human PK data: country, experimental groups (i.e. infected and healthy), study type (e.g. cohort, series), LASV strain, sample size, dose, frequency, route and duration of ribavirin, matrix for concentration measurement, PK findings.

PK modelling

PK modelling was carried out to determine the concentration profiles of the different ribavirin regimens currently in use to treat Lassa fever patients in order to compare against the in-vitro EC50/90 data. PK parameters were based on those provided by Preston et al [10]. A three-compartment disposition model, with bolus injection, was used for concentration-time profile simulations. The three different regimens were the McCormick regimen (loading dose of 33 mg/kg followed by 16 mg/kg QDS from day 1 to day 4, and then 8 mg/kg from day 5 to 10 [6]), the Irrua non-pregnant regimen (loading dose of 100 mg/kg followed by 25 mg/kg OD from day 2 to 7 and then 12.5 mg/kg day 8 to 10 in non-pregnant adults), and the Irrua pregnant regimen (loading dose of 100 mg/kg followed by 16 mg/kg QDS from day 2 to 5 and then 8 mg/kg TDS from day 6 to 10 in pregnant adults [4]).

Results

Pubmed returned 157 results and Embase returned 336 results; 350 articles remained after removal of duplicates. Eleven studies presented in-vitro data (Table 1), eight animal data (Table 2), two mechanism of action data, and one human PK data. No study reported animal PK data.

All eleven in-vitro studies demonstrated an inhibitory effect of ribavirin on LASV replication, although the concentration required to demonstrate an effect varied considerably across

Table 1. Summary of in-vitro data.

AUTHOR	YEAR	STRAIN	CELL	MOI	Ribavirin exposure	TIME ASSESSED	EC50	EC90 µg/mL	CC50	SI
Jahrling [20]	1980	Josiah	Vero	NA	With inoculation	96 hours	NA*	NA	NP	NP
			RAM	NA	With inoculation	96 hours	NA*	NA	NA	NP
Oestereich [81]	2016	Ba366	Vero	0.01	1 hr post inoculation	72 hours	6.47 µg/ml	8.22	NA*	
Mudhasani [82]	2015	Josiah	Hela	0.1	2 or 16 hr pre inoculation	24 hours	2.31 µg/ml	NA	> 49	> 21
Günther [15]	2004	AV	Vero	0.01	1 hr post inoculation	48 hours	9 µg/ml	14 µg/ml	> 35 ug/ml	NA
Cai [14]	2018	eGFR Josiah	Vero	0.1	NA	48 hours	NA	7.27 µg/ml	NA	NA
			A549	0.1	NA	48 hours	4.05 µg/ml		NA	NA
				0.1	"	72 hours	9.86 µg/ml		"	"
			Hela	0.1	"	48 hours	1.20 µg/ml		"	"
				0.1	"	72 hours	2.29 µg/ml		"	"
			Huh7	0.1	"	48 hours	3.37 µg/ml		"	"
				0.1	"	72 hours	8.45 µg/ml		"	"
			Vero	0.1	"	48 hours	8.65 µg/ml	1.50 µg/ml^	"	"
				0.1	"	72 hours	21.72 µg/ml		"	"
			Tong [83]	2018	Josiah	Vero	0.1	1 hr post inoculation	NA	NA**
Kim [84]	2019	eGFR Josiah	A549	0.1	2 hr pre inoculation	48 hours	NA	NA	NA	NA
Mudhasani [85]	2014	Josiah	Hela	1	2 hr pre inoculation	24 hours	1.66 ug/ml	NA	NA	> 29.4
Olschlager [16]	2011	AV	Vero	0.01	1 hr post inoculation	48 hours	16 ug/ml	29 ug/ml	NA*	NA
Petkevich [86]	1981	Josiah	Vero	NA	NA	72–96 hours	NA***	NA	NA*	NA
Welch [13]	2016	eGFR Josiah	Huh7	0.1	1 hr post inoculation	48 hours	0.60 ug/ml	NA	> 12 ug/ml	> 4.88

Ribavirin exposure refers to the addition of ribavirin relative to infection of cells with Lassa virus. Time assessed refers to the time point at which viral titres were assessed after infection. Hr = hour. RAM = Rhesus alveolar macrophages. NA = not available. NP = not performed.

* 50 µg/ml ribavirin completely inhibited replication from -3.3 log₁₀ in vero cells and from -1.3 log₁₀ in Rhesus alveolar macrophages.

** 33uM reduced virus titer by 0.5 log₁₀ pfu/mL and 100uM reduced virus titer by 1.7 log₁₀ pfu/mL relative to controls.

*** 50ug/ml reduced viral titre from 3.3 to 1.7 x 10(3) PFU/ml. 100 ug/ml and 200 ug/ml completely inhibited.

^ did not significantly affected cell viability. ^ unclear why the EC90 is lower than the EC50.

<https://doi.org/10.1371/journal.pntd.0010289.t001>

Table 2. Percentage of time above the lower and upper range of the EC50/90 and above the EC50/90 means for the different ribavirin regimens based on PK modelling.

Time above target (%)	EC50			EC90		
	0.6	7	22	7	15	29
24hr						
McCormick	100	12.5	1.9	12.5	5.5	1.0
Irrua non-pregnant	100	7.0	3.8	7.0	4.7	3.2
Irrua pregnant	100	10.5	4.6	10.5	6.3	3.5
48hr						
McCormick	100	13.2	1.7	13.2	5.3	0.5
Irrua non-pregnant	100	5.7	2.5	5.7	3.4	1.9
Irrua pregnant	100	12.5	3.2	12.5	5.8	1.8
72hr						
McCormick	100	14.3	1.8	14.3	5.5	0.3
Irrua non-pregnant	100	5.2	2.1	5.2	2.9	1.5
Irrua pregnant	100	14.1	2.8	14.1	5.9	1.2

<https://doi.org/10.1371/journal.pntd.0010289.t002>

experiments and as a result of MOI, LASV strain, cell type, and time point after infection at which viral replication was assessed (Table 1), as well as the timing and duration of ribavirin exposure. LASV strains used were Josiah (6), eGFR Josiah (2), AV (2), and Ba366 (1). Cell types were Vero (10) (an immortalised African green monkey kidney epithelial cell line), Rhesus alveolar macrophage (1), Hela (1) (an immortalised human cervical cancer cell line), A549 (1) (an immortalised human alveolar adenocarcinoma basal epithelial cell line), and Huh7 (2) (an immortalised human hepatocyte carcinoma cell line). There were no in-vitro data on the effect of ribavirin against LASV in human macrophages, human dendritic cells, human monocytes, or human endothelial cells, the cell types thought to be the most commonly infected by LASV in humans [11,12]. The EC50 of ribavirin ranged from 0.6 µg/ml to 21.72 µg/ml and the EC90 from 1.5 µg/ml to 29 µg/ml. The EC50 was consistently higher in Vero (primate) cells relative to other (human) cells. Two studies used the same experimental setups of Josiah eGFR LASV, Huh7 cells, MOI of 0.1, and timepoint at which viral replication was assessed of 48 hours—one reported an EC50 of 0.60 µg/ml (Welch et al)[13], the other an EC50 of 3.37 µg/ml (Cai et al)[14], though it is unclear if these two studies used the same timing of ribavirin exposure. Two studies used the same experimental setups of AV LASV, Vero cells, MOI of 0.01, timing of ribavirin exposure, and same timepoint at which viral replication was assessed of 48 hours—one reported an EC50 of 9 µg/ml and EC90 of 14 µg/ml (Günther et al) [15], the other an EC50 of 16 µg/ml and EC90 of 29 µg/ml (Olschlager et al) [16]. One paper reported a lower EC90 (1.50 µg/ml) than EC50 (8.65 µg/ml) with eGFR Josiah at 48 hours and an MOI of 0.1 (Cai et al)[14]. This is likely to represent a typographical error in the paper, since an EC50 of 1.5 results in a predicted EC90 of 8.6 (using an approximate hill coefficient of 0.8)[17]. The EC50 range was 0.6–21.72 µg/ml, with a mean of 6.8 µg/ml and the EC90 range (discounting the 1.50 from Cai et al) was 7.27–29 µg/ml, with a mean of 14.6 µg/ml. Fig 1A–1F are the PK profiles of the three main ribavirin regimens in use in patients, whilst Table 2 presents the time above the ranges and means of EC50/90 for the different regimens within the first 72 hours of starting therapy. Except for the lower range of the EC50 (0.6 µg/ml), each of the three regimens result in little time spent above the EC50/90.

All eight studies that presented animal data investigated the effect of ribavirin as post-exposure prophylaxis, but only two [18,19] amongst these investigated the effects of ribavirin as treatment (where the initiation of ribavirin was clearly defined in the article as having started after the onset of symptoms) (Table 3). Most studies used Josiah strain (7/8). Only one study used the aerosolised route of inoculation, whilst others either used intramuscular,

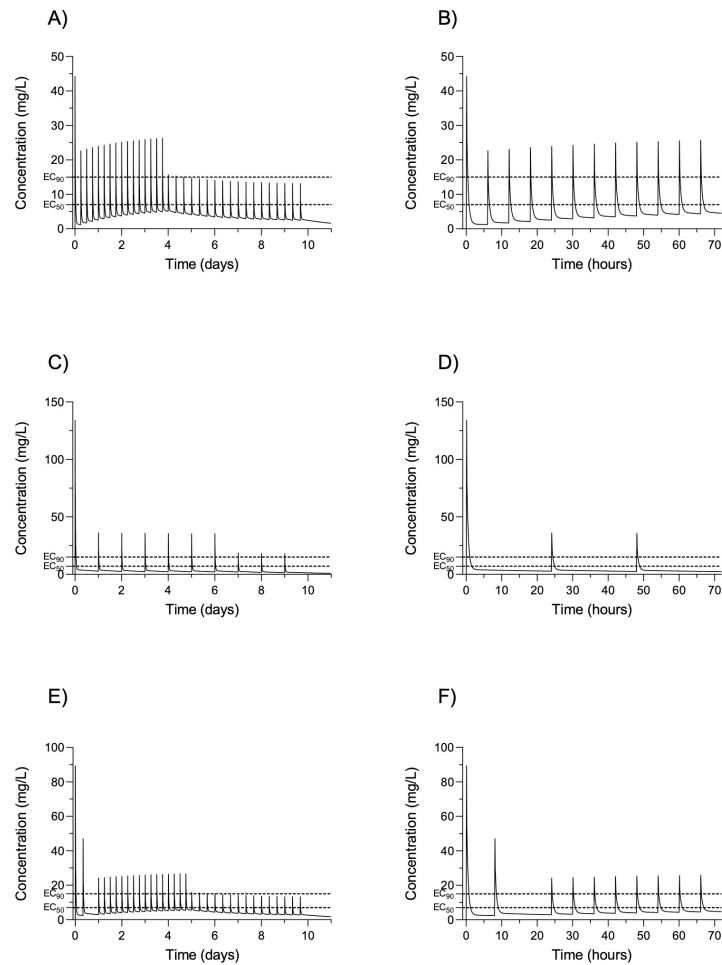


Fig 1. Pharmacokinetic simulations of the different ribavirin regimens. A) McCormick regimen day 0–10 and B) 0–72 hours. C) Irrua non-pregnant regimen day 0–10 and D) 0–72 hours. E) Irrua pregnant regimen day 0–10 and F) 0–72 hours. Dash lines represent mean EC50 and mean EC90.

<https://doi.org/10.1371/journal.pntd.0010289.g001>

subcutaneous or intraperitoneal routes and there were variations in inoculation dose. Samples sizes were small. Doses of ribavirin ranged from 20mg/kg to 230mg/kg for loading doses and 20mg/kg to 90mg/kg total daily maintenance doses in non-human primates (NHP), and from 25mg/kg to 160mg/kg total daily dose in rodents. There was no indication in any of these studies as to how these doses of ribavirin were derived, and there were no studies (within the domain of “Animal outcome” or “Animal PK”) on ribavirin PK in animal models of Lassa fever. In the NHP, prophylactic use of ribavirin resulted in 100% survival [18,20], 75% survival

Table 3. Summary of animal data.

