Improving patient centred research during infectious disease outbreaks

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Abstract

Emerging infectious diseases (EIDs) constitute an important global health security problem. During EID outbreaks, patient centred research can play a significant role in informing evidence based care for patients, in calibrating public health responses, and in directing effective policy and research. However, to date, this type of research has been limited in impact.

This thesis sets out to improve the value of patient centred research in combating EID outbreaks. It provides a structured analysis of what has previously constrained efforts to rapidly accumulate high quality evidence. It provides primary data from research conducted during an outbreak, and conducted in an outbreak vulnerable setting. And it provides recommendations that aim to facilitate high quality data collection in future events.

This thesis contains four results chapters. Chapter 2 systematically reviews elements of the research response to two EID outbreaks of public health importance. Chapter 3 provides findings of a phase II clinical trial of an investigational therapy for Ebola virus disease (EVD), contextualises the utility of this and comparable work in improving patient care, and discusses the operational feasibility of such work during an epidemic. Chapter 4 focuses specifically on improving one element - disease characterisation - during EID outbreaks. It achieves this through presenting a systematic analysis of bias in the characterisation of EVD and recommends how to prioritise data gathering for high risk pathogens. Chapter 5 exemplifies how clinical data collection practices can progress between outbreaks. It is the first stage of work undertaken to improve the clinical characterisation of communicable diseases in the vulnerable environment of refugee camps.

This thesis demonstrates progress towards having higher quality clinical research conducted during the time frame of an epidemic. Future work can focus on the most important barriers to accelerating research, now that these have been more clearly defined.

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List of Abbreviations

A(H1N1)pdm09 Novel H1N1 2009 Influenza A strain

AIDS . . . . . Acquired Immune Deficiency Syndrome

ALIMA . . . . The Alliance for International Medical Aid

BARDA . . . . Biomedical Advanced Research and Development Authority (US)

CDISC . . . . Clinical Data Interchange Standards Consortium

CEPI . . . . . Coalition for Epidemic Preparedness Innovations

CMV . . . . . Cytomegalovirus

COAST . . . . Children's Observations and Severity Tool

CFR . . . . . Case Fatality Rate

CRADA . . . Cooperative Research and Development Agreement

CRF . . . . . Case reporting form

Ct . . . . . Cycle-time

DDITD . . . . Donka Department of Infectious and Tropical Diseases

DFID . . . . . Department for International Development, United Kingdom

DNDi . . . . . Drugs for Neglected Diseases Initiative

DOD . . . . . Department of Defense

DTRA . . . . . Defense Threat Reduction Agency

DVISS . . . . . Dengue Virus Induced Shock Syndrome

EBOV . . . . . Ebolavirus

ECDC . . . . . European Centre for Disease Prevention and Control

EEID . . . . . Emerging and epidemic-prone infectious disease

ETC . . . . . Ebola Treatment Centre

EVD . . . . . Ebola Virus Disease

FEAST . . . . Fluid Expansion As Supportive Therapy

GCP . . . . . Good Clinical Practice

HIV . . . . . Human Immunodefiency Virus

HRZ . . . . . High Risk Zone in an Ebola Treatment Centre

HPAI . . . . . High Pathogenicity Avian Influenza

ICU . . . . . Intensive Care Unit

IDMC . . . . . Independent Data Monitoring Committee

IFRC . . . . . International Federation of the Red Cross

IMC . . . . . International Medical Corps

IND . . . . . Investigational New Drug

IPD . . . . . Individual Patient Data

ISARIC . . . . International Severe Acute Respiratory and Emerging Infection Consortium

IQR . . . . . Interquartile Range

IV . . . . . Intravenous

KEELPNO . . Hellenic Centre for Disease Prevention and Control

LNP . . . . . Lipid Nano-Particle

Lpol . . . . . L Polymerase

LPAI . . . . . Low Pathogenicity Avian Influenza

LRZ . . . . . Low Risk Zone in an Ebola Treatment Centre

MERS-CoV . Middle East Respiratory Syndrome Coronavirus

MMV . . . . . Medicines for Malaria Venture

MSF . . . . . Médecins sans Frontières

NAI . . . . . Neuraminidase Inhibitor

NGO . . . . . Non Governmental Organisation

NIH . . . . . National Institutes of Health (US)

NEWS . . . . National Early Warning Score

OSIR . . . . . Outbreak Surveillance, Investigation and Response

PBSL . . . . . Pharmacy Board of Sierra Leone

PDP . . . . . Product Development Partnership

PHEIC . . . . Public Health Emergency of International Concern

PHE . . . . . Public Health England

PHC . . . . . Public Health Canada

PLA . . . . . Peoples Liberation Army (China)

PO . . . . . Per oral

PPE . . . . . Personal Protective Equipment

R & D . . . . . Research and Development

RAPIDE . . . Rapid Assessment of Potential Interventions and Drugs for Ebola

SAR . . . . . Severe Adverse Reaction

SARS . . . . . Severe Acute Respiratory Syndrome

siRNAs . . . . Small Interfering Ribonucleic Acids

SUSAR . . . . Suspected Unexpected Serious Adverse Reaction

TDR . . . . . The UNICEF-UNDP-World Bank- WHO Special Programme for Research and Training in Tropical Diseases

UN . . . . . United Nations

US . . . . . United States of America

USAMRIID . The United States Army Medical Research Institute for Infectious Diseases

UNHCR . . . United Nations High Commissioner for Refugees

USD . . . . . United States Dollars

VHF . . . . . Viral Haemorrhagic Fever

VP35 . . . . . Viral Protein-35

WEF . . . . . World Economic Forum

WHO . . . . . World Health Organization

1

Introduction

Contents

1.1 General introduction

"And what are my tools to heal my patient?
A bed, three meals, fluids, tablets, antimalarials, painkillers...
in the end...when I get to them, I can only say
you have around 50 percent chance of dying
and I can do very little about it for you."
Médecins sans Frontières doctor, Sierra Leone (2014) [2].

During the Ebola Virus Disease (EVD) epidemic in west Africa (2013-6) there was clear frustration from clinicians that there were no specific treatment options available to improve survival, and from policy makers that an outbreak so quickly escalated to become a regional humanitarian crisis.

However, emerging infectious disease (EID, see box 1) outbreaks should not be unexpected. From its infancy, the field of public health has attempted to control infectious disease transmission - from the introduction of hand hygiene practices into hospitals[3], through to John Snow removing the Broad St pump handle in an effort to contain a cholera outbreak[4], and the remarkable gains in childhood survival made possible by mass vaccination campaigns[5]. These, and other successes led to some prominent public health experts foretelling an imminent end to the infectious diseases era during the mid 20$^{\text{th}}$century[6]. However, these predictions have proved optimistic. Despite continued improvements in communicable disease control, the 20$^{\text{th}}$century was also marked by EID outbreaks, including three influenza pandemics and the emergence of HIV/AIDs. In the 21$^{\text{st}}$century, several large scale epidemics have already threatened international health security. Two highly pathogenic novel coronaviruses (Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome Coronavirus (MERS Co-V)) have emerged and caused international outbreaks. The first influenza pandemic of the century pandemic A H1N1 2009 virus (A(H1N1)pdm09)), along with an EVD epidemic in west Africa, and the rapid spread of Zika virus and recognition of congenital Zika syndrome, have all prompted the declaration of a Public Health Emergency of International Concern (PHEIC) by the World Health Organization (WHO). Avian influenzas viruses have yet to cause widespread human infection, but these genetically promiscuous viruses require only further evolution to cause efficient human-to-human transmission[7–9].

Box 1. An emerging infectious disease is defined by a contemporary (<20 years) or predicted future rise in disease incidence, geographical distribution of infection, or pathogen virulence[10]. This definition includes diseases that have re-emerged after a period of inactivity.
Overlapping with this broad classification, the term emerging and epidemic-prone infectious disease (EEID) is used in this thesis to refer to the subset of diseases that have the potential to spread rapidly amongst a population.
Most emerging diseases that cause outbreaks are zoonotic and originate in wildlife, and are usually caused by viruses[11][12].

In fact, it is thought the rise in the frequency and diversity of infectious disease outbreaks is unprecedented[10, 11]. A variety of factors have likely contributed to escalating disease emergence and outbreak amplification. Encroachment of humans into natural habitats by expansion and intensification of agricultural practices (or other changes in land use) appears to be associated with the highest number of novel pathogen species[12]. Also important is the growth in human mobility (in terms of the volume of travel, speed of travel, and diversity of destinations) and international trade that increases the likelihood of a pathogen being carried into a naïve population by a vector or infected host[13]. Climate change and antimicrobial resistance are expected to play a central role in future disease emergence[14]. Propagation of outbreaks once spillover has occurred is facilitated by conditions of poverty, overcrowding, inadequate access to healthcare facilities, and poor-governance of public health activities. There are also geopolitical risks, including the risk that an EEID is used as a bioterrorism weapon[15, 16].

1.2 Importance of EID outbreaks

Without appropriate prevention, containment and management, the consequences of these outbreaks can be severe. Foremost, the impact is on the health and survival of those infected and their communities. By their nature, epidemics can be large in scale. The influenza A(H1N1)pdm09 pandemic is estimated to have infected between tens of millon and 200 million people[17]. The case fatality rate can be high - estimated to be 15% for SARS[18], 35% for MERS CoV [19], 40% for influenza A H7N9[20] and above 80% for some viral haemorrhagic fever (VHF) outbreaks[21, 22].

Healthcare ramifications extend beyond patients with confirmed disease. In the countries most affected by the EVD epidemic in west Africa, routine healthcare activities were halted due to unprecedented pressure on an already stretched systems, and exacerbated by the death or unavailability of healthcare workers. Patients were

also reluctant to seek care - admissions to remaining surgical facilities dropped by 70% in Sierra Leone during the latter months of 2014[23], consistent with data from Guinea showing a 40% reduction in primary health clinic attendance for the same period[24]. There were also precipitous drops in access to vertical programs for HIV, malaria, tuberculosis and maternal and antenatal care[24–27]. Fear of accessing care during an outbreak is not constrained to low income countries. As an example, evidence for reductions in healthcare utilization during the SARS outbreak spans across ambulatory, emergency and inpatient settings[28, 29], maternity care services[30] and hospice services[31].