PMID	YEAR	STRAIN	ANIMAL	RIBAVIRIN DOSE	RIBAVIRIN STARTED	DEATHS RIBAVIRIN	DEATHS CONTROLS	VIRAL TITRES	AST	TIME TO DEATH	TOXICITY
PROPHYLAXIS											
Jahrling [20]	1980	Josiah (1.2x10 ⁶ pfu SC)	Rhesus macaques	50 mg/kg loading, then 10 mg/kg tds 50 mg/kg, loading, then 10 mg/kg tds	Day 0 Day 5	0 (4) 0 (4)	6 (10)	↓ ↓	↓ ↓	NC NC	NR NR
Jahrling [18]	1984	Josiah (1.2x10 ⁶ pfu SC)	Cynomolgus macaques	75mg/kg loading, then 15mg/kg bd 75mg/kg loading, then 15mg/kg bd	Day 0 Day 4	0 (4) 0 (4)	13 (14)	↓ ↓	NA NA	NC NC	Anaemia Anaemia
Dvoretzkaia [19]	1990	Josiah (15–20 pfu AR)	Baboon hamadryas	Loading 20mg/kg, then 10mg/kg bd	Day 0	2 (5)	4 (4)	↓	NA	↑	NR
Fidarov [21]	1990	Josiah (3000 pfu IM)	Japanese macaques	Loading 75mg/kg, then 15mg/kg bd	Day 0	1 (4)	4 (4)	↓	NA	↑	NR
Cashman [87]	2011	Josiah (1000 pfu SC)	Hartley guinea pigs	25 mg/kg od	Day 0	8 (8)	7 (7)	↓	-	↑	NR
Safronetz [60]	2015	Josiah [^] (10 ⁵ TCID50 IP)	Hartley guinea pigs	50mg/kg od	Day 2	9 (9)*	6 (6)	↓	NA	↑	NR
Oestereich [81]	2016	Ba366 (1000 pfu IP)	<i>Ifnar-/-</i> B6 mice	80mg/kg od 80mg/kg od 80mg/kg bb	Day 0 Day 4 Day 4	5 (5) 5 (5) 4 (5)	8 (8)	- ↓ -	↓ ↓ ↓	↑ - ↑	NR NR NR
Lingas [22]	2021	Josiah (10 ⁴ TCID50 IM)	Cynomolgus macaques	Loading 30 mg/kg, then 10 mg/kg tds Loading 30 mg/kg, then 30 mg/kg od	Day 4 Day 4	4 (4)** 4 (4)**	8 (8)**	↓ ↓	NA NA	↑ ↑	NR NR
TREATMENT											
Jahrling [18]	1984	Josiah (1.2x10 ⁶ pfu SC)	Cynomolgus monkeys	75mg/kg loading, then 15mg/kg BD 150mg/kg loading, then 30mg/kg BD 230mg/kg loading, then 45mg/kg BD	Day 7 Day 7 Day 7	4 (8) 3 (4) 6 (6)	13 (14)	↓ NA NA	NA NA NA	↑ ↑ ↑	Anaemia Anaemia Anaemia
Dvoretzkaia [19]	1990	Josiah (15–20 pfu AR)	Baboon hamadryas	Loading 20mg/kg, then 10mg/kg BD	When feverish	4 (9)	4 (4)	↓	NA	↑	NR

NA not available. NC not applicable. NR none reported. SC subcutaneous. IM intramuscular. IP intraperitoneal. AR aerosolised. ↓ Reduced. ↑ Increased. - Unchanged.
* 3 of the 9 were euthanised due to clinical severity.

** Some animals euthanised due to clinical severity, numbers not indicated.

[^] Guinea pig adapted Josiah strain.

<https://doi.org/10.1371/journal.pntd.0010289.t003>

[21], 60% [19], and 0% survival [22] across the five studies (all Josiah strain). In the treatment NHP studies, ribavirin resulted in an approximate survival rate of 50% in one study (20mg/kg loading followed by 10mg/kg BD) [19], and survival rates of 50% at 75mg/kg loading followed by 15mg/kg and 0% at 230mg/kg loading followed by 45mg/kg BD in the other study [18]. Death at higher doses appeared to be due to severe anaemia [18]. In rodents, pre-exposure prophylaxis with ribavirin monotherapy had no effect on survival. There were no rodent studies that examined the effects of ribavirin treatment (as far as it was possible to deduce from the data and text as clearly having been started after symptom onset).

Only one study was identified which included human PK data in Lassa fever [23]. In this study, 5 patients received intravenous ribavirin and 4 received oral ribavirin. A competitive binding radioimmunoassay was used to measure ribavirin concentrations. Patients received either 1000mg QDS IV for 3 days or 1000mg/day PO for 10 days. Samples were collected 2.5 hours post dose, although it was unclear after which dose samples were collected, on which days, and how many samples were collected in total. Ribavirin concentrations ranged from 0.3–2.3 µg/ml (mean 0.8 µg) in the oral group and from 1.1–16.9 µg/ml in the IV group (mean 7.8 µg/ml). No PK parameters were presented due to the sparsity of the concentration data.

Two studies were identified which investigated the mechanism of action of ribavirin in Lassa. One in-vitro study investigated the effect of GTP depletion (a recognized mechanism of action of ribavirin against some other viruses via inhibition of inosine monophosphate dehydrogenase) on LASV replication [16]. Whilst ribavirin did inhibit viral replication, restoring the intracellular GTP pool by exogenous addition of guanosine had no effect on ribavirin's antiviral activity. The other study used mathematical models and experimental data in LASV-infected mice treated with ribavirin alone or in combination with favipiravir to explore different mechanisms of action for ribavirin [24]. Ribavirin appeared to protect infected cells from dying, thereby reducing the release of cell damage markers in the circulation, rather than impairing viral transmission, viral production, or enhancing the host's immune response.

Discussion

This systematic review reveals that the pre-clinical evidence-base for ribavirin use in the treatment of Lassa fever is conflicting and is largely insufficient by modern standards to warrant human trials in Lassa fever. Furthermore, no dose justification for the current ribavirin treatment regimens can be found.

The in-vitro data reviewed here show a wide range of EC_{50/90} for ribavirin against LASV, within and between different studies, even with similar experimental setups, and variations in EC_{50/90} according to cell type, MOI, and timing of ribavirin exposure. There was a 35-fold difference between the lowest and the highest EC₅₀ (range 0.6–21.72 µg/ml) and a 19-fold difference between the lowest and the highest EC₉₀ (range 1.5–29 µg/ml) (this lower range of the EC₉₀ should be interpreted with caution given that the EC₉₀ was lower than the EC₅₀ in the study which reported this). Based on PK modelling, as can be seen from Table 2 and Fig 1A–1F, these regimens do not come close to superseding the mean and upper range of EC_{50/90} for any significant amount of time.

Whilst all in-vitro studies demonstrated that ribavirin inhibited LASV replication, none of these studies used the human cell types thought to be most commonly infected by LASV and important in driving the pathophysiology of Lassa fever, namely macrophages, dendritic cells, monocytes and endothelial cells [11,12]. Whilst ribavirin has been shown to have broad spectrum antiviral effects across different viruses [25,26], these effects vary (and can be present or absent) according to the cell type, virus, concentration of ribavirin, and timing and duration of ribavirin exposure. Specifically, some cell types appear to be naturally resistant to the antiviral

effects of ribavirin. In some cells this appears to be due to reduced intracellular uptake of ribavirin as a result of decreased or absent transporter activity [27], whilst in others it appears to be due to differences in the intracellular metabolism of ribavirin [28]. For example, Shah et al showed that ribavirin inhibited vesicular stomatis virus (VSV) and Sendai virus (SV) in HeLa, HEp2 (human epidermal carcinoma cells) and BSRT7 cells (cells which are derived from BHK21) but had little effect on VSV and SV in BHK21 (Syrian golden hamster kidney fibroblast cells), Vero and A549 cells [28]. This effect was consistent across different ribavirin concentrations and exposures, and different MOI. This resistance to ribavirin's antiviral activity was not as a result of decreased uptake across the different cell types—all cell types showed similar levels of ribavirin uptake. Nor did this effect appear to be due to any differences in viral growth phenotype in different cells. Neither was there any suggestion of the emergence of viral resistance to ribavirin. However, the different cell types did show dramatic differences in the long-term accumulation of ribavirin. Specifically, BHK21, Vero and A549 cells showed markedly decreased levels of ribavirin accumulation, indicating differences in intracellular metabolism. In our review, we identified the EC50 of ribavirin for LASV as being consistently higher in Vero cells relative to other cells. More generally, ribavirin has effects on cellular physiology independent of viral infection, and this effect depends again on ribavirin exposure and cell type. For example, ribavirin can either have no effect, increase, or decrease macrophage nitric oxide production depending on the concentration, timing and duration of exposure [29,30]. Nitric oxide is thought to play a key role in the vascular pathophysiology of LASV [31,32]. Therefore, overall, it should not be assumed that ribavirin has anti-LASV effects in human mononuclear cells and/or endothelial cells in general or at equivalent doses to those currently used in humans without direct evidence, which is currently lacking. This needs to be tested experimentally. Going forward, standardization of experimental protocols more generally for in-vitro studies of anti-LASV agents would also be extremely helpful for comparison within and between agents.

The single published PK study of ribavirin in Lassa fever does not present sufficient ribavirin concentration data to be able to estimate ribavirin PK parameters in the context of Lassa fever [23]. The wide range of different EC50/90 also make it unclear which concentrations of ribavirin to aim for in any phase 2 PK/PD ribavirin modelling studies, a necessary step which has been suggested prior to any future trials of ribavirin [33]. However, as can be seen from Fig 1A–1C, which is a PK model of serum ribavirin concentrations using the “McCormick dosing” regimen based on PK parameters obtained from phase I studies of ribavirin in healthy volunteers [10], the plasma concentration of ribavirin falls rapidly after administration. According to this model, concentrations are not maintained above the majority of the documented EC50, let alone EC90, for most of the time during therapy. The findings are similar for modelling of the Irrua regimens. Concentrations probably need to be maintained above the EC90, and for most of the time (e.g. > 80% of the time), at least early on in treatment, for effective, rapid, and sustained viral suppression. However, ribavirin is a pro-drug and its antiviral effects are mediated by one or more of its active metabolites (ribavirin monophosphate, diphosphate and triphosphate) [34]. Therefore, likely of more relevance are the intracellular concentrations of ribavirin's metabolites, specifically in human mononuclear cells and/or endothelial cells. Indeed, this may be another explanation for why there is variation in the EC50/90 of ribavirin in the different experimental setups. The relationship between serum ribavirin concentrations and cellular metabolite concentrations depends, in part at least, on cell type and the differential expression of the enzymes responsible for the uptake and conversion of ribavirin into its metabolites as well the metabolism of the metabolites themselves. For example, Page et al found the ratio of ribavirin monophosphate, diphosphate and triphosphate to be 4:1:40 in human fibroblasts, 3:1:8 in lymphoblasts and 1:5:17 in erythrocytes [35].

Furthermore, different mechanisms of action have been proposed and investigated for the different ribavirin metabolites across different viruses [36]. Therefore, robust in-vitro data are needed on the EC50/90 of ribavirin as well as its metabolites in LASV-infected human mononuclear cells and/or endothelial cells. Future human PK/PD studies should also therefore involve the measurement of intracellular ribavirin metabolite concentrations in peripheral blood mononuclear cells (PMBC).

In both the in-vitro and animal studies, the most frequently used strain of LASV was Josiah. Josiah was isolated in Sierra Leone [37], whilst AV was identified in Côte d'Ivoire [38] and Ba366 in Guinea [39]. LASV has multiple different strains across seven distinct lineages. Lineages I-III and VI exist in Nigeria, whilst IV, V and VII exist in other West African countries [37–41]. Nigeria experiences by far the highest annual number of cases of Lassa fever in comparison to other West African countries, and yet there exists no in-vitro data on the effect of ribavirin on LASV replication from Nigerian lineages (nor animal data). There is high nucleotide sequence diversity across the different LASV lineages and strains [42]. It has been hypothesised that LASV strain may impact disease phenotype and severity in humans [43], and some animal data using different strains at identical doses and routes of inoculation do show differences in disease phenotype and outcome [44,45]. Although there were large differences in EC50/90 according to LASV strain in the different in-vitro studies we reviewed, there were no two studies that used the same combination of cell type, MOI, and timing of ribavirin exposure, but different LASV strains. Therefore, it is unknown whether the differences in EC50/90 between studies with different strains is potentially a result of LASV strain or the other described factors. This is worth investigating experimentally. It is not inconceivable that ribavirin may impact different LASV strains differently given the various hypothesised antiviral mechanisms of action of ribavirin (see below). There is up to 25% sequence variation in the LASV small segment (S) and 32% sequence variation in the large (L) segment across LASV lineage genomes [42,46]. The L segment encodes the viral RNA-dependent RNA polymerase [47], inhibition of which is one proposed mechanism of action of ribavirin [48,49]. The S segment encodes the nucleoprotein (NP). NP participates in the suppression of host interferon-type-I, as does the Z protein (encoded by L), both of which allow LASV to evade host immune sensing [50–52]. Data exists showing differences in the ability of NP across different strains to differentially inhibit hRIG-I function (an innate antiviral immune protein involved in interferon-type-I production) [53]. Modulation of interferon-stimulated gene responses is another hypothesised mechanism of action of ribavirin in viral infections [54,55]. In summary, data showing differences in ribavirin EC50/90 (and indeed other pre-clinical studies of compounds with antiviral activity against LASV) across different LASV strains might have important implications for Lassa fever treatment and implications for the choice of ribavirin dosing to test in an international clinical trial of ribavirin across different countries in West Africa. Such an international trial is preferred to a trial in a single country because of the sample sizes required. If LASV strain does impact ribavirin EC50/90, this could introduce significant heterogeneity.

The animal data supporting ribavirin as a treatment against symptomatic LASV disease have significant limitations. Only two studies clearly identified ribavirin being started after symptom onset, whilst the other six studies investigated ribavirin as prophylaxis (of which three were rodent models of Lassa—it is unclear how well these rodent models recapitulate human disease). There are many examples of anti-infective agents having a prophylactic effect on survival in animal models (or in-vitro for that matter and then no effect in animal models) but little or no effect on survival when started after symptom onset, for example favipiravir in the context of Ebola [56], and hydroxychloroquine and lopinavir/ritonavir in the context of COVID-19 [57,58]. Notably, in Lassa fever patients the median duration from symptom onset

to presentation at a health care facility is approximately 6 days (IQR 4–10 days) and the median duration to drug administration is approximately 8 days (IQR 7–13 days) [59]. The different NHP studies used different species of NHP, routes of inoculation of LASV, and different doses of ribavirin. Therefore, direct comparison between studies is not possible. The four NHP prophylaxis studies performed in 1980/90s showed a survival benefit whereas the one recent study [22] showed no survival benefit at all. This recent study used a similar dosing regimen as McCormick et al (30 mg/kg loading, then 10mg/kg TDS [6]), despite the fact the equivalent conversion dose in NHP would be substantially higher. This is a markedly lower loading dose than the 1980/90s studies (apart from the one study which used the aerosolized route of inoculation). The NHP model used in the recent study and the NHP models used in the 1980/90s appear to have similar disease course, which approximates that seen in humans (namely hepatitis, renal failure, respiratory distress and shock). Therefore, it is possible, though unclear (there were also differences in LASV inoculation dose and route), that the difference in survival is due to the lower dosing regimen. In contrast to several of the NHP studies, the prophylactic studies in rodents showed no effect of ribavirin on survival. Although in one of these rodent studies, guinea pigs treated with ribavirin were free of symptoms whilst ribavirin was continued but only succumbed to infection when ribavirin treatment ended. It is unclear whether continuing ribavirin in this specific study would have resulted in survival [60]. These rodent studies either used genetically modified rodents or required adaptation of the LASV to recapitulate severe disease, and it is unknown what effect this has on the drug efficacy for the reasons described above. Further, given that some cell types appear resistant to ribavirin's antiviral effects, it cannot be assumed that the absence of an effect in rodents represents an equivalent absence of effect in other species. We did not identify any in-vitro studies in our review that investigated the antiviral activity of ribavirin against LASV in rodent cell lines.

Amongst the two therapeutic studies, one used the aerosolised route of inoculation with one dosing regime and the other used the intramuscular route with three different dosing regimens. A total of 27 NHP received ribavirin across these two studies. Survival benefits were not that impressive, with an approximately 50% survival rate in the lower dose groups and 0% survival in the higher dose groups. This is, again, in the context of ribavirin being started early on in the disease course (e.g. on the day of fever development). At the higher doses, ribavirin appeared to directly result in death due to severe haemolytic anaemia, rather than failure to prevent progression of disease due to Lassa fever. Haemolytic anaemia is a recognised side effect of ribavirin due to the absence of 5'-nucleotidase and alkaline phosphatase in erythrocytes and therefore the accumulation of RTP, which results in oxidative membrane damage [61,62]. Ribavirin treatment results in a severe drop in haemoglobin in approximately 20% of Lassa fever patients [63]. This, in the context of a disease where patients are prone to bleeding [63] and in healthcare settings where there is often limited availability of blood transfusion [64]. The effect of ribavirin on erythrocyte survival is dose cumulative—RTP accumulates in circulating erythrocytes until dysfunctional erythrocytes are removed from the circulation [61]. Many people suffer from anaemia in West Africa as a result of malaria and nutritional deficiencies [65], and the risk of ribavirin induced morbidity (and potentially mortality) secondary to anaemia is even higher in such a population. Importantly, approximately 30% of Lassa fever patients require blood transfusions as a result of anaemia and this is rarely secondary to frank haemorrhage [63]. Specifically, defining to what extent anaemia is due to ribavirin therapy would be important to accrue before any trial as this would have important safety considerations. Overall, the animal data supporting ribavirin in Lassa fever are very limited as well as conflicting. Whether such data would support taking ribavirin into clinical trials in humans by today's regulatory standards, especially given toxicity concerns, is unclear.

Three different ribavirin regimens are used to treat Lassa fever in West Africa. The McCormick regimen, based on the one clinical trial that exists in Lassa fever (which notably suffers from numerous methodological flaws, including the use of historic controls, inclusion of pregnant women in the control group but not the ribavirin group, and post hoc merging of treatment groups [7]), uses a loading dose of 33 mg/kg followed by 16 mg/kg QDS from day 1 to day 4, and then 8 mg/kg TDS from day 5 to 10 [6]. Two other regimens are in use, primarily in Nigeria. The Irrua regimen uses a loading dose of 100 mg/kg followed by 25 mg/kg OD from day 2 to 7 and then 12.5 mg/kg day 8 to 10 in non-pregnant adults, and a loading dose of 100 mg/kg followed by 16 mg/kg QDS from day 2 to 5 and then 8 mg/kg TDS from day 6 to 10 in pregnant adults [4]. Some clinicians continue ribavirin beyond 10 days. There is no published data on how these three different ribavirin regimens were derived and it is unclear when the Irrua regimen began to be used in Nigeria. The total ribavirin dose across ten days is 417 mg/kg for the McCormick regimen, 287 mg/kg for non-pregnant Irrua regimen, and 476 mg/kg for the pregnant Irrua regimen. By comparison, the total dose across ten days in the Jahrling et al study [18] in *Cynomolgus* monkeys (in which ribavirin resulted in death) was 375 mg/kg at the lowest dose, 750 mg/kg at the medium dose and 1130 mg/kg at the highest dose, which, using FDA guidance [66], is equivalent to 122 mg/kg, 243 mg/kg, and 264 mg/kg respectively in humans. It is notable that the three different total ribavirin doses currently in use in humans exceed the NHP equivalent dose that resulted in severe haemolytic anaemia and 100% mortality. The highest dose used across the different regimens is in pregnant women (almost double the highest NHP equivalent). This regimen (which is without any basis) is in the context of there being no trial data for ribavirin in pregnant women at all (because they were excluded from the ribavirin arms but not the control arms in McCormick et al [6]) and in the context of ribavirin having known severe teratogenic effects [67,68]. This NHP data, and the recent analysis of previously unpublished human data suggesting that ribavirin may actually increase mortality in some Lassa patients [7] (see below), raise critical concerns about the safety of ribavirin in Lassa fever patients.

There are sparse data on any potential mechanisms of action of ribavirin in Lassa fever. Across multiple different viruses, numerous different antiviral mechanisms of action for ribavirin have been investigated and proposed [36], mediated by ribavirin's metabolites (monophosphate, diphosphate and triphosphate). Broadly speaking these can be divided into inhibition of (A) viral replication via 1. inhibition of IMPDH [69], 2. inhibition of mRNA capping [70], 3. induction of host spermidine-spermine acetyltransferase [71], 4. binding to viral RNA-dependent RNA polymerases and chain termination [49], 5. promotion of viral mutagenesis and error catastrophe [72], or (B) modulation of cellular immune function via modulation of 1. TH1 and TH2 balance [73], 2. modulation of interferon stimulated gene expression (55). The only robust clinical data for ribavirin in any viral infection in humans is for hepatitis C, where ribavirin is only effective at inducing viral clearance in combination with interferon- α [74,75]. Its mechanism of action in humans with hepatitis C appears not to be antiviral [76], despite in-vitro data demonstrating robust inhibition of hepatitis C replication (49). Rather, its effect appears to be immune modulatory [77]. The in-vitro antiviral effect of ribavirin against other specific viruses has also failed to translate into clinical success in human trials [78–80]. In this review, we identified a rodent study which suggested that ribavirin limited hepatitis in Lassa fever via a cell protective effect [24]. It is noteworthy that analysis of the previously unpublished US Army Lassa fever cohort data raises the possibility that ribavirin may actually increase mortality in patients with hepatic aspartate aminotransferase < 150 UI/mL [7]. Only in patients with AST > 300 UI/mL did ribavirin appear to be protective (although this does need to be interpreted with caution because of possible bias). One possibility is that ribavirin's effect in patients with high AST levels (if real) could be due to immune-modulatory or cell

protective effects. Further data supporting a non-antiviral effect of ribavirin in Lassa fever would be important to accrue as this might be a rationale for the testing of combination therapy with an effective antiviral agent in clinical trials.

Conclusion

This review summarizes the available pre-clinical data on ribavirin in Lassa fever and highlights several critical issues, both in terms of efficacy and safety, which together with concerns regarding the clinical data in humans add extra credence to the proposition that the routine use of ribavirin in Lassa fever should be reconsidered. Convincing the medical community that the use of a drug that has been standard clinical practice for decades may in fact be ineffective, or indeed be harmful, is challenging. However, further data is needed to support the continued use of ribavirin in Lassa fever. Specifically, the following steps should ideally be taken before any expensive and challenging clinical trials in which ribavirin's effectiveness is directly investigated or in which ribavirin is used as a control arm in trials of new antivirals: 1. in-vitro experiments investigating the antiviral activity of ribavirin and its active metabolites against LASV in human PBMC and/or endothelial cells across different LASV strains with standardization of MOI and timing of ribavirin exposure, 2. in-vitro experiments investigating the mechanism of action of ribavirin and its active metabolites in LASV infected human PBMC and/or endothelial cells, 3. at least one further in-vivo experiment in NHP investigating an equivalent ribavirin dosing regimen to that currently used in humans in West Africa 4. PK and PD studies of ribavirin in humans with Lassa fever investigating any correlations between ribavirin and ribavirin metabolite concentrations and viral load and expression of interferon stimulated genes and other relevant genes and proteins [88,89].

It is possible that we may be harming more patients than we are benefiting with the continued use of ribavirin in Lassa fever. Discussions need to be had about the continued use of ribavirin in Lassa fever and adequate investments made if the question of ribavirin's effectiveness is to be answered definitively.

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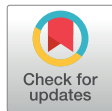
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RESEARCH ARTICLE

A standardised Phase III clinical trial framework to assess therapeutic interventions for Lassa fever

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OPEN ACCESS

Citation: Olayinka AT, Bourner J, Akpede GO, Okoeguale J, Abejegah C, Ajayi NA, et al. (2022) A standardised Phase III clinical trial framework to assess therapeutic interventions for Lassa fever. *PLoS Negl Trop Dis* 16(1): e0010089. <https://doi.org/10.1371/journal.pntd.0010089>

Editor: Michael R. Holbrook, NIAID Integrated Research Facility, UNITED STATES

Received: June 4, 2021

Accepted: December 14, 2021

Published: January 6, 2022

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Data Availability Statement: All relevant data are within the manuscript and its [Supporting Information](#) files.

Funding: This work was supported by the UK Foreign, Commonwealth and Development Office and Wellcome (215091/Z/18/Z) and the Bill & Melinda Gates Foundation (OPP1209135). This project is part of the EDCTP2 programme supported by the European Union (RIA2016E-1612 - African coalition for Epidemic Research, Response and Training (ALERT)). The funders

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Abstract

Background

Only one recommendation currently exists for the treatment of Lassa fever (LF), which is ribavirin administered in conjunction with supportive care. This recommendation is primarily based on evidence generated from a single clinical trial that was conducted more than 30 years ago—the methodology and results of which have recently come under scrutiny. The requirement for novel therapeutics and reassessment of ribavirin is therefore urgent. However, a significant amount of work now needs to be undertaken to ensure that future trials for LF can be conducted consistently and reliably to facilitate the efficient generation of evidence.

Methodology

We convened a consultation group to establish the position of clinicians and researchers on the core components of future trials. A Core Eligibility Criteria (CEC), Core Case Definition

had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

(CCD), Core Outcome Set (COS) and Core Data Variables (CDV) were developed through the process of a multi-stakeholder consultation that took place using a modified-Delphi methodology.

Results

A consensus position was achieved for each aspect of the framework, which accounts for the inclusion of pregnant women and children in future LF clinical trials. The framework consists of 8 core criteria, as well as additional considerations for trial protocols.

Conclusions

This project represents the first step towards delineating the clinical development pathway for new Lassa fever therapeutics, following a period of 40 years without advancement. Future planned projects will bolster the work initiated here to continue the advancement of LF clinical research through a regionally-centred, collaborative methodology, with the aim of delineating a clear pathway through which LF clinical trials can progress efficiently and ensure sustainable investments are made in research capacity at a regional level.

Author summary

Lassa fever (LF) is an acute viral haemorrhagic fever endemic to West Africa, causing an estimated 500,000 new infections and 10,000 deaths per year. At present, no treatment has been developed to specifically treat LF and only one prospective clinical trial has been conducted to evaluate the current treatment recommendation. Before a new era of LF clinical trials can begin, it is important to develop standardised methods and tools to ensure that trials are conducted in a consistent way and can generate reliable, comparable data. The aim of this project was to initiate the first step to improve the comparability of LF studies by developing a standardised Phase III clinical trial methodology for LF therapeutics that could be applied in low-resource settings. Through multi-stakeholder consultation, we established a consensus position of clinicians and researchers on the core components of future Phase III clinical trials: Core Eligibility Criteria (CEC), Core Case Definition (CCD), Core Outcome Set (COS) and Core Data Variables (CDV).