There are also repercussions for economic development and stability. Modelling suggests that the cost of pandemics this century may exceed $ 6 trillion USD[32]. The direct costs of an outbreak include those spent on hospitalizations or other medical care, public health control measures, and incurred through loss of income secondary to death or disability. In a globalized world with interdependent economies, indirect costs (due to impacts on travel, trade, investor confidence, and consumer behaviour) are increasingly important. Indeed, these costs may eclipse direct costs, as was the case for SARS when they were estimated to be $40 billion USD. These economic consequences, can, in turn, create further vulnerability to outbreaks through excerbating poverty. As an example, the World Food Program indicates an additional 200,000 people experienced food insecurity during the EVD epidemic in west Africa [33].

1.3 New initiatives for EID outbreaks

The relevance and potential impacts of emerging infectious disease outbreaks in the modern world are clear. Despite this, there is concern that the world remains ill placed to prevent or respond to the next significant outbreak[17]. The west Africa EVD epidemic has re-invigorated interest in epidemic preparedness and control

and in response the landscape of EEID management is rapidly changing. It is hoped that the lessons learned from this outbreak will herald a new era of agile and effective clinical intervention and public health control.

A plethora of reports and commentaries have analysed the response to that epidemic. In general, there is criticism of the lack of preparation for an outbreak in a region at high risk for zoonotic diseases, acknowledgement of failures in international leadership for the outbreak, and critique of the delayed recognition that the outbreak was spiralling out of control. Organisations that provided clinical care decried the lack of available vaccines or specific treatments for the disease and lamented their inadequacy in being unable to provide sufficient clinical expertise[2].

Report name	Recommendations promoting clinical research
WHO: Ebola Interim Assessment Panel [34]	Recommendation 16: WHO should play a central convening role in research and development efforts in future emergencies, including the acceleration of the development of appropriate diagnostics, vaccines, therapeutics and medical and information technology.
Harvard University and the LSHTM: Independent Panel on the global response to Ebola [35]	Recommendation 6: Develop a framework of rules to enable, govern and ensure access to the benefits of research. Recommendation 7: Establish a global fund to finance, accelerate and prioritise R & D[36].
National Academy of Medicine: Commission on a Global Health Risk Framework for the Future [32]	Recommendation D1: WHO should establish an independent Pandemic Product Development Committee...to galvanize acceleration of relevant R & D, define priorities, and mobilize and allocate resources. Recommendation D2: WHO should work with global R & D stakeholders to catalyse the commitment of a $1 billion per year to maintain a portfolio of projects in drugs, vaccines, diagnostics. Recommendation D3: the...committee should convene regulatory agencies, industry stakeholders, and research organizations to a) commit to adopting R & D approaches during crises that maintain consistently high scientific standards. b) define protocols and practical approaches to engage local scientists and community members in the conduct of research c) agree on ways to expedite medical product approval, manufacture and distribution...pre-approval of clinical trial designs, mechanisms for intellectual property management, data sharing and property liability.
UN: High-Level Panel on the Global Response to Health Crises [37]	Recommendation 13: WHO coordinates the prioritization of global R & D efforts for neglected diseases that pose the greatest threat of turning into health crises. Recommendation 16: WHO leads efforts to assist developing countries in building research and manufacturing capacities for vaccines, therapeutics and diagnostics.
GOAL Global: Ebola Lessons Learned Report [38]	Recommendation: Identify and implement strategies to reduce the amount of time between identifying a research priority and implementing activities to gather data. Recommendation: Improve local capacity to identify research needs, develop protocols, implement research activities. Recommendation: Identify funding for clinical research, trials.
MSF: 'Pushed to the limit and beyond' Report [2]	A practical plan to sustain research and development for vaccines, treatments and diagnostic tools must be developed. These will be key in protecting the region from current or future resurgences of similar outbreaks.
House of Commons Science and Technology Committee: UK lessons from Ebola [39]	Recommendation 7b: We urge the government to...investigate the public health, economic and regulatory feasibility of establishing investigational stockpiles of vaccines that would be ready for Phase 2 trials during an outbreak. Recommendation 14: We recommend that the Chief Medical Officer urgently takes forward the work...to negotiate new processes for embedding research in the emergency response. This should establish protocols for facilitating research that positively contributes to the emergency response.

Table 1.1: Research specific recommendations contained in reports commissioned following the West Africa EVD epidemic.

In response, a general consensus now exists that future epidemic control activites require an emphasis on health system strengthening, improved global governance, greater accountability of leadership, and clarification of the roles and responsibilities of the UN system[40]. Importantly, for the first time there is also widespread endorsement (table 2.1) for research and development (R & D) to be strengthened and recognised as an integral component of coordinated outbreak management.

This call to action has been further supported by the implementation of two significant initiatives that focus on research. Firstly, WHO has announced their R & D blueprint for action to prevent epidemics, and within this programme has specified infectious diseases that should be prioritised for further research given their public health relevance and the absence of effective countermeasures[41, 42]. In addition a new global coalition (The Coalition for Epidemic Preparedness Innovations (CEPI)) (cepi.net) has been launched to advance vaccine development for epidemic threats by way of increased advocacy, funding, and convening of stakeholders.

It should be noted that the importance of research is not an unforeseen revelation and some scientists have made sustained progress in advocating this agenda over the last few decades. However, the post-Ebola epidemic landscape provides unprecedented opportunity to accelerate these efforts in the setting of multi-sector support and political momentum. The scope of this thesis, will therefore focus on clinical research as a component of EEID management.

1.4 Role of patient centred research

Within the broad spectrum of science relevant to EEID management, the potential contributions, risks, and limitations of patient centred research (box 2) is an area of active debate. Patients are the primary source of much of the information (e.g. clinical presentation and outcomes) and materials (e.g. pathogens and antibodies)

that are vital for both clinical and public health decision-making; for advancing basic scientific understanding; and for evaluating the products of enhanced diagnostic, drug and vaccine development pipelines. In this thesis it is argued that thinking should therefore converge on the patient and that the needs of all disciplines should be addressed within a strengthened and unified framework.

Box 2. In this thesis the term 'patient centred research' refers to research where patients are the primary source of the clinical data, biological samples, experiential narrative, or other key component of analysis that is required to conduct the research according to its aims. It is a term that is specific to inclusion of patients with the disease of interest, and so for epidemic science is useful terminology to distinguish from population based research.

The role of patient-centred research during outbreaks - improving patient outcomes

Confidence in the care provided to patients is dependent on the quality of the underlying evidence, and 'evidence-based practice' whereby the best available research evidence is incorporated into clinical care, is a core aspiration of modern medicine. However, during epidemics, decisions such as which drugs, fluids or supportive care strategies to offer patients are usually made on an ad hoc basis by the treating clinician, or from guidelines that approximate from other diseases and experiences[43, 44]. In the least, the consequence is that survival rates are slow to improve, but also means that we may be doing unintentional harm. The trial of Fluid Expansion As Supportive Therapy (FEAST) for critically ill children, which found that giving fluid boluses to children with impaired perfusion in resource-limited settings in Africa actually increased mortality, was a clear demonstration of the potential dangers of plausible extrapolation given that this treatment has been long standing and well accepted treatment for septic shock[45].

Therefore, all patients, irrespective of the location and circumstances of their illness,

deserve evidence-based care. Yet, for recent notable outbreaks including SARS, pandemic influenza and EVD, we have yet to identify an effective therapeutic agent for the infection. This vulnerability continues. Despite over 1500 avian influenza A(H7N9) cases since 2013 (correct as of September 13, 2017)[46], there is only one registered treatment trial on clincialtrials.gov (NCT02095444). This represents a major global vulnerability, especially given the recent emergence of highly pathogenic avian influenza (HPAI) A(H7N9)[47] and the relative lack of treatment options now that neuraminidase inhibitor resistance has been identified in both HPAI and LPAI strains[47, 48]. There are also only two clinical treatment trials registered for MERS Co-V (NCT02845843, NCT02190799), although cases have now been reported for 4 years. There has also been a failure to gather evidence on the effectiveness of readily available and widely used supportive care measures. For example, when treating EVD there remains no robust evidence on the optimal intravenous fluid resuscitation strategy, the use of vitamin K, or the provision of loperamide for diarrhoea; all practices that were adopted to varying extents during the west Africa epidemic (appendix 1).

The primary aim of patient centred research is therefore to improve patient survival, reduce the severity and length of illness, and to alleviate suffering by way of evidence based improvement in clinical care.

The role of patient-centred research during outbreaks - helping to control the epidemic

Patients with EEIDs deserve to benefit from the fruits of research as much as any other patient, yet the broader societal benefits of clinical research are even greater in the context of outbreaks. A well-focused and calibrated public health response to an epidemic can save lives and money. The west Africa EVD epidemic is set to become a notorious case study of the consequences of under-reaction. However,

even when faced with an outbreak of what we think is a well-characterised infection, there is always a need to critically re-evaluate received wisdom and to be sceptical of initial impressions. Influenza is a good case in point. The initial public health response to the 2009 influenza A/H1N1 pandemic is widely considered to have been poorly calibrated owing to excessive early estimates of the case fatality rate based on data from Mexico City and Winnipeg[49, 50]. What was initially thought to be a severe novel influenza turned out to be no more severe than an average seasonal influenza[51]. The resulting expenditure on antivirals and vaccines has been widely criticised and illustrates the social and economic imperative for a rigorous approach to assessing disease severity, that explicitly considers biases that are inherent in surveillance and reporting systems. As another example, due to the limited natural history data for EVD, it has only recently been established that fever is absent in approximately 10% of patients[52, 53]. However, fever was used for the entirety of the west Africa epidemic as often the solitary indicator for screening at airports and checkpoints, and as part of the case definition for Ebola virus testing[54, 55] - with obvious implications for transmission control. In addition, limitations in collecting and analysing biological specimens from patients have resulted in inadequate understanding of transmission risks. For example, despite 22 prior EVD outbreaks and around 2000 cases, it was also only in 2015 that the risk of sexual transmission was confirmed[56]. There have been few, if any, comparable and comprehensive sampling studies done for other high-threat epidemic-prone diseases, even those with predictable seasonal outbreaks such as Crimean-Congo haemorrhagic fever (CCHF). During the most recent PHEIC, the Zika virus outbreak, the poor availability of well-characterised patient-derived samples has impeded the development and validation of crucial assays for patient diagnosis[57].