Introduction

Lassa fever (LF) is an acute viral haemorrhagic illness endemic to West Africa, causing an estimated 500,000 new infections and 10,000 deaths per year. [1] The main animal reservoir of the Lassa virus is the multimammate rat, *Mastomys natalensis*, through which transmission to humans can occur either from direct contact with the rat or indirect contact with contaminated food or household items. [2] Human-to-human transmission is most common in healthcare staff working in resource-limited settings where the supply of high quality protective equipment is suboptimal, preventing consistent adherence to infection prevention and control measures. [3]

The incubation period of LF is wide-ranging, with the onset of symptoms occurring typically between 6 and 21 days. [2] Symptoms are often non-specific and most commonly include fever alongside headache, vomiting and abdominal pain, with a small proportion of patients

presenting with clinically severe, life-threatening symptoms, such as seizure, breathing difficulty and shock. [4,5] In hospitalised cases, the case fatality rate (CFR) is reported to be 24–27% [6,7] overall and 34% for pregnant women [8]—however in a recent cohort study taking place in a research setting, the CFR for hospitalised cases was 13% for adults and 6% for children. [9]

Despite a weak evidence base, the primary treatment for LF is ribavirin in conjunction with supportive care. [4,10] This recommendation is largely based on the results of a single prospective clinical trial that was conducted in the 1980s, which has since generated concern about its methods, analysis and the safety of ribavirin when used to treat mild cases of LF. [11–13] The requirement for novel therapeutics, a small number of which have been identified as candidates to be tested, is therefore urgent. [14–16]

However, before a new era of LF clinical trials can begin, robust methods must be developed to ensure that trials are conducted in a consistent way and can generate reliable, comparable data. Given that clinical trial registries show no record of plans to conduct Phase III clinical trials for LF therapeutics in 2021, an opportunity has arisen to set the groundwork for well-conducted trials to begin.

The aim of this project is to improve the comparability of LF studies and accelerate the evaluation of LF therapeutics by outlining the framework—or establishing the foundations—on which future trials can be conducted. We convened a consultation group to establish the position of clinicians and researchers—those who have previous experience of LF patient care or research and who are most likely to be involved in the conduct of future trials—on the core components of LF clinical trials. Specifically, the group were asked to consider the fundamental requirements that a Phase III pivotal trial should incorporate in its eligibility criteria, case definition, outcome measures and data variables to evaluate new and existing therapeutics (Box 1).

This project represents the first step towards delineating the clinical development pathway for future LF therapeutics with the aim of guiding wider discussions within the West Africa Lassa fever Consortium (WALC). The WALC plans to build on the work undertaken in this project and expand the scope of this consultation to develop a pre-positioned protocol for Phase II/III clinical trials through consultation with researchers, industry, clinicians, regulators and ethics boards.

The development of COS is supported by the Core Outcome Measures in Effectiveness Trials (COMET) Initiative [17] and the methodology for developing COS is described in the

Box 1 –Definitions of the consultation outputs

The definitions of the four key outputs of the consultation are as follows:

Core Eligibility Criteria (CEC)—the characteristics of the study population

Core Case Definition (CCD)—how to identify a patient with confirmed Lassa fever

Core Outcome Set (COS)—what outcomes to measure in order to assess treatment efficacy

Core Data Variables (CDV)—list of recommended data variables for collection in all patients to standardise the characterisation of Lassa fever

COMET Handbook. [18] The group also recognised the value of developing additional outputs (CEC, CCD and CDV) that would improve the comparability of LF clinical trials.

Methodology

The project was conducted in two stages. First, a systematic review was carried out to describe the clinical characteristics and outcomes of LF and to guide the consultation process—the results of which have been reported separately. [5] These results generated an initial list of potential inclusion and exclusion criteria, case definitions, patient outcomes and data variables that were included in the first round of the consultation survey. Second, a stakeholder consultation was established to consider the key elements of a clinical trial framework for LF.

We have reported our methodology according to the COS-STAR (Core Outcome Set-STAR Standards for Reporting) Statement. [19]

Stakeholder consultation

A stakeholder group was established to collaborate on the development of the core criteria for future trials through a consensus-driven, modified Delphi process (Fig 1).

The consultation group consisted of experts in the field of LF, who had experience of LF patient care and/or who had been involved in clinical research for LF. The Nigeria Centre for Disease Control (NCDC) and the International Severe Acute Respiratory and emerging Infection Consortium (ISARIC) assembled the initial list of individuals and organisations that would form the consultation group. This group was expanded following additional proposals from invited members. In total 43 individuals were invited to participate in the consultation group, of whom four declined. The membership of the group covered 16 organisations across 6 countries (Fig 2). Of the 39 individuals who agreed to participate, 5 were involved in the central coordination, development and analysis of the consultation surveys and therefore did not complete the questionnaire to avoid bias.

Due to restrictions imposed as a result of the COVID-19 pandemic, it was not possible for the consultation to take place face-to-face as originally planned. The consultation therefore took place remotely over the course of one initial kick-off meeting, two modified Delphi consultations (with two follow-up meetings to discuss results) and one final consensus meeting.

The consultation was conducted using a modified Delphi methodology. The overarching structure of the consultation mirrored a typical Delphi methodology, in that: an expert panel was formed and two rounds of questionnaires were administered with the second questionnaire evolving from the responses to the first questionnaire; and a summary of the responses in each round was anonymously shared with the wider group.

The consultation departed from the traditional structure of a Delphi methodology as a small pilot of the first questionnaire was conducted ahead of the consultation to aid the development of relevant, clear questions and pre-defined responses. Further feedback on the clarity of the first questionnaire was also gathered at the consultation kick-off meeting. Panel meetings were also held between each survey round, in which members could discuss the complex themes that emerged from the survey. A consensus meeting was also held in lieu of a third questionnaire round for panel members to discuss the items that had not gained consensus in the first two rounds and cast final votes on items for inclusion in the framework. In these meetings, it was not possible to ensure the anonymity of participants during discussions; although anonymity was maintained throughout the voting process. The questionnaire used in the second round also used mixed response and scoring types.

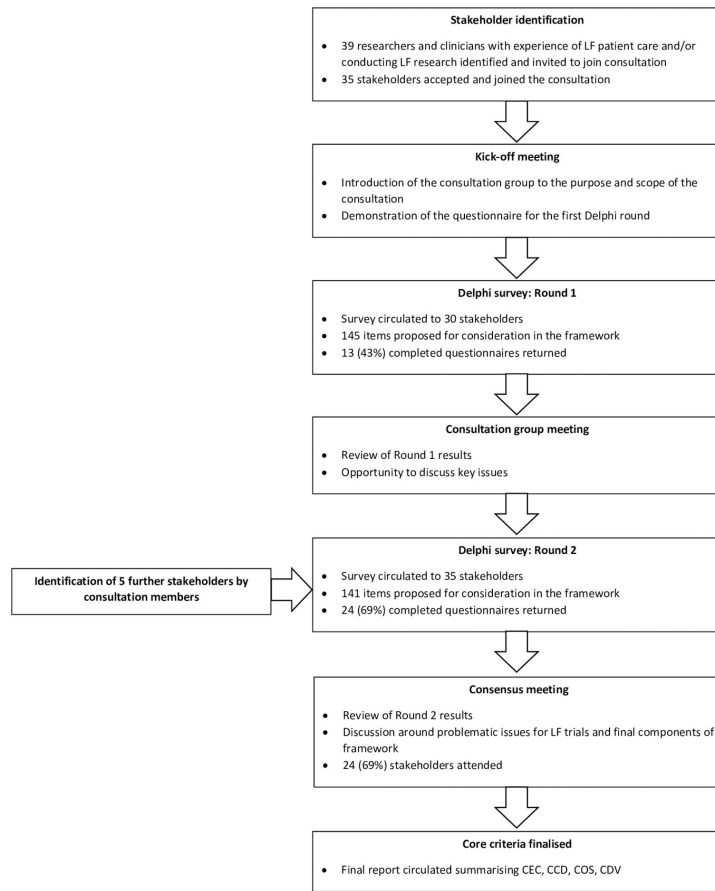


Fig 1. Flowchart illustrating the consultation process.

<https://doi.org/10.1371/journal.pntd.0010089.g001>

Kick-off meeting

The initial kick-off meeting was organised to explain the structure and scope of the consultation to the participating stakeholders and to demonstrate the questionnaire. Stakeholders were given the opportunity to provide feedback and raise questions about the consultation process. As a result of this meeting, minor modifications were made to the wording and formatting of the questionnaire to ensure clarity.

Delphi survey

A Delphi-type survey was used to gain consensus on the CEC, CCD, COS and CDV from the consultation group. The questionnaire was developed as an Excel spreadsheet on which

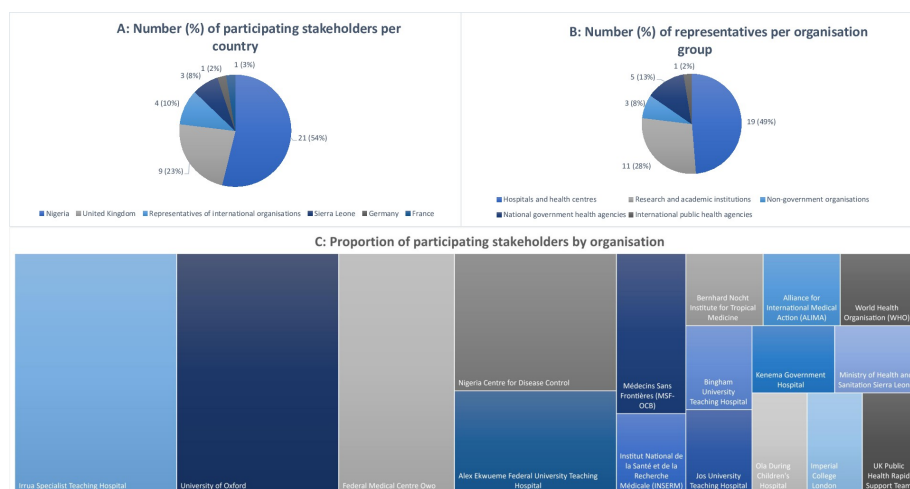


Fig 2. Participating stakeholder summary.

<https://doi.org/10.1371/journal.pntd.0010089.g002>

stakeholders could respond to the items proposed under each output using a Likert scale, provide additional items for inclusion and provide explanatory comments. An Excel-based questionnaire was chosen over online survey tools to allow participants flexibility to complete the questionnaire over multiple sessions and to maximise participation from members in locations where there weak or unstable internet connections exist.

Consensus was determined when 60% or more of the stakeholders selected the same response. Where 60% or more of the stakeholders stated “Strongly Agree” or “Agree”, items were taken forward to the next Delphi round for further assessment. Where 60% or more of the stakeholders stated “Strongly Disagree” or “Disagree”, items were excluded from the framework. At the end of two Delphi rounds, all remaining items gaining $\geq 60\%$ agreement for inclusion in the framework were taken forward to a consensus meeting for discussion and a final voting round where inclusion in the framework was determined by the highest vote above 60%.

The first questionnaire contained 145 items—whose inclusion was based on the results of the systematic review—for consideration across all four outputs and was circulated to all stakeholders for completion (S1; S2).

Limited decisions were made in the first round to refine the items for inclusion in the framework. To aid the decision-making process, the questionnaire was redeveloped using a mix of scoring systems for the second round and contained 141 items for consideration (S3).

All additional items proposed by stakeholders were eligible for inclusion in the next round of the consultation.

Following the analysis of each round, a study report was circulated to all stakeholders and included an anonymised summary of the results (% of stakeholders selecting each score, the group’s mean score and the scoring range) and additional comments provided by the group. These results were also presented to the group at post-survey meetings held between each round during which panel members could discuss issues raised during the survey.

Consensus meeting

A consensus meeting was held in lieu of a third Delphi-type round, in which the results of the second round were presented and stakeholders discussed and voted on the remaining items to be included in the framework. The final points requiring discussion were as follows: definition of 'clinical diagnosis of LF'; primary outcome measure and endpoint; measurement instruments for the assessment of 'Unfavourable Outcome'. Based on feedback from Round 2, workable proposals to navigate the key challenges around these discussion points were developed by the central coordination team as a starting point for discussion.

Following discussion, each proposal was presented using Zoom's polling function on which stakeholders cast an anonymous vote (S2). The poll could be adapted based on discussions and relaunched if amendments were considered necessary. Once all votes were cast, the results were shared (% votes for each option) and stakeholders were given the opportunity to discuss further. The selection gaining the highest number of votes over 60% was included in the framework.

Analysis

All analyses were performed using Excel. The results of each round were presented using descriptive statistics: N and % of each response per item.

All explanatory comments were presented to stakeholders for further consideration.

Results

Round 1

The questionnaire was circulated to 30 stakeholders for completion, 13 of whom returned a completed questionnaire by the deadline, representing a 43% response rate.

Of the 145 items included in this questionnaire, 108 (74%) met the consensus criteria and were taken forward in to the second round of the consultation (**Tables A–D in S1 Data**). 45 new items were proposed for consideration in the next round (**Table E in S1 Data**).

There were no differences in the scoring for pregnant women and children in Round 1, but additional suggestions were provided for these populations in the comments section (**Tables A–D in S1 Data**).

The results of Round 1 and the comments formed the basis of the discussions in the post-survey consultation meeting, during which the group agreed to reformulate the questionnaire and invite further key stakeholders.

Round 2

In Round 2, 5 stakeholders were added to the group. The questionnaire for Round 2 was therefore circulated to 35 stakeholders in total, 24 (69%) of whom returned a completed questionnaire.

Core eligibility criteria. Of the 33 items proposed for the Core Eligibility Criteria in Round 2, 10 items achieved consensus (**Tables G and H in S1 Data**). Across both rounds it was evident that stakeholders did not favour a restrictive list of exclusion criteria, but stated a preference for inclusive eligibility criteria that would not exclude patients on the basis of disease severity or comorbidity or limit recruitment.