Many of these aspects of an appropriate public health response are dependent on high-quality data and samples from patients. For example, reliable illness severity data are required to predict the number of infected and ill people and then scale

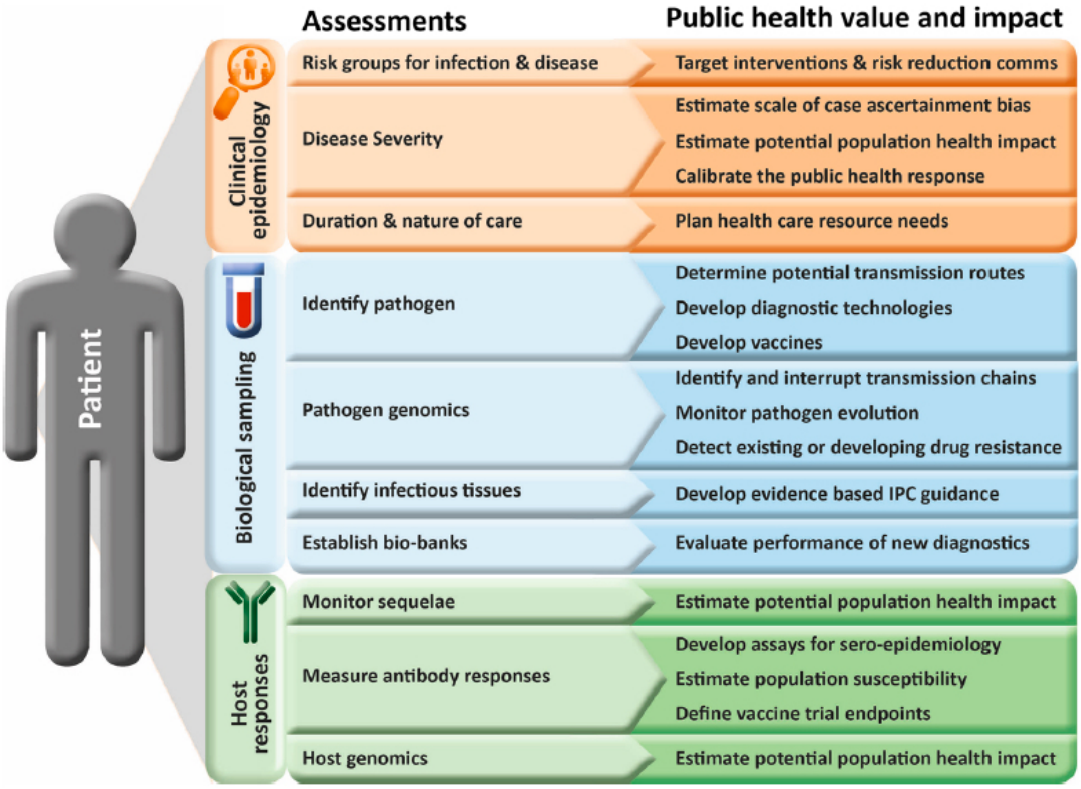

Figure 1.1: The public health value and impact of patient based assessments

the response appropriately; groups at high risk for infection or poor prognosis need to be identified for targeted preventative and treatment interventions; genetic sequencing of pathogens from biological samples can provide critical information on transmission pathways, evolutionary pressures and drug resistance; and the characterisation of immunological responses is a prerequisite for developing the laboratory tools for critical sero-epidemiologic and vaccine immunogenicity studies. Figure 1.1 summarises the public health value of some of the key parameters that can only be derived from patients.

It is clear that there is significant room for improvement in the systematic collection of data and biological samples from patients, with the explicit aim of improving the evidence-base for public health decision-making.

Limitations and criticisms of patient-centred research

Despite these over-arching benefits, there has been contention regarding the importance of this research in comparison to the immediate humanitarian needs of patients. Clinical care providers have worried that research may stretch capacity unrealistically, and in doing so, divert attention away from patient care[58, 59]. The unique circumstances in which this research is conducted has also led to disagreement over a variety of research design questions, such as the most valid and appropriate trial design, and the ethics of patient randomization, or enrolment of high risk groups such as pregnant women and children.

There has also been widespread criticism of some of the perceived failures of patient centred research. A prominent critique is that research efforts to date have reaped a 'thin harvest'[60]- namely that despite existing work, the natural history of EEIDs remain poorly understood, and that very few countermeasures are available. The research response has been characterised as slow and underwhelming, lagging behind public health interventions[61]. However, almost always these criticisms have been provided in the context of expert commentary, or narrative analysis of the shortcomings of individual projects. Furthermore, there is limited understanding of the specific factors that are the bottleneck to a faster, more capable response.

It is possible to make progress toward the ambitious goal of an accelerated, high yield patient oriented research response to EEIDs. What is required is a structured analysis of the factors that have previously impeded implementation, the identification of those factors that may be tractable, and efforts to design and evaluate new models of working.

1.5 Aims and structure

The overarching aims of the work presented in this thesis are to:

1. Provide a structured analysis of the quantity, quality and impact of patient centred research in recent EID outbreaks of public health importance.
2. Conduct clinical research during an EID outbreak and draw lessons for improvement in the field.
3. Develop, test and recommend methods and tools that facilitate implementation of patient oriented research during present and future EID outbreaks.

Chapter 2 examines the clinical research response to the influenza A(H1N1)pdm09 pandemic, and the 2003 SARS outbreak. By using systematic review methodology, it provides quantitative metrics of the volume and timing of literature reporting patient treatment. The most significant benefit of this approach is that allows for a more evidenced based analysis of the commendations and criticisms of clinical research during outbreak compared with existing opinion based recommendations.

Chapter 3 reports clinical research that was conducted during the west African EVD epidemic. This chapter includes the primary findings of a phase II clinical trial to test the efficacy of an experimental EVD therapeutic (TKM-130803(TKM)). The importance of this trial is contextualised in two further sections of the chapter. The first explores how trial groups were able to mount a research response within the time-frame of the epidemic. It uses data from the TKM trial to describe operational innovations that facilitated rapid initiation of the trial and explores remaining regulatory and operational constraints that are likely to affect future trials. The second section suggests ways in which the west Africa outbreak can shape innovation for outbreak R & D.

Chapter 4 examines why improvements in one component of outbreak clinical research (clinical characterisation of the disease) are needed and provides suggestions to achieve this. There are two avenues of exploration. Firstly, a systematic review of clinical characteristics of patients with EVD during the west Africa epidemic demonstrates how inadequate clinical data collection risks creating a biased characterisation of the illness, and discusses the potential implications of this bias on providing an evidence base for patient care. I also provide a framework in which to assess clinical characterisation of outbreak diseases, and prioritise knowledge gathering for high risk pathogens.

Chapter 5 demonstrates how progress for patient centred research can be made during inter-epidemic periods. It reports original data from a clinical audit of patient consultations in refugee camps in Greece. Refugee camps are a high risk environment for an infectious disease outbreak. This audit evaluated the extent to which clinicians assessed the risks, severity, and presenting symptoms of patients presenting with potentially communicable diseases and how this might affect outbreak surveillance and investigation in this setting.

Publication

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2

The patient centred research response to previous EID outbreaks

Contents

2.1 Preface to the chapter

The first objective of this thesis is to provide a structured analysis of the contributions and shortcomings of patient centred research in recent emerging infectious disease outbreaks. There are two reasons that this is necessary:

1. Criticisms of patient centred research during outbreaks have almost always been made in the context of opinion pieces[60–62]. While these are often produced by experienced practitioners in the field and seem reasonable in their overall assessment, their generalised nature means that they are unable to identify specific modifiable bottlenecks.

2. Likewise, reports commissioned in response to previous EID outbreaks call for ambitious improvements in the research response, but are unable to provide a credible road map without a quantitative evidence base[32, 34, 35].

This chapter contributes to this objective by providing systematic reviews that focus on the timing, quantity, and quality of patient centred research during two outbreaks of public health importance.

2.1.1 Context

This is not the first bibliometric work to examine research output in this setting. Xing *et al*[63] reviewed epidemiological literature for SARS in two highly affected cities, Hong Kong and Toronto. Their major finding was that research dissemination was delayed - only 22% of manuscripts were submitted for publication, and 7% were published before the outbreak concluded. A review of randomized trials of influenza A(H1N1)pdm09 vaccination found that research was difficult to complete - only a minority (29% at the time of assessment) of registered clinical trials were

published [64]. Another review focused on the geographical origins of MERS-CoV research but did not include any temporal analysis[65]. These reviews are helpful in describing the general categories of literature published and by detailing publication timelines. However, because data were not extracted from the methods or results sections of articles they are unable to describe content, or aggregate articles by any variable other than publication time. They also do not evaluate reporting quality.

Several focused systematic reviews also exist with the objective of evaluating treatment effects for patients treated during outbreaks, including for influenza A(H1N1)pdm09[66] and SARS[67, 68]. In order to meet their objective, these systematic reviews used restrictive selection criteria to exclude biased, or poor quality evidence. This means they were not designed to broadly evaluate evidence generation during the outbreaks - including research that was planned but not completed, or data in manuscripts further down the hierarchy of evidence (such as case series). They also do not focus on contextualising the work undertaken, and so there is rarely comparison to the outbreak epidemiology, or exploration of barriers and facilitators to undertaking research within the timeframe of the outbreak.

2.1.2 Rationale for methodology

For these reasons, the review methodology contained in this chapter is a hybrid between a bibliometric review and a focussed systematic review. While we include bibliometric analysis, there is more extensive data extraction from articles so that we can also interrogate the information contained in (or missing from) the research and discuss the contribution of the research to improving patient care.

For this body of work I selected the 2009 influenza A(H1N1)pdm09 pandemic, and the 2003 SARS outbreak for examination. There are a number of reasons. Firstly, these two pathogens remain relevant for current EEID preparedness efforts;

pandemic influenza constitutes the most severe threat of all known EEIDs, and the lack of adequate countermeasures for, and the risk of another novel coronavirus outbreak means that it constitutes one of the WHO priority diseases needing R & D actions[41]. These two outbreaks were also large enough that a substantive body of literature was developed in response. Analysis of a more recent outbreak is not possible due to the length of expected delays in publication. We estimated that as a minimum, we would need to select an outbreak that occurred over five years ago based on literature[69–71] that indicates the delay between completion of data colllection and publication to be years (as an example one report estimates delays of 4-5 years for trials with positive results, and 6-8 years for trials with negative results[70]), even for EEID focussed publications[63, 72] where faster publication might be anticipated. The two outbreaks had very different epidemiological profiles and affected different geographical regions to a greater or lesser extent. Therefore the research response was expected to differ - providing a more well rounded view of the capabilities of the global research infrastructure in responding to an outbreak.