Stakeholders agreed that inclusion should be based on a combination of clinical diagnosis and laboratory confirmation. The group also agreed that clinical diagnosis should be based on a list of pre-specified signs and symptoms, of which four (fever, sore throat, hearing loss and bleeding) achieved a consensus from a proposed list of 20. While consensus was achieved

according to the analysis plan for these items, the feasibility of identifying eligible patients for a clinical trial based on these four criteria alone was questioned by the analysis team. The earlier systematic review indicated that a low proportion of patients presented with these symptoms (except fever) on admission: sore throat– 44%; hearing loss– 4%; bleeding– 18%. [5] Due to potential low inclusions based on these criteria, the analysis team decided to raise this issue with the stakeholders at the next meeting.

Core case definition. Three of the eight proposed items achieved a consensus (Tables I–K in S1 Data). All stakeholders agreed that a confirmed case was to be defined on the same basis as the inclusion criteria (clinical diagnosis + laboratory confirmation). All stakeholders also agreed that laboratory confirmation of LF should be conducted using RT-PCR alone and 76% of the stakeholders agreed that sites without access to RT-PCR should not participate in a clinical trial.

Core outcome set. Of the 21 proposed outcome measures included in the survey, 4 achieved a consensus for consideration as a primary outcome measure: survival/mortality; progression to severe disease (as defined by presence of either renal failure, encephalopathy, shock, respiratory failure); unfavourable outcome (mortality + progression to severe disease); pregnancy outcome (for pregnant patients only) (Table L in S1 Data). In addition, stakeholders were asked to select one preferred primary outcome measure (Table M in S1 Data). While no individual primary outcome measure was selected by more than 60% of the stakeholders, “survival/mortality” was selected by 59%. One stakeholder commented that using “survival/mortality” as the primary outcome measure for a clinical trial may not be feasible due to the large sample size required to detect a treatment effect. The primary outcome measure and the feasibility of “survival/mortality” were therefore further debated at the next meeting.

The definitions of four clinical syndromes for inclusion in the characterisation of ‘progression to severe disease’ were also suggested. Two of the proposed syndromes achieved a consensus for their definitions (Acute Kidney Injury and Acute Respiratory Distress Syndrome) (Tables N and O in S1 Data). However, revisions were required for encephalopathy and shock due to the lack of an existing standardised definition and feasibility concerns for the proposed measurement instrument, respectively (Tables P and Q in S1 Data).

Core data variables. Finally, 21 core data variables were identified carried forward in to the consensus meeting (Table S in S1 Data).

Consensus meeting. Of the 35 stakeholders invited to attend the meeting, 24 participated representing an attendance rate of 69%.

Core eligibility criteria. Polling for the definition of ‘clinical diagnosis’ (or ‘clinical suspicion’ in section 1.1) was launched twice as no consensus was achieved in the first poll (Table U in S1 Data).

Following discussion and polling, 64% of the stakeholders agreed that clinical suspicion of LF should be based on “history of fever or presence of fever unresponsive to treatment for common illnesses AND at least one of the following: headache/weakness/back pain/joint pain/ dizziness/sore throat/bleeding/ abdominal pain/ vomiting/diarrhoea/vaginal bleeding/abortion or miscarriage/ unexplained intrauterine death/unexplained breast engorgement) (Table V in S1 Data). Members of the group specialising in obstetrics revised the wording relating to pregnancy-specific signs and symptoms following the meeting (Table 1).

Core outcome set. Sample size simulations were shown to the stakeholders to guide discussions about the feasibility of including survival/mortality as the primary outcome measure. It was widely agreed that the sample sizes presented would not be achievable for LF and, upon voting, the majority of stakeholders (83%) agreed that ‘unfavourable outcome’—a composite

Table 1. Core outputs and definitions included in the final framework.

Item	Criteria	Additional considerations
Core Eligibility Criteria		
Clinical suspicion of LF (non-pregnant adults and children)	Non-pregnant adults and children should be enrolled in clinical trials based on clinical suspicion of LF, defined as: <ul style="list-style-type: none"> • History of fever or presence of fever unresponsive to treatment for common illnesses • AND at least one of the following: headache; weakness; back pain; joint pain; dizziness; sore throat; bleeding; abdominal pain; vomiting; diarrhoea; seizures; haematuria or proteinuria on dipstick urinalysis 	At the point of enrolment, a sample should be taken for case confirmation by RT-PCR (see section 1.2) with results returned within 48 hours. Upon receipt of a negative test result for Lassa fever, patients should be immediately removed from the study.
Clinical suspicion of LF (pregnant women)	Pregnant women should be enrolled in clinical trials based on clinical suspicion of Lassa fever, defined as: <ul style="list-style-type: none"> • History of fever or presence of fever unresponsive to treatment for common illnesses • AND at least one of the following: headache; weakness; back pain; joint pain; dizziness; sore throat; bleeding; abdominal pain; vomiting; diarrhoea; breast swelling or engorgement; unexplained pregnancy loss (miscarriage or intrauterine death); seizures; haematuria or proteinuria on dipstick urinalysis 	
Inclusion criteria	Confirmed cases of Lassa fever	See Core Case Definition
Exclusion criteria	<ul style="list-style-type: none"> • Patients receiving end-of-life care for other concomitant conditions • Patients involved in another clinical trial 	Additional exclusion criteria will be based on study design and treatments involved.
Core Case Definition		
Definition of a confirmed case of LF	Patients who have a positive result on RT-PCR for Lassa fever	Sites that do not have the facilities or capacity to conduct RT-PCR do not qualify for conducting Phase III clinical trials.
Core Outcome Set		
Primary Outcome Measure	Unfavourable Outcome: a composite outcome consisting of mortality or deterioration from baseline assessed at Day 14	See Tables 2 and 3
Secondary Outcome Measures	<ul style="list-style-type: none"> • Presence of severe anaemia (defined according to: WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System (2011) Geneva, World Health Organization (WHO/NMH/NHD/MNM/11.1)) • Hearing loss • Pregnancy outcome • Time between start of treatment and discharge/recovery • Time between treatment start and delivery/miscarriage (only for trials enrolling pregnant patients) • Time between delivery/miscarriage and hospital discharge/recovery (only for trials enrolling pregnant patients) 	
Core Data Variables		
Signs, symptoms and assessments critical to evaluate treatment safety and/or efficacy	<ul style="list-style-type: none"> • Date of symptom onset • Date of contact with a confirmed case (optional) • Frank bleeding • Pregnancy complications (including vaginal bleeding in pregnancy and excessive bleeding in labour) • Level of consciousness (ACVPU or GCS, as specified in Tables 2 and 3) • Seizure • Temperature • Blood pressure • Creatinine • Urine output (if creatinine testing is not available) • Partial pressure of oxygen (PaO₂) • Fraction of inspired oxygen (FiO₂) • Oxygen saturation (SpO₂) • Blood urea nitrogen • Aspartate aminotransferase • Alanine transaminase • Pulse • Potassium • Haemoglobin • Point of care ultrasound (pregnant patients only) 	Data on other signs and symptoms (that meet the CTCAE definition for Grade 3 or Grade 4) or assessments that were conducted from the point of inclusion until patient outcome should also be collected.

<https://doi.org/10.1371/journal.pntd.0010089.t001>

primary outcome measure—is a more feasible primary outcome measure (**Table W in S1 Data**). 84% of stakeholders agreed that the assessment of the primary outcome measure should take place at 14 days post-enrolment (**Table X in S1 Data**).

Table 2. Definition of Unfavourable Outcome (adults).

Body system	Renal	Respiratory	Cardiovascular	Nervous
Pathology	AKI (Acute kidney injury)	ARDS (Acute respiratory distress syndrome)	Shock	Encephalopathy
Assessment method	Creatinine, Urine output	Arterial blood gas (ABG) analysis: PaO2 or Pulse oximetry: SpO2	Blood Pressure: Mean Arterial Pressure (MAP)	ACVPU [21]
Acceptable definitions	SOFA [22] 0–4 (creatinine test preferred; where creatinine testing is not available, urine output is acceptable provided it is measured accurately)	SOFA [22] 0–4 1. If ABG available: PaO2/FiO2 2. if ABG not available: SpO2/FiO2	1. SOFA [22] 0–4 (if inotropes used, dose recorded; if not available tick box 'not available')	ACVPU [21] + seizure

<https://doi.org/10.1371/journal.pntd.0010089.t002>

Measurement instruments for the four pathologies characterising 'unfavourable outcome' (Acute Kidney Injury, Acute Respiratory Distress Syndrome, shock, and encephalopathy) were proposed for evaluation.

64% of the group agreed that Acute Kidney Injury (AKI) should be assessed on the basis of either the Sequential Organ Failure Assessment (SOFA) score [20] or urine output alone (where creatinine testing is not possible) (Table Y in S1 Data).

68% of the stakeholders agreed that Acute Respiratory Distress Syndrome (ARDS) should be assessed by SOFA score using one of two methods (partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) or oxygen saturation (SpO2)/FiO2)) (Table Z in S1 Data).

The majority of stakeholders (76%) agreed that shock can be assessed by SOFA score (with the option to not record inotropes if unavailable) (Table AA in S1 Data).

Following discussions and multiple rounds of voting, stakeholders agreed that encephalopathy should be assessed on the basis of 'ACVPU + seizures' (Table AB in S1 Data).

The definition of 'unfavourable outcome' for each syndrome will be delineated in future work.

The group also agreed that an adapted assessment system for paediatric patients was required.

The final CEC, CCD, COS, CDV are presented in Tables 1–3.

Discussion

This project represents the first step towards delineating the clinical development pathway for new Lassa fever therapeutics, following a period of 40 years without advancement.

We have presented the consensus position of clinicians and researchers, from a range of health agencies, humanitarian organisations and academic institutions, on the core criteria for future pivotal clinical trials for LF. The multi-stakeholder approach is critical to prepare the groundwork for the collaborative and coordinated progression of LF trials and ensure the buy-in of potential investigators and treatment centres. Establishing these partnerships is crucial to avoid a piecemeal approach to conducting trials for a disease that requires an efficient coordinated research strategy. Prioritising an efficient clinical trial pathway will also optimise the use of the limited resources that are available to conduct studies of this nature.

The breadth of this framework also highlights the need to ensure key populations—specifically, pregnant women and children, who are disproportionately affected by LF and face increased risks—are included in the clinical development pathway for new therapeutics.

[8,24]

Table 3. Definition of Unfavourable Outcome (children).

Body system	Renal	Respiratory	Cardiovascular	Nervous
Pathology	AKI (Acute kidney injury)	ARDS (Acute respiratory distress syndrome)	Shock	Encephalopathy
Assessment method	Creatinine, Urine output	Arterial blood gas (ABG) analysis: PaO ₂ or Pulse oximetry: SpO ₂	Blood Pressure: Mean Arterial Pressure (MAP)	Paediatric GCS [23]
Acceptable definitions	pSOFA [22] 0–4 (creatinine test preferred; where creatinine testing is not available, urine output is acceptable provided it is measured accurately)	pSOFA [22] 0–4 1. If ABG available: PaO ₂ /FiO ₂ 2. if ABG not available: SpO ₂ /FiO ₂	1. pSOFA [22] 0–4 (if inotropes used, dose recorded; if not available tick box 'not available')	Paediatric GCS [23] +/- seizure (single prolonged >15 min, or multiple)

<https://doi.org/10.1371/journal.pntd.0010089.t003>

Limitations

The scope of this project was limited to collating the perspectives of clinicians and researchers. To increase the utility and specificity of the core criteria outlined above, obtaining input from regulators and ethics boards will be critical in future work. In particular, identifying suitable primary outcome measures for pivotal clinical trials for diseases, such as Lassa fever—that have low, sporadic case notification and low mortality, and which may be hampered by suboptimal surveillance systems—is challenging and will benefit from collaboration with these groups. The use of hard endpoints—those that are typically used in pivotal trials like mortality—are not feasible for LF due to the large sample size required. Therefore, discussions will need to be initiated about the acceptability of composite and surrogate endpoints.

Equally, due to restrictions enforced as a result of the COVID-19 pandemic, it was not possible to involve survivors of LF in the development of the framework at this stage. Funding has been secured to complete this activity and collect data on patients' expectations of LF clinical trials and therapeutics towards the end of 2021.

Future work

The West Africa Lassa fever Consortium (WALC) will progress the work described here through a multi-stakeholder, platform approach that aims to build capacity in regional research centres and support the sustainable uptake of future clinical trials by local research groups.

The stakeholders and scope of the consortium have been expanded beyond this project to include national and international regulators, ethics boards, health agencies and social scientists who, alongside clinicians and researchers, will generate a clinical development plan and a pre-positioned protocol for future pivotal Phase II/III LF trials, among other outputs. The core criteria developed within the scope of this project will therefore be reviewed by the consortium to obtain the necessary specificity for implementation in pharmaceutical trials aiming to establish the safety and efficacy of new drugs.

Social science studies have been incorporated in to the WALC's workplan to enhance the acceptability and uptake of the consortium's outputs among both patients and clinicians. The project will engage survivors of LF on topics such as their patient journey to enhance the eligibility criteria and inform the development of clinically relevant primary and secondary outcome measures.

Planned future work will also involve working directly with regulatory authorities to identify a clear regulatory pathway for the inclusion of pregnant women and children in clinical trials for novel therapeutics.

Overall the WALC aims to bolster the work initiated in this project to continue the advancement of LF clinical research through a regionally-centred, collaborative methodology and to delineate a clear pathway through which LF clinical trials can progress efficiently ensuring sustainable investments are made in research capacity at a regional level.

Supporting information

S1 Table. Delphi survey: Round 1.
(XLSX)

S2 Table. Extended methodology.
(DOCX)

S3 Table. Delphi survey: Round 2.
(XLSX)

S1 Data. Consultation results.
(DOCX)

Acknowledgments

The authors would like to thank Maurizio Solca for their input in to the development of the primary outcome measure.