2.1.3 Objectives of the chapter

The primary objectives of the reviews, along with their underlying rationale are described below in table 2.1. To summarise, section 2.2 is a systematic review of manuscripts, and clinical trial records that describe clinical outcomes for hospitalised patients who were treated for SARS, and section 2.3 is an analogous review for the influenza A(H1N1)pdm09 pandemic.

Aim	Reason
1. Quantify the volume of data that described patient treatment, stratified by research type	The volume of research gives an indication of the scale of the response mounted. Comparisons of different types of research (clinical trial, hypothesis driven observational study, case series) describes the quality of evidence available
2. Document the time taken to initiate and complete this clinical research and compare this to the outbreak epidemiology	The faster that clinical research is commenced, the greater the pool of potential participants, and the greater the likelihood of enrolling a sufficient sample size and completing within the timeframe of the outbreak.
3. Document the time taken to submit and publish this research and compare this to the outbreak epidemiology	Research can only influence patient treatment in the current outbreak by providing enhanced evidence if it is available within the time-frame of the outbreak
4. Describe the extent to which manuscripts report key clinical parameters, including those needed for stratification of treatment effect (the age of patients, the pregnancy status of patients) or indicate the quality of reporting of treatment effect (adverse events due to treatment)	Exhaustive quality assessment of the manuscripts cannot be undertaken in this review, due to the volume of data and diversity in type of article. We have selected key parameters for assessment that would be necessary to know in order to evaluate a treatment effect.
5. Describe the outcomes of clinical research that was prospectively registered	This expands discussion around limits to conducting high quality research as we can comment on the proportion of planned research that was able to complete.

Table 2.1: Objectives of systematic reviews included in chapter 2.

2.1.4 Author's contribution

For the work contained in section 2.2 I conceived the work, undertook preliminary selection of all manuscripts for inclusion, produced the database and standard operating procedures for data extraction, and undertook the majority of English language data extraction. I trained and supervised two research assistants who assisted with data extraction (Nzelle Kayem (English language) and Jenny Hsieh (Chinese language)). I undertook all data analysis, produced all figures and tables, and wrote the manuscript.

For the work contained in section 2.3 I conceived the work, designed the review methodology, undertook all data collection and compiled all the data. I undertook all data analysis, produced all figures and tables, and wrote the manuscript.

Publication

Abbreviated forms of work in this chapter are drafted for journal submission upon thesis hand-in. The provisional publications are as follows:

Rojek, A. M., Kayem, N., Hsieh, J., & Horby, P. W. (2017). Clinical evidence accumulation to inform treatment of Severe Acute Respiratory Syndrome. in prep.

Rojek, A. M. & Horby, P. W. (2017). A systematic review of reporting the treatment of hospitalised H1N1 pandemic influenza cases. in prep.

2.2 Research response to the SARS outbreak

2.2.1 Introduction

The SARS outbreak that began in late 2002 is an excellent model for assessing evidence generation. It was the first outbreak of SARS in humans and so there was no existing guidance on patient treatment. Early rationalisation of treatment was based on broad spectrum agents with efficacy in similar syndromes. Later during the outbreak, treatment was guided by emerging in-vitro and animal data. However, despite some evidence accrual, the scientific community was unable to produce definitive safety or efficacy data for any treatment by the resolution of the outbreak[67].

The first consequence of this was that treatment strategies for affected patients were ad-hoc and diverged depending on interpretation of poor quality evidence. For example, ribavirin was discarded as a treatment in Canada, but continued to be favoured in China and Hong Kong throughout the outbreak[73]. The ongoing consequence is that there remains no licensed therapy, or high quality treatment guidelines available for a disease that is at risk of re-emergence.

Therefore, despite the opportunity to produce evidence during the outbreak, WHO continues to list highly pathogenic novel coronaviruses as priority diseases that require urgent research and development to prevent epidemics[41].

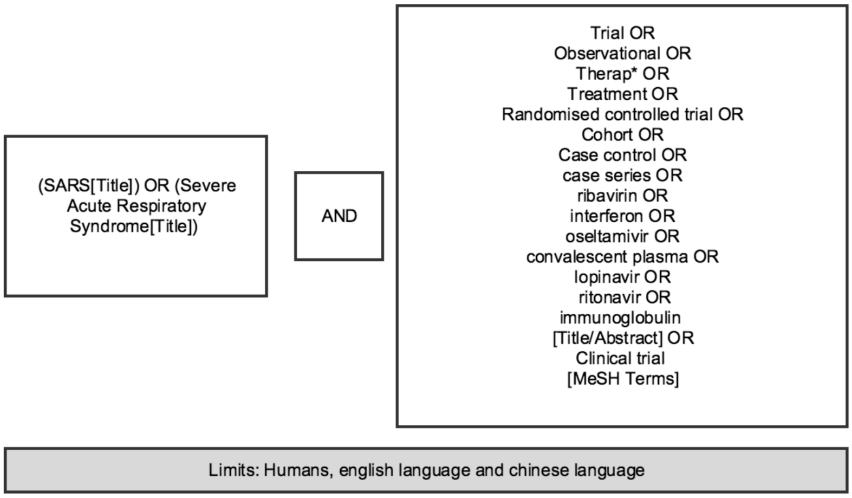


Figure 2.1: Search strategy for systematic review of published literature.

The objectives of this systematic review are to a) clarify the volume and type of evidence that was generated during the outbreak, b) identify when planned clinical trials failed to materialise c) quantify the time taken to initiate, complete and publish research and discuss the relevance of these parameters to disease epidemiology at the time and ongoing efforts to produce high quality evidence.

2.2.2 Methods

We conducted a systematic analysis of treatment of patients with acute SARS infection. Two types of data were included: articles from the peer reviewed literature, and clinical trial registration records. The methods were prospectively registered (PROSPERO record number CRD42016039549).

Published literature search

The search strategy was devised in consultation with an experienced research librarian. PubMed was searched according to the strategy contained in figure2.1. There was no date restriction for inclusion.

As one of the review objectives was to investigate the number of patients who received treatment without enrolment in a clinical trial, we included observational research and descriptive case studies or case series, as well as interventional trials. We included both prospectively and retrospectively gathered data. There was no minimum sample size. Articles must have reported acute clinical outcome(s) of patients (defined as length of hospitalisation, intensive care admission or length of stay, medical complication, requirement for mechanical ventilation or other intensive care treatment, or mortality).

We excluded articles if the number of patients receiving specific therapies was not defined (including use of the general term 'antiviral therapy' without clarification). We defined treatment as pathogen directed therapy, or host directed therapy where the specific indication was listed as SARS. This included use of corticosteroids and immunoglobulins, but not general supportive measures (intravenous fluids, antipyretics, intensive care bundles). We included suspected, probable (according to the prevailing WHO or US CDC case definition at the time) and laboratory confirmed cases in light of the unavailability of diagnostics early during the outbreak. Where a patient cohort (defined by same sample size, enrolment period, and study location) were presented in more than one paper, the smaller duplicate was excluded.

Clinical trial registry search

The clinical trials registries clinicaltrials.gov and WHO International Clinical Trials Registry Platform were searched using the term 'Severe Acute Respiratory Syndrome' or 'SARS' in the topic field. Included submissions were those available in the database as of the 31st of October 2016. We included clinical trials and observational research using the same inclusion and exclusion criteria described above.

Epidemiology data

All SARS epidemiological information was manually extracted from WHO's cumulative number of probable cases of SARS, publicly available at http://www.who.int/csr/sars/country/en/.

Data extraction

Data extraction was performed by three reviewers (English language: AR, NK, Chinese language: JH) according to the pre-specified inclusion and exclusion criteria. All decisions and reasons were electronically stored using online systematic review software(rayyan.qcri.org).

For published literature, we extracted the name of the paper, type of research, number of patients included, diagnostic method, patient outcome measures, patient treatment(s), reporting of treatment adverse events, information on inclusion of high risk groups (pregnant women, elderly (defined as older than 65 years), children (defined as younger than 18 years)) and dates of patient enrolment and publication (submission, acceptance, publication). Where dates were sought, but only described as month, year, (e.g. patients were enrolled from June to December) the 1st day of the month was used for analysis.

For clinical trial registries, the information available differs. Therefore data capture included the type of study, date of submission to the registry, predicted and actual values for the first and final dates of enrolment and sample size, whether high risk groups (as defined above) were included, the geographical location of the studies, the completion status of the work, and any corresponding publications.

Statistical analysis

Descriptive statistics are presented as frequencies for categorical variables and medians with interquartile range for continuous variables. Clinical trial results and published literature are reported separately. Chinese medicines were presented as a single class of drug as the individual components were rarely described. Quantitative analysis of combination therapy was not possible due to variation in reporting practices in the literature. The R statistical software (version 3.3.2) and Microsoft Excel for Mac (version 15.21.1) were used for statistical analysis.

2.2.3 Results

Findings from published literature

81 studies are included in the final quantitative synthesis (figure 2.2). 52 articles were English language, and the remaining 29 were written in Chinese language.

In total, 8433 patients are described in the review. The median number of patients in each paper is 44 (IQR 14-103). In total, 15 276 treatment courses are described, although it is not possible to distinguish in what combinations patients received multiple therapies. The most frequently administered treatment was corticosteroids (table 2.2).

Of the 81 articles included, seven articles are interventional trials (n = 367, representing 4.3% of patients), 12 are prospective observational studies (n = 813, accounting for 9.6% of patients), and 62 contained only retrospective data (n = 7253, accounting for 86.0% of patients) (see figure 2.3).

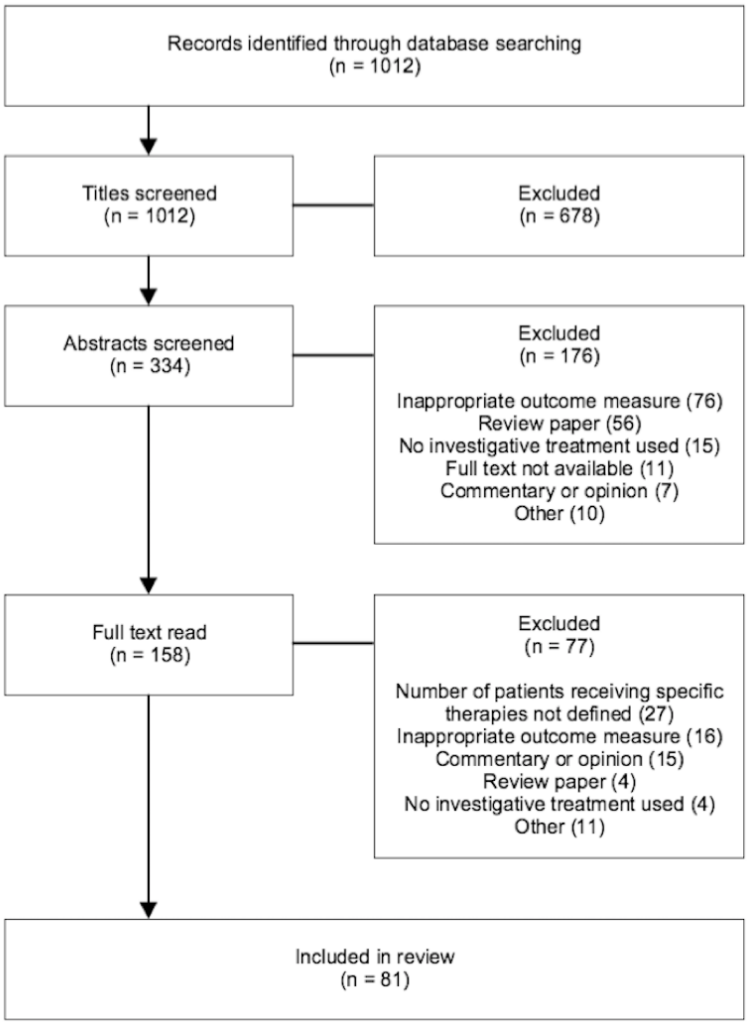


Figure 2.2: Prisma flow diagram describing screening, eligibility and inclusion for peer reviewed literature.

Time to open enrolment in prospective research

The first official reports on a cluster of atypical pneumonia to WHO occurred on 11 February, 2003[18]. The median enrolment opening date for the interventional trials was 60 days later (IQR 45-68 days), and enrolment closed at a median 109 days (IQR 101-125 days) later.

For prospective observational studies, the median day of first patient enrolment was 36 (IQR 28-42) days following WHO notification, and the median day of last

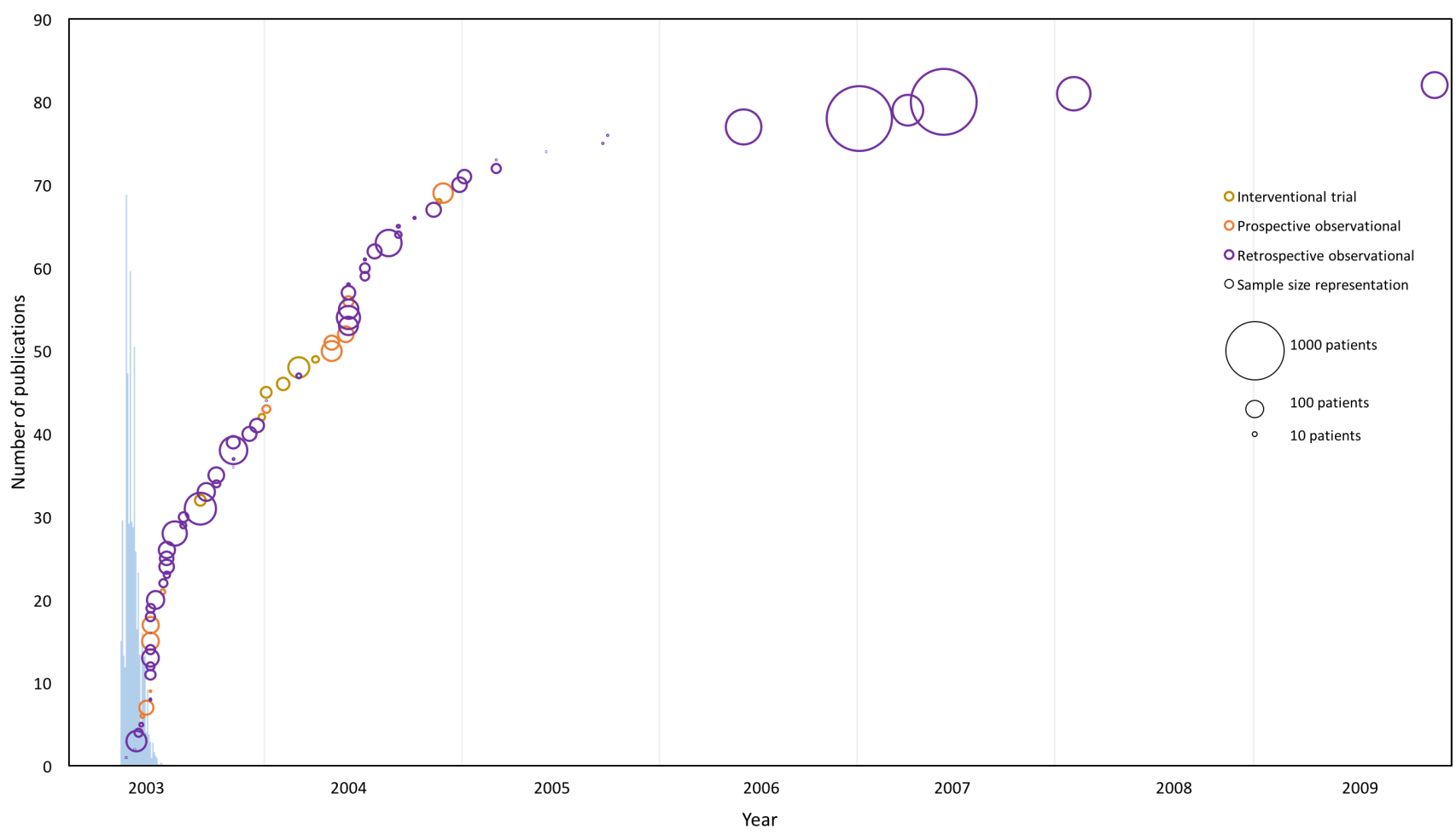


Figure 2.3: Timeline of publication of manuscripts, according to publication type and number of patients included. Data overlays the epidemic curve (disease incidence by date) according to publicly available data from WHO.

Treatment name	Number of publications reporting use	Total number of patients receiving treatment*	Median (interquartile range) number of patients receiving drug per publication**
Corticosteroids	78	6931	41 (13-80)
Ribavirin	61	5767	40 (10-96)
Immunoglobulins	23	772	15 (3-41)
Chinese medicines^	15	498	31 (16-38)
Thymosins	5	497	60 (41-133)
Oseltamivir	10	422	15 (1-81)
Lopinavir/ritonavir	4	141	32 (17-50)
Interferons	4	135	41 (32-42)
Convalescent plasma	8	117	2 (1-9)

Table 2.2: Volume of treatment courses described in the literature for patients with SARS. * Some patients received more than one treatment. ** when publication describes use of that drug. Chinese medicines are presented as one class due to difficulty determining active ingredients.

patient enrolment was 77 (IQR 46-103) days following WHO notification.

Publication timelines

The last chain of SARS transmission was broken on 5 July, 2003[18]. To influence clinical care, the results of publications needed to be available before this date. No interventional trials published before this date. 50% of prospective studies were published, and 32% of articles describing retrospective data were published.

The publication timeline for all articles is shown in figure 2.3. The median delays from closing enrolment to submission was 133 days (IQR71-274, n = 21), from submission to acceptance was 84 days (IQR 59–122, n=25), and acceptance to publication 90 days (IQR 44–158, n=56) where information was available for that comparison. The median delay from closing enrolment to publication was 208 days (IQR 61-403, n=65).

Region	Total SARS patients*	Clinical trial data		Prospective observational data		Retrospective observational data	
		Articles (n)	Patients enrolled (n)	Articles (n)	Patients enrolled (n)	Articles (n)	Patients described (n)
China (Mainland)	5327	5	193	2	202	28	2477
Hong Kong	1755	1	152	5	488	23	3603
Taiwan	346	0	0	3	109	4	109
Canada	251	1	22	1	14	5	560
Singapore	238	0	0	0	0	4	504

Table 2.3: Geographical distribution of publications reporting patient outcomes for patients treated for SARS. *according to publicly available WHO data.

Geographical representation

Of the 30 geographical regions (27 independent countries and 3 administrative regions of China (Mainland, Hong Kong, Macau) that experienced SARS patients, 5 regions published literature. The geographical distribution of publications is described in table 2.3.

Because the period of SARS transmission varied by region, we also stratified analysis of patient enrolment dates and publication dates by region. Table 2.4 describes the proportion of WHO reported patients in a given country that were treated before the first opportunity for enrolment in clinical research (for countries that published literature). Table 2.5 describes the proportion of patients in each country that were treated before study data were publicly available to inform treatment.

Region	Clinical trials			Prospective observational studies		
	Date first trial opens to enrolment	Cumulative *n* by that date	Proportion of total *n* ineligible due to delay	Date first study opens to enrolment	Cumulative *n* by that date	Proportion of total *n* ineligible due to delay
Mainland China	28/03/2003	806	15.1%	01/02/2003	Recruiting before WHO reporting commenced	
Hong Kong	24/03/2003	260	14.8%	09/03/2003	Recruiting before WHO reporting commenced	
Taiwan	none	n/a	n/a	08/03/2003	Recruiting before WHO reporting commenced	
Canada	11/04/2003	98	39.0%	23/03/2003	9	3.6%

Table 2.4: Influence of delays to prospective data collection on possible enrolment numbers for prospective research. n is the number of patients with probable or confirmed SARS in the region.