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Annexe 3 : « supplementary data from EVISTA publication »

Necessary additional data

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Annexes

1. Supplementary tables

Table S1. Signs, symptoms, and vital signs at baseline and follow-up

	N	Baseline* n (%)	Follow-up** n (%)
Any type of bleeding symptom	711	116 (16%)	275 (39%)
Epistaxis	700	25 (4%)	56 (8%)
Hematemesis	698	22 (3%)	74 (11%)
Hematuria	699	8 (1%)	24 (3%)
Hemoptysis	697	7 (1%)	31 (4%)
Melena	700	26 (4%)	97 (14%)
Purpura	696	3 (0%)	7 (1%)
Conjunctival bleeding	699	18 (3%)	49 (7%)
Gingival bleeding	699	37 (5%)	85 (12%)
Venous puncture point bleeding	696	45 (6%)	139 (20%)
Any type of neurologic symptom	711	88 (12%)	264 (37%)
Agitation	700	44 (6%)	174 (25%)
Coma	701	50 (7%)	201 (29%)
Focal deficiency	696	7 (1%)	28 (4%)
Seizure	702	15 (2%)	103 (15%)
Confusion	700	43 (6%)	115 (16%)
Photophobia	657	10 (2%)	23 (4%)
Any type of systemic symptom	711	611 (86%)	669 (94%)
Anorexia	695	364 (52%)	484 (70%)
Asthenia	702	543 (77%)	640 (91%)
Articular pain	697	230 (33%)	288 (41%)
Headache	696	310 (45%)	400 (57%)
Myalgia	696	209 (30%)	309 (44%)
Any type of pulmonary symptom	711	180 (25%)	351 (49%)
Dyspnea	700	70 (10%)	225 (32%)
Cough	697	80 (11%)	162 (23%)
Chest pain	670	57 (9%)	89 (13%)
Any type of digestive symptom	711	525 (74%)	610 (86%)
Diarrhea	699	352 (50%)	525 (75%)
Dysphagia	695	148 (21%)	237 (34%)
Hiccup	699	23 (3%)	62 (9%)
Jaundice	701	11 (2%)	28 (4%)
Nausea	698	278 (40%)	404 (58%)
Vomiting	698	305 (44%)	453 (65%)
Abdominal pain	694	323 (47%)	451 (65%)
Splenomegaly / Hepatomegaly	684	12 (2%)	24 (4%)
Vital signs			

Respiratory rate \geq 24 breaths per minute	672	330 (49%)	383 (57%)
Heart rate \geq 110 per minute	673	211 (31%)	441 (66%)
Body temperature $>$ 38.5 °C	684	155 (23%)	408 (60%)
Diastolic Arterial Pressure $<$ 60 mmHg	642	191 (30%)	489 (76%)
Systolic Arterial Pressure $<$ 90 mmHg	644	139 (22%)	370 (57%)
SpO ₂ $<$ 92%	665	95 (14%)	333 (50%)
Mean Arterial Pressure $<$ 65 mmHg ⁽¹⁾	635	94 (15%)	343 (54%)
Glycaemia $<$ 50 mg/dl	649	77 (12%)	229 (35%)
Level of consciousness (ACPVU classification): ⁽²⁾	677		
Alert		594 (88%)	439 (65%)
Impaired		83 (12%)	238 (35%)

Footnotes to Table S1

*Baseline = on admission

**Follow-up = entire follow-up, including admission

⁽¹⁾ Mean Arterial pressure = $2/3$ Diastolic Arterial Pressure + $1/3$ Systolic Arterial Pressure

⁽²⁾ ACPVU classification: A: Alert; C: new confusion; V: responsive to voice; P: responsive to pain, U: Unresponsive

Table S2. Association of selected baseline characteristics with death

	All (N = 711)	Died (N = 323)	Survived (N = 388)	P
Age				0.560
$<$ 2 yr	44 (7%)	21 (7%)	23 (6%)	
2 to 5 yr	41 (6%)	22 (7%)	19 (5%)	
6 to 12 yr	48 (7%)	20 (7%)	28 (8%)	
13 to 18 yr	53 (8%)	27 (9%)	26 (7%)	
19 to 49 yr	406 (60%)	174 (57%)	232 (63%)	
\geq 50 yr	84 (12%)	42 (14%)	42 (11%)	
Sex				0.140
Female	409 (58%)	196 (61%)	213 (55%)	
Male	302 (42%)	127 (39%)	175 (45%)	
Patient-reported vaccination with rVSV				$<$ 0.001
No	358 (67%)	177 (77%)	181 (60%)	
Yes	175 (33%)	53 (23%)	122 (40%)	
Time from vaccination to admission (days)				0.183
$>$ 10	41 (31%)	15 (41%)	26 (27%)	
\leq 10	93 (69%)	22 (59%)	71 (73%)	
Time from 1st symptom to admission (days)				0.001
$<$ 5	384 (55%)	140 (45%)	244 (64%)	
6 to 9	237 (34%)	129 (41%)	108 (28%)	
\geq 10	76 (11%)	45 (14%)	31 (8%)	
Number of symptoms at admission				0.001
\leq 5	428 (60%)	143 (44%)	285 (73%)	
6 to 10	253 (36%)	151 (47%)	102 (26%)	
$>$ 10	30 (4%)	29 (9%)	1 (0%)	

mNEWS2 ⁽¹⁾				0.001
≤ 4	357 (72%)	124 (60%)	233 (81%)	
> 4	139 (28%)	84 (40%)	55 (19%)	
Hematology				
Hemoglobin, g/dl				0.672
< 10	29 (10%)	10 (8%)	19 (10%)	
≥ 10	272 (90%)	110 (92%)	162 (90%)	
Hematocrit, %				0.194
< 30	22 (7%)	12 (10%)	10 (6%)	
≥ 30	276 (93%)	105 (90%)	171 (94%)	
Platelets, G/l				<0.001
< 150	158 (54%)	40 (35%)	118 (66%)	
≥ 150	136 (46%)	75 (65%)	61 (34%)	
Leucocytes, G/l				<0.001
< 4	59 (20%)	5 (4%)	54 (30%)	
4 to 10	103 (34%)	22 (18%)	81 (45%)	
> 10	138 (46%)	92 (77%)	46 (25%)	
Renal function				
Creatinine, mg/dl				0.001
< 1.2	277 (51%)	54 (23%)	223 (71%)	
1.2 to 4	149 (27%)	82 (35%)	67 (21%)	
> 4	122 (22%)	96 (41%)	26 (8%)	
Liver function				
ALT, U/l				0.001
< 47	118 (22%)	15 (7%)	103 (33%)	
47 to 235	164 (30%)	35 (15%)	129 (41%)	
> 235	262 (48%)	178 (78%)	84 (27%)	
AST, U/l				<0.001
< 38	16 (4%)	4 (5%)	12 (4%)	
38 to 190	138 (37%)	15 (17%)	123 (42%)	
> 190	224 (59%)	69 (78%)	155 (53%)	
Bilirubin, mg/dl				0.001
< 1.6	433 (85%)	142 (70%)	291 (94%)	
1.6 to 2.9	58 (11%)	50 (25%)	8 (3%)	
> 2.9	20 (4%)	11 (5%)	9 (3%)	
Electrolytes and biochemistry				
CRP, mg/l				<0.001
< 50	283 (55%)	77 (35%)	206 (69%)	
≥ 50	234 (45%)	142 (65%)	92 (31%)	
CPK, U/l				<0.001
< 400	194 (37%)	37 (17%)	157 (53%)	
400 to 800	86 (17%)	39 (18%)	47 (16%)	
> 800	239 (46%)	144 (65%)	95 (32%)	
Sodium, mmol/L				0.004
< 130	183 (33%)	96 (41%)	87 (27%)	
130 to 145	353 (64%)	132 (56%)	221 (70%)	
> 145	15 (3%)	6 (3%)	9 (3%)	
Potassium, mmol/L				<0.001

< 3.5	75 (16%)	30 (17%)	45 (16%)	
3.5 to 5	303 (65%)	86 (48%)	217 (75%)	
> 5	91 (19%)	65 (36%)	26 (9%)	
Amylase, U/l				<0.001
< 100	245 (46%)	69 (30%)	176 (58%)	
100 to 150	118 (22%)	46 (20%)	72 (24%)	
> 150	171 (32%)	113 (50%)	58 (19%)	
Corrected calcemia, mmol/l ⁽²⁾				0.021
< 2.20	167 (30%)	86 (36%)	81 (26%)	
2.20 to 2.45	260 (47%)	99 (42%)	161 (51%)	
> 2.45	127 (23%)	52 (22%)	75 (24%)	
Albumine, g/l				0.001
< 25	166 (30%)	122 (51%)	44 (14%)	
25 to 35	324 (58%)	106 (45%)	218 (69%)	
> 35	66 (12%)	10 (4%)	56 (18%)	
Glycemia, mg/dl				<0.001
< 73	102 (18%)	59 (25%)	43 (14%)	
73 to 118	304 (55%)	107 (45%)	197 (62%)	
> 118	150 (27%)	72 (30%)	78 (25%)	
Virology				
EBOV RT-PCR NP Ct value				<0.001
≤ 22	356 (54%)	252 (84%)	104 (29%)	
> 22	299 (46%)	48 (16%)	251 (71%)	
EBOV RT-PCR GP Ct value				<0.001
≤ 22	83 (13%)	82 (27%)	1 (0%)	
> 22	561 (87%)	217 (73%)	344 (100%)	

Footnotes to Table S2

⁽¹⁾ NEWS2 score: National Early Warning Score 2nd version; mNEWS2: modified NEWS2 score without respiratory rate

⁽²⁾ Corrected calcemia = measured calcemia – 0.025*(albuminemia – 40); measured calcemia in mmol/L, albuminemia in g/L

Table S3. Selected baseline characteristics, treatment and outcome by vaccination status

	Not vaccinated (N = 358)	Vaccinated (N = 175)	p.overall
Baseline, median [IQR]			
Age (years)	25 [16;39]	30 [23;40]	0.004
Time from 1st symptom to admission (days)	5 [3;7]	3 [2;4]	<0.001
mNEWS2	3 [2;5]	3 [2;4]	0.026
Creatinine, mg/dl	1.3 [0.7;3.8]	1.0 [0.8;2.4]	0.151
ALT (U/l)	268 [62;663]	116 [48;380]	<0.001
Leucocytes (G/l)	11 [6;22]	6 [4;15]	0.001
EBOV RT-PCR NP Ct value	22 [19;27]	24 [21;29]	<0.001
Treatment, n (%)			

Investigational treatment received	312 (87%)	171 (98%)	<0.001
Outcome			
Dead, no. (%)	177 (49%)	53 (30%)	<0.001
Time from admission to death (days), median [IQR]	3 [1;4]	4 [2;7]	0.035
Alive, no. (%)	181 (51%)	122 (70%)	<0.001
Time from admission to discharge (days), median [IQR]	18 [14;23]	16 [14;20]	0.015

Table S4. Treatment and outcome for children (5 years or less at admission)

	≤ 28 days* (N = 6)	29 days to 2 yr (N = 52)	3 to 5 yr (N = 27)
Treatment, n (%)			
Investigational treatment received	6 (100%)	40 (77%)	19 (70%)
MAb114	4 (67%)	18 (47%)	8 (42%)
Regeneron	0 (0%)	12 (32%)	4 (21%)
Remdesivir	2 (33%)	4 (11%)	3 (16%)
Zmapp	0 (0%)	4 (11%)	4 (21%)
Outcome			
Dead, no. (%)	2 (33%)	27 (52%)	14 (52%)
Time from admission to death (days), median [IQR]	9 [9;9]	1 [1;4]	1 [1;6]
Alive, no. (%)	4 (67%)	25 (48%)	13 (48%)
Time from admission to discharge (days), median [IQR]	26 [13;40]	19 [13;27]	15 [11;22]

Table S5. Selected baseline characteristics, treatment and outcome by viral load at admission

	Np Ct ≤ 22 (N = 310)	Np Ct > 22 (N = 345)	p.overall
Baseline, median [IQR]			
Age (years)	25 [14;39]	28 [19;39]	0.077
Time from 1st symptom to admission (days)	5 [3;7]	3 [2;7]	<0.001
mNEWS2	4 [2;6]	3 [1;4]	<0.001
Creatinine, mg/dl	2.0 [0.9;5.0]	0.9 [0.6;1.8]	<0.001
ALT (U/l)	532 [269;906]	80 [39;216]	<0.001
Treatment, n (%)			
Investigational treatment received	249 (81%)	329 (95%)	<0.001
Outcome			
Dead, no. (%)	232 (75%)	68 (20%)	<0.001
Time from admission to death (days), median [IQR]	2 [1;4]	3 [2;7]	0.001
Alive, no. (%)	78 (25%)	277 (80%)	<0.001
Time from admission to discharge (days), median [IQR]	24 [19;32]	16 [13;20]	<0.001

Table S6. Selected baseline characteristics and outcome by treatment status

	No investigational treatment (N = 87)	Investigational treatment received (N = 623)	p.overall
Baseline, median [IQR]			
Age (years)	21 [10;35]	28 [18;39]	0.014
Time from 1st symptom to admission (days)	5 [3;9]	4 [2;7]	0.001
mNEWS2	6 [4;9]	3 [2;5]	<0.001
Creatinine, mg/dl	3.2 [1.1;7.3]	1.1 [0.7;3.5]	0.003
ALT (U/l)	538 [196;933]	201 [57;560]	0.006
EBOV RT-PCR NP Ct value	19 [17;21]	23 [20;28]	<0.001
Outcome			
Dead, no. (%)	79 (91%)	243 (39%)	<0.001
Time from admission to death (days), median [IQR]	1 [1;1]	3 [2;6]	<0.001
Alive, no. (%)	8 (9%)	380 (61%)	<0.001
Time from admission to discharge (days), median [IQR]	8 [5;10]	17 [14;22]	<0.001

Table S7. Selected baseline characteristics and outcome by treatment program

	MEURI (N = 170)	PALM RCT (N = 453)	p.overall
Baseline, median [IQR]			
Age (years)	30 [20;39]	26 [17;39]	0.120
Time from 1st symptom to admission (days)	4 [2;7]	4 [2;7]	0.290
mNEWS2	3 [2;4]	3 [2;5]	0.337
Creatinine, mg/dl	1.1 [0.7;2.1]	1.1 [0.7;3.8]	0.835
ALT (U/l)	156 [68;391]	216 [52;622]	0.235
Treatment, n (%)			
Mab114	50 (30%)	147 (32%)	
Regeneron	61 (37%)	130 (29%)	
Remdesivir	28 (17%)	94 (21%)	
Zmapp	25 (15%)	82 (18%)	
Outcome			
Dead, no. (%)	52 (31%)	191 (42%)	0.011
Time from admission to death (days), median [IQR]	3 [2;5]	3 [2;6]	0.069
Alive, no. (%)	118 (69%)	262 (58%)	
Time from admission to discharge (days), median [IQR]	17 [14;20]	18 [15;23]	0.036

Table S8. Selected baseline characteristics and outcome by treatment

	MAb114 (N = 197)	REGN-EB3 (N = 191)	REMEDSIVIR (N = 122)	ZMAPP (N = 107)
Baseline, median [IQR]				
Age (years)	27 [13;38]	28 [20;38]	30 [19;42]	26 [19;40]
Time from 1st symptom to admission (days)	4 [2;7]	4 [2;6]	3 [2;6]	4 [2;6]
mNEWS2	3 [2;4]	3 [2;5]	3 [2;4]	3 [2;4]
Creatinine, mg/dl	1.0 [0.7;3.2]	1.2 [0.7;3.5]	1.2 [0.7;3.3]	1.2 [0.8;4.7]
ALT (U/l)	224 [58;580]	202 [67;528]	184 [48;514]	172 [45;664]
Outcome				
Dead, no. (%)	68 (35%)	66 (35%)	54 (44%)	54 (50%)
Time from admission to death (days), median [IQR]	3 [2;5]	2 [1;5]	4 [2;6]	3 [2;6]
Alive, no. (%)	129 (65%)	125 (65%)	68 (56%)	53 (50%)
Time from admission to discharge (days), median [IQR]	17 [14;22]	18 [15;21]	18 [15;21]	18 [15;23]

2. PREDS Score development and validation

Step 1: model coefficients, means/proportions, adjusted 28-day survival rate in the training sample

Risk factor	Regression coefficient	Mean or proportion
ALT at admission (+/-2 days) > 5N (U/L)	0.9097955	0.4444444
Creatinine at admission (+/-2 days) (mg/dL)	0.1710014	2.3788530
mNEWS2 > 4	0.8385998	0.3835125
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	0.9401694	0.4193548
Age at admission (categorical) (years)	0.4066104	0.1362007
Delay between 1st symptom onset and admission (categorical) (days)	0.3674715	0.5268817

Means are reported for continuous variables. For categorical variables, the proportions of patients on the non-reference category are reported.

Adjusted Kaplan-Meier estimate of the average 28-day survival rate in the training sample = 0.9044

Step 2: Risk factors, categories, and reference values

Risk factor	Categories	Reference
Creatinine at admission (+/-2 days) (mg/dL)	<= 1.3	0.75
Creatinine at admission (+/-2 days) (mg/dL)	> 1.3	6.26
ALT at admission (+/-2 days) > 5N (U/L)	0	0.00
ALT at admission (+/-2 days) > 5N (U/L)	1	1.00
mNEWS2 > 4	0	0.00
mNEWS2 > 4	1	1.00
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	0	0.00
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	1	1.00
Age at admission (categorical) (years)	< 50	0.00
Age at admission (categorical) (years)	>= 50	1.00
Delay between 1st symptom onset and admission (categorical) (days)	< 4	0.00
Delay between 1st symptom onset and admission (categorical) (days)	>= 4	1.00

To determine the reference values for the categories of creatinine (i.e., mid-points), we use the 1st percentile and the 99th percentile to minimize the influence of extreme values.

Step 3: Referent risk profile Reference value

Risk factor	Categories	Reference	Referent risk profile
Creatinine at admission (+/-2 days) (mg/dL)	<= 1.3	0.75	0.75
Creatinine at admission (+/-2 days) (mg/dL)	> 1.3	6.26	0.75
ALT at admission (+/-2 days) > 5N (U/L)	0	0.00	0.00
ALT at admission (+/-2 days) > 5N (U/L)	1	1.00	0.00
mNEWS2 > 4	0	0.00	0.00
mNEWS2 > 4	1	1.00	0.00
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	0	0.00	0.00
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	1	1.00	0.00

Age at admission (categorical) (years)	< 50	0.00	0.00
Age at admission (categorical) (years)	>= 50	1.00	0.00
Delay between 1st symptom onset and admission (categorical) (days)	< 4	0.00	0.00
Delay between 1st symptom onset and admission (categorical) (days)	>= 4	1.00	0.00

Step 4: Distance from base category

We now compute how far each category of each risk factor is from the base category in terms of regression units using $\beta_i(W_{ij}-W_{iREF})/\beta_i(W_{ij}-W_{iREF})$

Risk factor	Categories	Reference	Referent risk profile	Regression coefficient	Distance
ALT at admission (+/-2 days) > 5N (U/L)	0	0.00	0.00	0.9097955	0.0000000
ALT at admission (+/-2 days) > 5N (U/L)	1	1.00	0.00	0.9097955	0.9097955
Age at admission (categorical) (years)	< 50	0.00	0.00	0.4066104	0.0000000
Age at admission (categorical) (years)	>= 50	1.00	0.00	0.4066104	0.4066104
Creatinine at admission (+/-2 days) (mg/dL)	<= 1.3	0.75	0.75	0.1710014	0.0000000
Creatinine at admission (+/-2 days) (mg/dL)	> 1.3	6.26	0.75	0.1710014	0.9422178
Delay between 1st symptom onset and admission (categorical) (days)	< 4	0.00	0.00	0.3674715	0.0000000
Delay between 1st symptom onset and admission (categorical) (days)	>= 4	1.00	0.00	0.3674715	0.3674715
mNEWS2 > 4	0	0.00	0.00	0.8385998	0.0000000
mNEWS2 > 4	1	1.00	0.00	0.8385998	0.8385998
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	0	0.00	0.00	0.9401694	0.0000000
EBOV RT-PCR Ct value at admission (+/-2 days) <= 22 (Cycles)	1	1.00	0.00	0.9401694	0.9401694

Step 5: Set the constant, B

We now define the constant for the points system, or the number of regression units that will correspond to one point. Here we let B reflect the increase in risk associated with a 2-unit (mg/dL) increase in creatinine.

B = 0.3420028

Step 6: Determine points associated with each of the categories of the risk factors

We now determine the points associated with each category of each risk factor using

$$\text{Points}_{ij} = \beta_i(W_{ij} - W_{iREF}) / B$$

Note: the results of the operation above are rounded to the nearest integer to obtain the points.

Risk factor	Categories	Reference	Referent risk profile	Regression coefficient	Distance	Points
ALT at admission (+/- 2 days) > 5N (U/L)	0	0.00	0.00	0.9097955	0.0000000	0
ALT at admission (+/- 2 days) > 5N (U/L)	1	1.00	0.00	0.9097955	0.9097955	3
Age at admission (categorical) (years)	< 50	0.00	0.00	0.4066104	0.0000000	0
Age at admission (categorical) (years)	>= 50	1.00	0.00	0.4066104	0.4066104	1
Creatinine at admission (+/- 2 days) (mg/dL)	<= 1.3	0.75	0.75	0.1710014	0.0000000	0
Creatinine at admission (+/- 2 days) (mg/dL)	> 1.3	6.26	0.75	0.1710014	0.9422178	3
Delay between 1st symptom onset and admission (categorical) (days)	< 4	0.00	0.00	0.3674715	0.0000000	0
Delay between 1st symptom onset and admission (categorical) (days)	>= 4	1.00	0.00	0.3674715	0.3674715	1
mNEWS2 > 4	0	0.00	0.00	0.8385998	0.0000000	0
mNEWS2 > 4	1	1.00	0.00	0.8385998	0.8385998	2
EBOV RT-PCR Ct value at admission (+/- 2 days) <= 22 (Cycles)	0	0.00	0.00	0.9401694	0.0000000	0
EBOV RT-PCR Ct value at admission (+/- 2 days) <= 22 (Cycles)	1	1.00	0.00	0.9401694	0.9401694	3

Step 7: Determine risks associated with point totals

We now determine the risks that are associated with each point total. The first step is to determine the theoretical range of the point totals based on the point system computed in Step 6. In this system, the theoretical range of point totals is 0 to 13.

We now need to attach a risk estimate to each point total using the Cox Proportional Hazards model:

$$p^{\wedge} = 1 - S_0(t) \exp(\sum_{i=1}^p \beta_i X_i - \sum_{i=1}^p \beta_i X_i^{\wedge})$$

The point total, when multiplied by the constant (B) approximates $\sum_{i=1}^p \beta_i X_i - \sum_{i=1}^p \beta_i X_i^{\wedge}$. We also need to add back the creatinine value we considered the base ($c = 0.75$).

$$\sum_{i=1}^p \beta_i X_i \approx \beta_{\text{creatinine}}(0.75) + B(\text{total}) - \sum_{i=1}^p \beta_i X_i^{\wedge} = \beta_{\text{creatinine}}(0.75) + B(\text{total})$$

We also need to compute $\sum_{i=1}^p \beta_i X_i^{\wedge}$, which can be done once using the regression coefficients and the means (or proportions) of the risk factors given in Step 1.

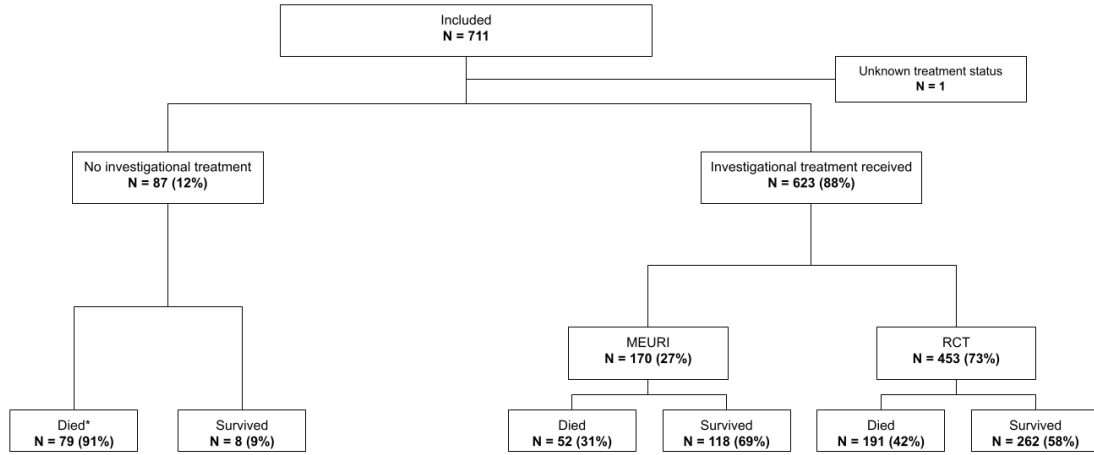
The result of the operation is 1.7760136.