Region	Clinical trials			Prospective observational data			Retrospective observational data		
	First data published	Cumulative *n* by that date	Proportion of total *n* with no evidence	First data published	Cumulative *n* by that date	Proportion of total *n* with no evidence	First data published	Cumulative *n* by that date	Proportion of total *n* with no evidence
Mainland China	01/09/2003	5327	100%	01/06/2003	5328	100.%	01/06/2003	5328	100%
Hong Kong	01/03/2004	1755	100%	17/05/2003	1710	97.4%	17/04/2003	1297	73.9%
Taiwan	none	N/A	N/A	01/06/2003	676	100%*	01/01/2005	346	100%
Canada	24/12/2003	251	100%	24/06/2003	249	99.2%	06/05/2003	148	59.0%
Singapore	none	N/A	N/A	none	N/A	N/A	16/07/2003	206	86.6%

Table 2.5: Influence of delays to publication on availability of evidence to influence care. n is the number of patients with probable or confirmed SARS in the region. * of revised WHO count

Quality reporting

Clinical trials: Four quality indicators were used for assessment of clinical trials (table 2.6). Of the seven included trials, only one reported a single primary outcome. Only one trial was prospectively registered. Two trials reported their planned enrolment size. Three trials reported on drug adverse events (including when they were absent).

Title	Language	Comparison (*n* patients)	Primary outcome	Was this trial registered?	Was the planned sample size reported?	Were drug adverse events reported^?
Controlled clinical study on 49 patients of SARS treated by integrative Chinese and western medicine	Chinese	Ribavirin + corticosteroids (29) vs Above + Chinese medicines (20)	No single primary outcome. Abstract lists: time to resolution of symptoms, use of 'hormone' treatment, time to improve peripheral WBC and lymphocytes, time to resolution of CXR findings.	No evidence	No	No
Interferon alfacon-1 plus corticosteroids in SARS: a preliminary study	English	Corticosteroids (13) vs Above + interferon alfacon-1(9)	No single primary outcome. Abstract states: "Clinical parameters, including oxygen saturation and requirement, laboratory measures, and serial chest radiography results".	No evidence	No	Yes
Clinical study on treatment of SARS with Integrative Chinese and western medicine approach	Chinese	Corticosteroids (24) vs Above + Chinese medicines (24)	No single primary outcome. Abstract states: development of illness, time of using corticosteroid, and absorption time of pulmonary inflammatory lesions	No evidence	No	No
Clinical Study on Treatment of SARS by integrative Chinese and Western Medicine	Chinese	Ribavirin, immunoglobulin, corticosteroids, thymosins vs Above + Chinese medicines (31)	No single primary outcome. Abstract states: "improving clinical symptoms, absorption of inflammation in lung, oxygen saturation and the dosage of corticosteroid used."	No evidence	No	Yes
Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings.	English	Ribavirin and corticosteroids (111) (historical) vs Above + lopinavir/ritonavir (41)	A composite adverse outcome defined as ARDS or death, measured at day 21.	No evidence	No	Yes
Clinical observation on treatment of SARS with combination of Chaihu droplet pill and Huoxiang Zhengqi droplet pill	Chinese	Ribavirin and corticosteroids (11) vs Above + Chinese medicines (11)	No single primary outcome. Abstract states "to investigate the changes of the clinical indexes such as creatinine kinase, lactate dehydogenase and serum sodium levels"	No evidence	Yes	No
Inhalation of nitric oxide in the treatment of SARS: a rescue trial in Beijing	English	Ribavirin and corticosteroids (8) vs Above and nitrous oxide* (6)	No single primary outcome. Outcomes include arterial oxygenation, and need and extent of mechanical ventilation.	Yes, with Chinese FDA	No	No

Table 2.6: Description of clinical trials reporting treatment for SARS[74–80], with selected indicators for trial reporting quality (clinical trial registration detailed, reports planned enrolment size, describes clear primary outcome, reports drug adverse events). ^includes reporting that no adverse events were observed. **nitrous oxide is not quantified in the remainder of the paper as a SARS treatment, because it is a host directed therapy where the indication was ARDS.

All included articles: With respect to reporting of clinical co-variates of patient outcome, there was no consistent methodology used in the reporting on patients at the extremes of age and so we were unable to assess to what extent these populations were included and reported. No pregnant women were known to be enrolled in clinical trials, and only 1 pregnant woman is known to have been included in prospective observational research. However, reporting of pregnancy status was incomplete for all 7 treatment trials and 7/11 prospective observational studies. With respect to reporting of safety information, 37% (n=30) of articles reported if there had been adverse effects from treatment. In 67% of these reports (n=20), adverse effects or severe adverse events due to medications were reported.

Clinical trial registry for SARS studies

69 registration records were reviewed, and 3 met inclusion criteria (selection details are in figure 2.4). The details of the relevant clinical trial records are contained in table 2.7. None of the three studies were registered, or began enrolment during the outbreak. No study has been published, although one study appears to still be open for enrolment.

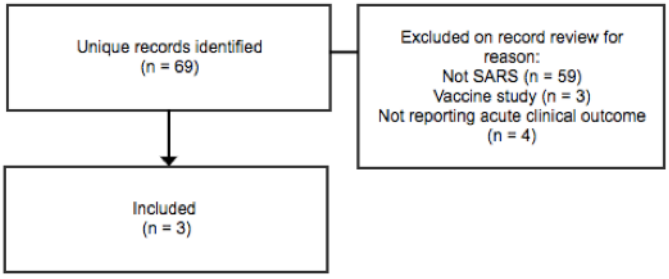


Figure 2.4: Decision tree for inclusion of clinical trial registration records.

Registration details*	Study detail	Anticipated enrollment	Progress	Results published
00578825 (Dec 2007)	Clinical trial: double blinded RCT of lopinavir/ritonavir plus ribavirin vs. placebo. Outcome measures: safety and efficacy (measured by disease severity).	340	Unknown status	No
00073086 (Nov 2003)	Prospective cohort study: clinical characterisation of SARS Outcome measures: unclear	200	Withdrawn prior to enrolling	No
01056185 (Jan 2010)	Prospective cohort study: clinical characterisation of SARI (including SARS) Outcome measures: morbidity, mortality, predictors of severity.	1000	Ongoing recruitment, began August 2009	No

Table 2.7: Clinical trial registration records for studies planning to enrol SARS patients and investigate acute clinical outcomes. *NCT registration number and date of registration

2.2.4 Discussion

Highly pathogenic novel coronaviruses are a priority disease for research and development to prevent further epidemics[41]. Previous work has decried the overall clinical research response to the SARS outbreak as inadequate to determine the safety or efficacy of treatments[67]. This systematic review explores more thoroughly what type and volume of evidence was generated, and when, to help streamline patient centred research in future outbreaks. We detail a research response that was early to start but late to publish, and heavily dependent on retrospective data.

Descriptions of patient treatment

We found details of over 15 000 treatment courses – a large volume given the total number of patients in the outbreak numbered just over 8000[81]. The most frequently prescribed agents were readily available, repurposed drugs (corticosteroids and ribavirin). These agents were selected early during the outbreak on the basis of the congruency between clinical presentation of SARS and bronchiolitis obliterans[82]. This syndromic approach is common for EID outbreaks – in fact, the early management of the subsequent MERS-CoV outbreak was partially based on the perceived clinical responsiveness to therapies for SARS[68]. Chinese medicines were widely used in China, probably facilitated by the endorsement of the Chinese Centers for Disease Control and Prevention[83].

However, we found that most descriptions of treatment use were (86% of patients) contained in retrospective observational studies, rather than prospective trials or prospective, hypothesis led, observational studies. Variation in timing, dosage and indication for therapy even within study cohorts means that this observational data cannot be meaningfully compared. Furthermore, because these studies were not designed to examine treatment efficacy or safety, reporting of essential parameters (such as the presence of adverse effects) was poor. We are not the first to report the paradox of outbreak patient treatment data accruing quickly under compassionate use, without stringent safety assessment and reporting mechanisms because of the impediments of a trial regulatory infrastructure intended to prevent harm[84].

Delays to opening trials

We found that the prospective research that was published began enrolment quickly. The median time to opening recruitment of prospective observational trials was just over one month following the first official report of atypical pneumonia to

WHO, and it was two months for interventional trials. When analysed at a country level, this means that relatively few patients (as a proportion of the official case count in the country, although this will underestimate cases in China) missed the opportunity for enrolment in a trial due to delays. These timelines to trial initiation compare favourably to those during the EVD outbreak in west Africa (2013-6)[62] and influenza A(H1N1) pdm09 (section 2.3). The data are inconsistent with a recent report that describes a median time of 225 days to initiate outbreak and pandemic observational research [85]. While this result is optimistic, it is probably confounded by two factors. The first is that some clinicians treating patients in the first affected regions were aware of an unusual cluster of patients before confirmation to international public health authorities (evident given some began recruitment prior to this notification). In addition, there is likely a publication bias, where trials that were fastest to open enrolment were most likely to recruit a sufficient sample size, and most likely to successfully publish. It is also possible that there were regional differences the complexity of regulatory processes required to initiate a trial.

Delays to publication

We found that the majority of manuscripts were not published until after the end of SARS transmission, meaning that the data were not available in time to influence patient treatment – especially when this was assessed on a regional basis. This finding is in agreement with a systematic review of epidemiology publications for the SARS outbreak that found only 7% were published during the outbreak[63]. Of the various components that contributed to these delays, the time taken to submit the manuscript appeared to delay publication longer than the peer review period, or time to publish following acceptance, although only a small proportion of articles reported these dates. The reasons for author delay may have included prior submission to a preferred journal, or less motivation to publish rapidly as the outbreak waned. Potential solutions to this delay really depend on the extent to

which previous rejections play a role, in which case reasonable propositions such as funder or academic body mandated publication deadlines may be unachievable.

Geographical distribution and collaboration

We found that the five geographical regions that reported the most number of SARS cases published patient treatment data in the literature, but another 25 affected regions did not. No publication included patients treated in more than one region. This failure to report the global picture of patient treatment is important because many EID outbreaks affect a broad geographical distribution. For example the 468 confirmed human cases of avian influenza A (H5N1) to date have been distributed across 16 countries[86]. The solutions to this include a greater commitment from researchers to produce and use minimum agreed data standards and case reporting forms that allow international data to be combined, and continued facilitation of collaborative work through international consortia[87–90].

Quality assessment

The minority of clinical trials met any of our quality assessment criteria. The reason for this is unclear, perhaps it reflects enthusiasm from journals to publish original data during a high profile event. The reporting standards may also differ between journals in different languages. While we only assessed for two quality indicators in all articles (stratification of covariates of outcome, and reporting of adverse events), again reporting was poor and in this case will partially reflect the diversity of the reasons for reporting data.