Substituting these components along with the estimate of the average 28-day survival (also shown in Step 1) into the Cox model, we generate the following table:

Point total	Risk estimate
0	0.0191524
1	0.0268564
2	0.0375994
3	0.0525223
4	0.0731388
5	0.1014040
6	0.1397401
7	0.1909525
8	0.2579227
9	0.3429130
10	0.4463236
11	0.5649241
12	0.6901263
13	0.8078201

3. Supplementary figures

Figure S1. Treatment flowchart



*Died 24h or less after ETC admission: N = 64 (81%)

Figure S2. Kaplan Meier estimate of the probability of death, according to individual risk score factors at admission

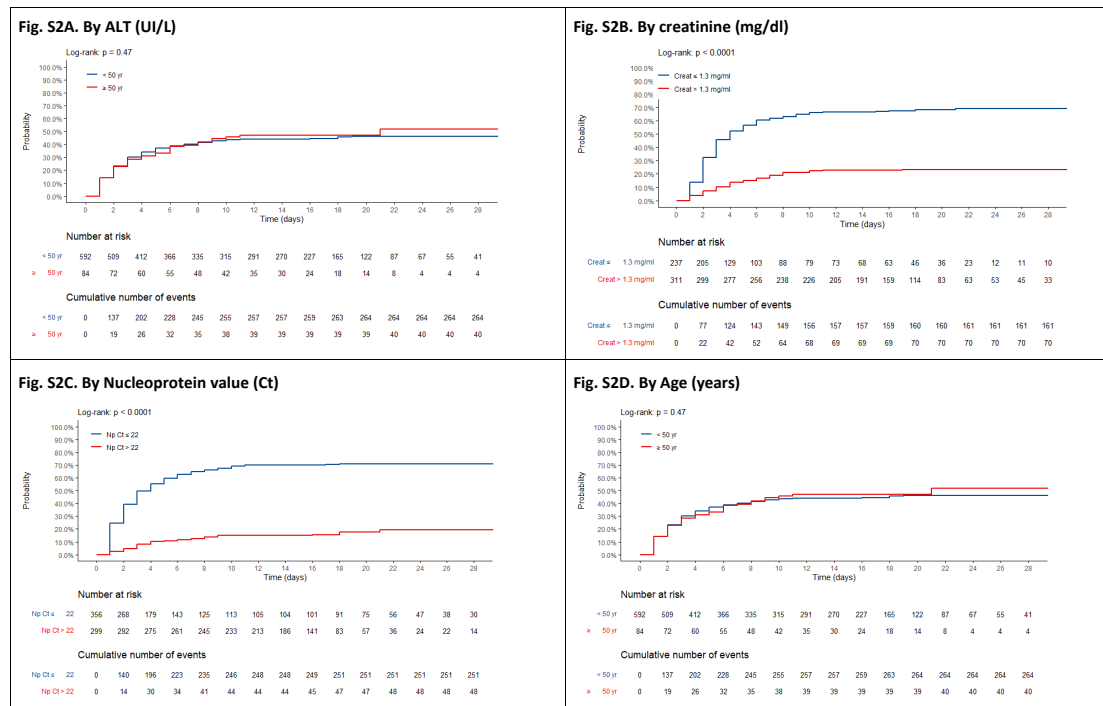


Fig. S2E. By time since 1st symptom (days)

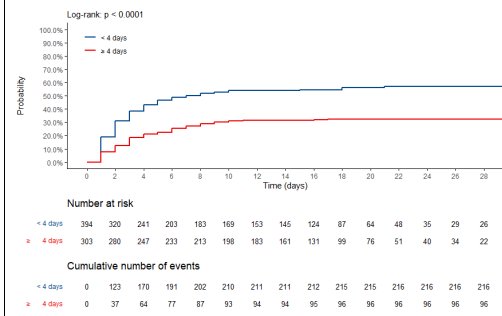


Fig. S2F. By mNEWS2 score

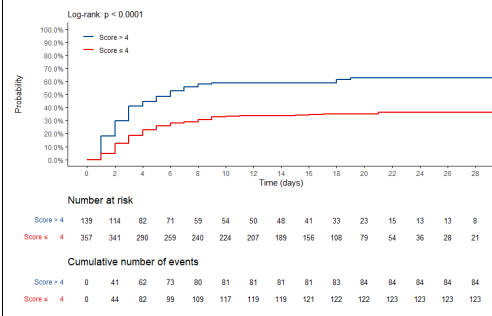
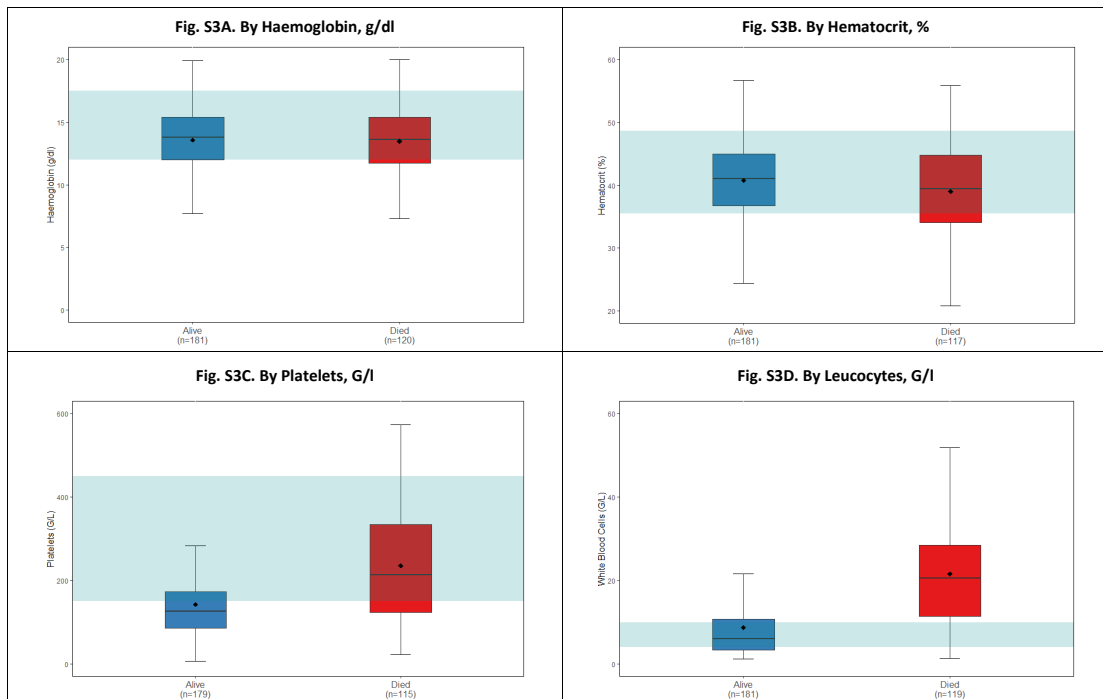
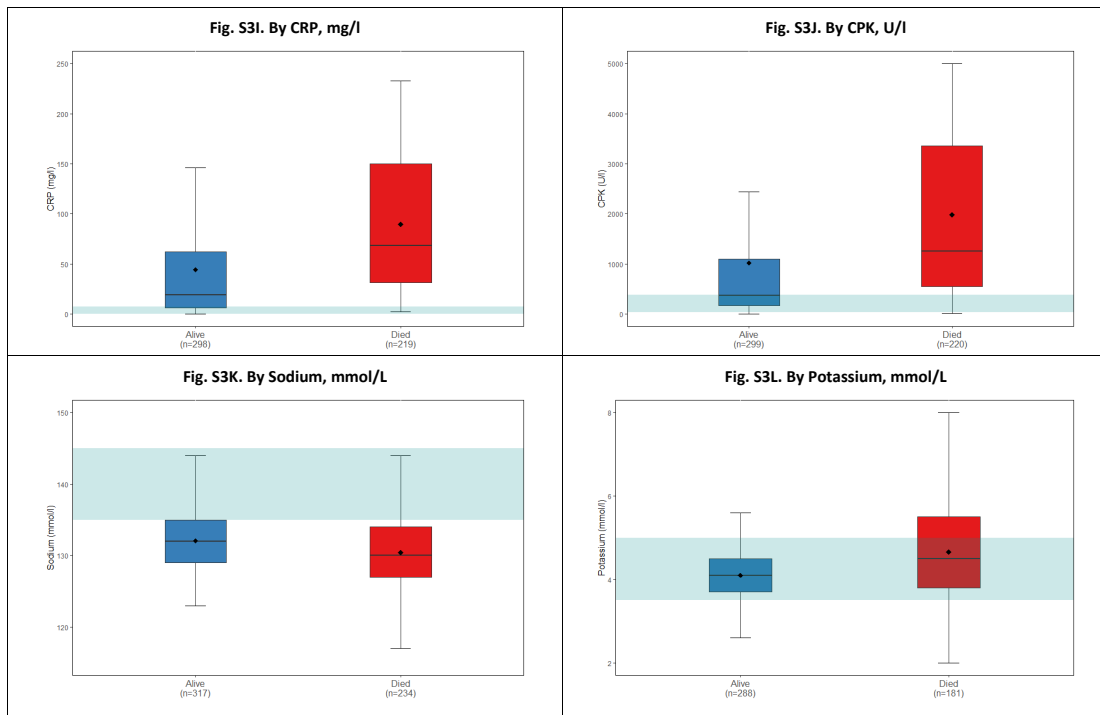
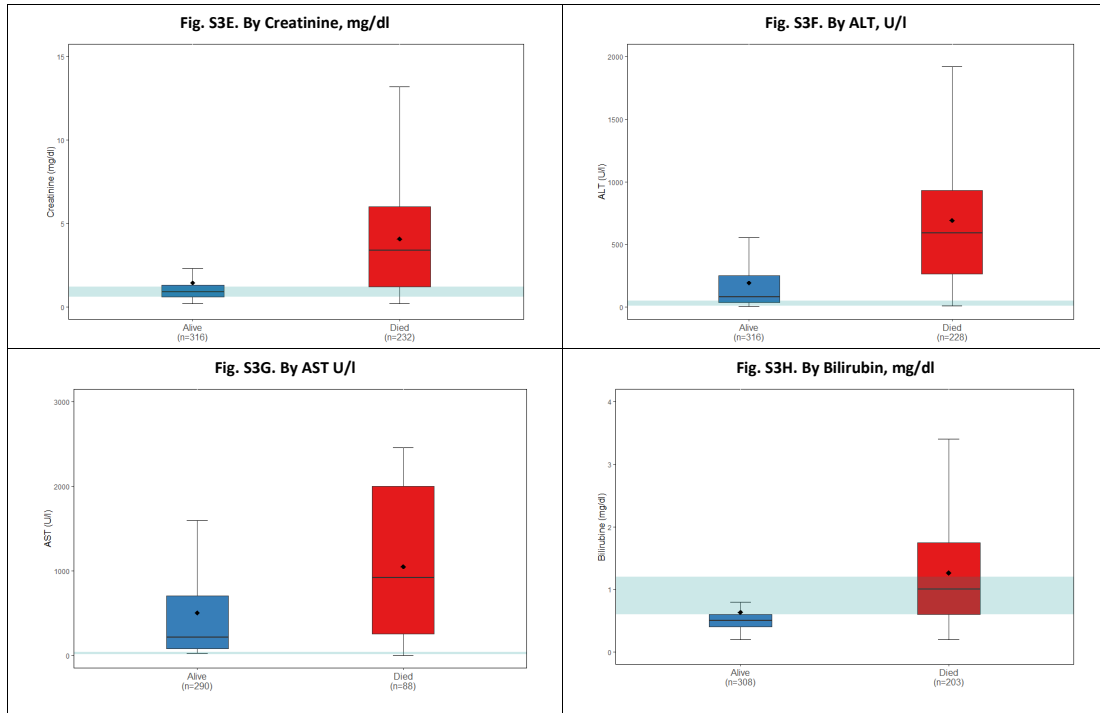


Figure S3. Boxplot distribution of main biological variables at admission by death (light blue = normal value)





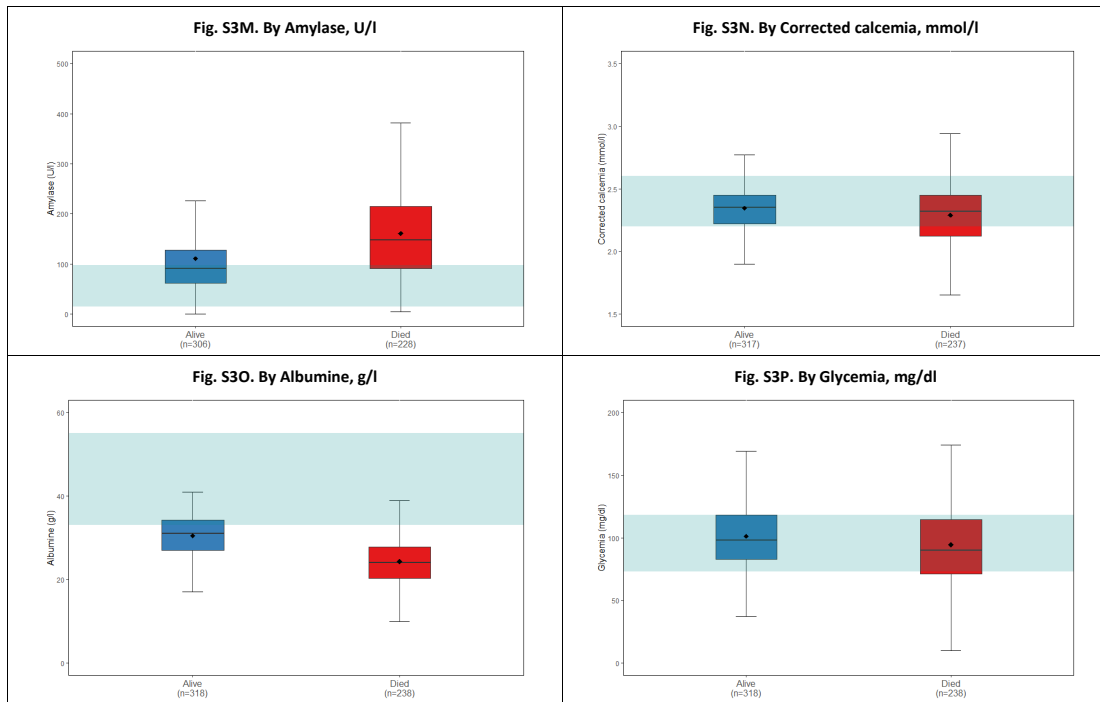


Figure S4. Predictive model calibration plot

Hosmer-Lemeshow Goodness of Fit Test

Chi Square = 12.9

P-value = 0.1153