Limitations

Publication bias is inherent in the methods used by this systematic review. We can only describe evidence that was successfully generated and reported. Undoubtedly other scientists attempted to mount studies or trials but were unable to within the timeframe of the epidemic. For example, in one narrative report of their timeline to launch of a planned RCT of ribavirin during the outbreak, Muller*et al* describe that during the period before approval of their research protocol, 149 out of 249 potential patients had already been treated, and an inability to recruit patients contributed to the decision to abandon the trial[73]. The magnitude of the hidden research that was planned, commenced, or completed without publication, is unclear. Likewise, the number of registered clinical trials was small, but probably underestimates the true number of trials because the outbreak preceded mandatory clinical trial registration.

There is duplicate patient data reported in this review. This appears to stem from small cohorts of patients being later included in larger retrospective studies. Our inability to identify the source of this duplication is, in itself a criticism of the research response. The risk of duplicate data publication without clear identification is that indications of a treatment effect, or futlity, or potential harm are over-estimated by clinicians using the literature to rationalise treatment for their patients. We included suspected cases in this review (these could not be differentiated from probable and confirmed cases in the literature) and this also contributes to the large patient cohort we describe when compared to WHO.

Conclusions

During the SARS outbreak, many patients were provided medical treatment without high quality evidence to justify use of those treatments. During the outbreak it was faster to accrue information outside a clinical trial setting, and the continued

reliance on these retrospective cohorts with variation in treatment regime and clinical data capture now means that optimal clinical management strategies for novel coronaviruses remain unclear. To prepare for future outbreaks of a novel coronavirus, barriers to implementation of clinical trials need to be overcome. Where trials remain infeasible, a greater commitment to prospective, standardised clinical data collection of a quality sufficient to meet research needs is required.

2.3 Research response to the influenza A(H1N1) pdm09 pandemic

2.3.1 Introduction

Influenza pandemics constitute one of the most important threats to global health security[91]. Within the last 100 years, there have been four pandemics, including the 1918-9 "Spanish" influenza pandemic that caused over 50 million deaths[92]. Another pandemic is likely (with a projected likelihood of between 5-50% in the next five years) [91] and could be severe in health and economic impact (in the UK alone, up to 750 000 additional deaths could occur[91]). Despite this evident risk, there is clear consensus from the international community that preparedness is inadequate[17, 32, 37, 93]. This present inter-pandemic period is therefore critical – it provides an opportunity to assess recent pandemic management and strengthen preparedness.

One important element of pandemic mitigation is prophylaxis and treatment of patients. For an influenza pandemic antiviral therapies should be a key medical countermeasure because strain specific vaccine production takes months. However, there is significant controversy about whether neuraminidase inhibitor (NAI) use is supported by adequate safety and efficacy data [94, 95]. There are clear implications for the well-being of patients who are administered these drugs, and the influence of treatment on pandemic control. There are also economic consequences. To ensure ready supply of antivirals, many countries now stockpile these drugs, with an estimated expenditure of $1.3 billion USD (United States) and $675 million USD (UK) for this purpose during the 2009 pandemic[96, 97]. Accruing better evidence to inform the use of NAIs continues to be a high priority for pandemic preparedness[98].

The objective of this systematic review is to investigate how safety and efficacy data for treatment of severe A(H1N1)pdm09 accrued during the outbreak. We

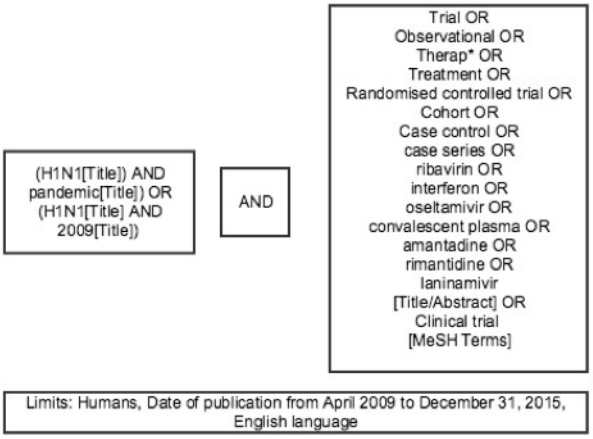


Figure 2.5: Search strategy for systematic review of published literature.

review the quantity and timing of publication of clinical trials of treatments, but also information where patients were treated outside a formal trial setting (case studies or series, and observational studies), or when research was registered but not completed, as these represent opportunites lost to gather high quality evidence. This data can be used to target future research and development efforts for pandemic influenza.

2.3.2 Methods

A systematic search was conducted to identify hospitalised patients receiving treatment for A(H1N1)pdm09 during the pandemic. Two types of evidence were searched: peer reviewed publications and clinical trial registration records. The review was prospectively registered (PROSPERO database record CRD42016039549).

Published literature search

Relevant literature was identified through a search of PubMed, using the search terms contained in fig 2.5.

To capture information on the number of patients who received treatment outside

of a trial, case studies, case series, and observational research were included in addition to interventional research. The single exception was to limit literature describing the use of oseltamivir to publications describing 10 or more patients, because case reports were abundant. Included research described hospitalised patients and reported acute clinical outcomes (defined as length of hospitalisation, intensive care admission or length of stay, medical complication, requirement for mechanical ventilation or other intensive care treatment, or mortality).

Only laboratory confirmed patients were included in the review. While A(H1N1) pdm09 was the prevailing strain during the outbreak, significant differences in how probable cases were defined between articles meant a consistent inclusion method based on other criteria was not possible. For observational studies we included articles only if enrolment opened between April 1 2009 (when the virus strain was first identified) and was completed by August 10 2010 (the declaration of the end of the PHEIC by WHO). This limitation was necessary to differentiate research conducted during the pandemic compared with routine seasonal influenza reporting once A(H1N1) pdm09 became a circulating stain. This criteria did not apply for clinical trials (where there could be no confusion with seasonal reporting) - therefore A(H1N1)pdm09 clinical trials that began during the pandemic but kept their enrolment status as open following the end of the PHEIC were included. We excluded articles if description of treatment was non numeric, or the treatment name was not provided (including use of the general term 'antiviral therapy'). We defined treatment as pathogen directed therapy, or host directed therapy where there was a specific indication for A(H1N1)pdm09. We excluded all descriptions of intensive care interventions including corticosteroids and extracorporeal membrane oxygenation for pragmatic reasons. When a single patient cohort (same sample size, enrolment period, author(s) and study location) were presented in more than one paper, duplicates were excluded (the larger cohort was used).

Clinical trial registry search

Two clinical trial registry searches were undertaken. The purpose of the first was to examine research that was planned in response to the pandemic. Clinicaltrials.gov was searched using the condition 'H1N1' and dates were restricted to following the onset of the pandemic. In comparison, a second search identified pre-existing influenza studies that were able to enrol A(H1N1)pdm09 infected patients.

Search 1: Search of clinical trial records for A(H1N1)pdm09 influenza records. Clinicaltrials.gov was searched using the term "H1N1". Included submissions were those submitted after the date of onset of the pandemic, that began recruitment during the pandemic period, and were available on the database as of the 31st of December 2015. We included clinical trials and observational research enrolling patients with confirmed A(H1N1)pdm09. Trials must have reported on one or more outcomes of drug safety, efficacy, pharmacokinetics, or patient clinical outcomes. There was no minimum anticipated, or actual sample size. We excluded submissions where A(H1N1)pdm09 influenza was not specifically identified as a strain of interest. Registration records infrequently defined the hospitalisation status of patients, and so a subgroup analysis limiting enrolment to when records were explicit about enrolling only 'severe' or hospitalised patients is also shown.

Search 2: Search of clinical trial records for influenza records. Clinicaltrials.gov was searched using the term "influenza" with Boolean operators to exclude "vaccine", "vaccination", "prophylaxis", and "diagnostic". Included submissions could begin recruitment before or during the pandemic (so long as they remained open to enrolment for some period during the pandemic period) and were available on the database as of the 31st of December 2015. Analysis is stratified as to whether the studies were ongoing at the time of the pandemic onset (April 1 2009). We

included clinical trials and observational research enrolling patients with confirmed influenza. Trials must have reported on one or more outcomes of drug safety, efficacy, pharmacokinetics, or patient clinical outcomes. There was no minimum anticipated, or actual sample size.

Data extraction

Data extraction was performed by one reviewer (AR) according to the pre-specified inclusion and exclusion criteria. Decisions were recorded using electronic systematic review software (rayyan.qcri.org), available to the other author (PWH).

For published literature, I extracted the type of research, the location of research, patient outcome measure(s) described, the number of patients, the dates that patients were enrolled, the number of patients receiving A(H1N1)pdm09 specific treatments, reporting of adverse events, reporting of drug resistance, the dates for manuscript submission, acceptance and first publication(including e-publication), and information on inclusion of some high risk[99] groups - pregnant women, the elderly (defined as older than 65 years), and children (defined as younger than 18 years). Where dates were only described to the closest month, the 1st day of the month was used for analysis. Any resulting bias will contract timelines for the time to conduct research relative to the pandemic, but may overestimate the time taken to publish work.

For registered clinical trials, I included the type of study, date of submission to the registry, predicted dates of enrolment and sample size (and actual values when known), whether high risk groups (as defined above) were included, the geographical location of the research, the completion status of the work, and the date of first publication (including e-publication). These details were cross checked against any corresponding publications.

Statistical analysis

Descriptive statistics are presented as frequencies for categorical variables and median with interquartile range for continuous variables. The findings from the published literature and trial registries are reported separately. Analysis of the literature was stratified by country of origin, and by research type. Chinese medicines are presented as a single class because individual components could not be differentiated. Assessment of combination therapy was not possible due to variable reporting practices in the literature. The R statistical software (version 3.3.2) and Microsoft Excel for Mac (version 15.21.1) were used for statistical analysis.

2.3.3 Results

Findings from published literature

Summary: Patients hospitalised with A(H1N1)pdm09 were often given specific treatments for their infection and a variety of agents were used. There are 160 articles included in this review (figure 2.6) that describe 39, 577 hospitalised patients and 33 869 treatment courses (table 2.8). Twelve different treatments were used, with oseltamivir being most common. The median number of treatment courses described in each article is 63 (IQR 22-193).

Little prospective research was undertaken. Of the 160 articles included, two articles are interventional trials[100, 101] (n = 73, representing 0.2% of total reported patients), 28 are prospective observational studies (n = 6102, accounting for 15.4% of total patients), 129 articles are retrospective observational studies or case reports (n = 33342, 84.2% of total patients), and one paper enrolled patients both prospectively and retrospectively (n = 98, 0.24% of patients).

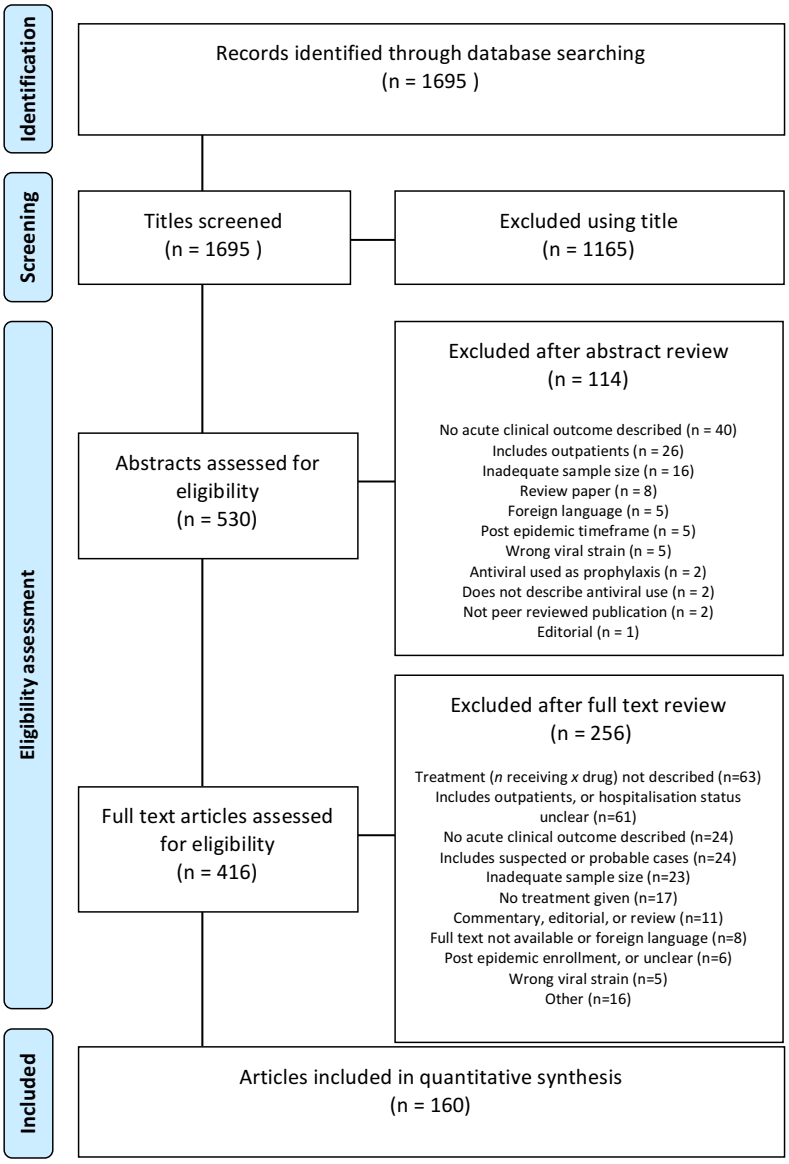


Figure 2.6: Prisma flow diagram describing screening, eligibility and inclusion for peer reviewed literature.

Time required to initiate research: The viral strain was identified on April 1, 2009. The median delay to first patient enrolment for prospective observational studies was 102 days (61-172 days). The two clinical trials began enrolment after a delay of 244 days and 275 days.

Treatment name	Number of publications reporting use	Total number of patients receiving treatment*	Median (IQR) number of patients receiving treatment per publication**	FDA drug approval status for use in influenza in 2009
Oseltamivir	154	31737	63 (21-184)	Approved for acute uncomplicated influenza, expanded under EUA April 2009
Zanamivir	54	368	2 (1-9)	Approved for acute uncomplicated influenza, expanded under EUA April 2009
Peramivir	14	403	1 (1-3)	Unapproved, eIND in April 2009, EUA October 2009
Amantadine	11	86	3 (2-13)	Approved for acute uncomplicated influenza, but resistance to A(H1N1)pdm09 demonstrated
Rimantidine	5	32	3 (1-13)	Approved for acute uncomplicated influenza, but resistance to A(H1N1)pdm09 demonstrated
Ribavirin	5	34	2 (1-6)	Not approved for influenza
Intravenous Immunoglobulin	4	44	4 (3-13)	Not approved for influenza
Chinese medicines	3	1051	245 (151-498)	Not approved for influenza
Convalescent Plasma	2	52	26	Not approved for influenza
Macrolides^	1	31	31	Not approved for influenza
Sirolimus	1	19	19	Not approved for influenza
Statins^	1	12	12	Not approved for influenza

Table 2.8: Volume of treatment courses described in the literature for hospitalised patients with A(H1N1)pdm09 during the pandemic period. * Some patients received more than one treatment. ** when publication describes use of that drug. eIND = emergency investigational new drug authorisation. EUA = emergency use authorization.

Time required to publish research: For prospective observational studies, the median number of days that enrolment stopped after virus identification was 274 (IQR 195-313). This was 223 days before the end of the PHEIC (August 10, 2010), but at a time when case numbers were falling. The two clinical trials did not close to enrolment until 699 and 944 days after virus identification (corresponding with March and November 2011).

The publication of all articles over time is shown in figure 2.7. No (0/2) interven-

tional trials were published before the end of the PHEIC (they were published in August 2013 and February 2014). 25% (6/28) of prospective observational studies, and 22% (26/130) of retrospective or mixed enrolment research was completed and published by the end of the PHEIC. The median date of publication for all articles was March 18 2011 (IQR September 28 2010 – October 24 2011). This was 213 days after the end of the PHEIC.

Overall the median delay between final patient enrolment (or inclusion) and publication date was 444 days (IQR 281 – 684). The median delay was 302(IQR 142 – 534) days between final patient enrolment and article submission, 93 days (IQR 63 – 144) between submission and acceptance, and 56 days (IQR 24 – 94) between acceptance to publication, where data existed for these intervals.

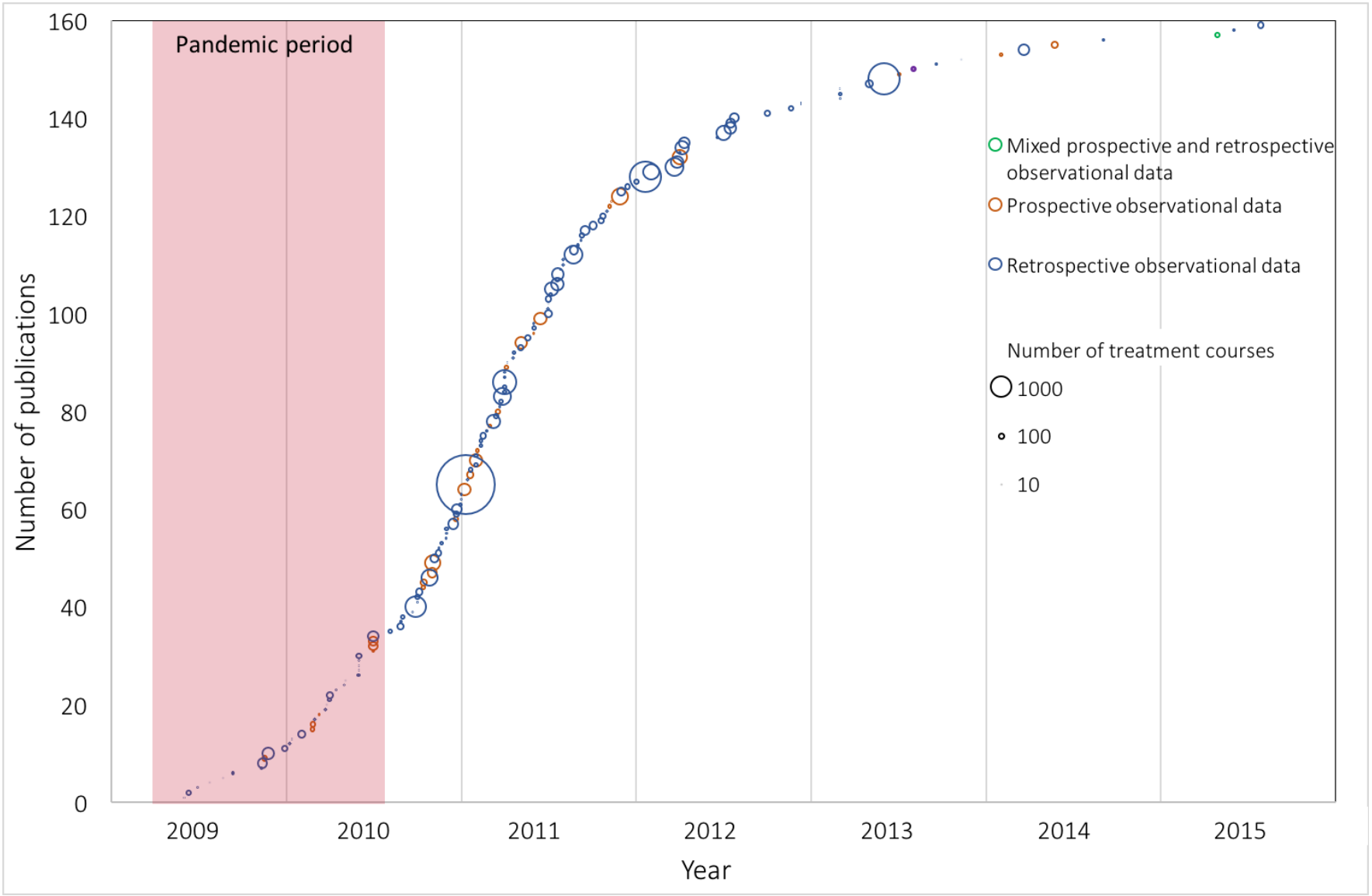


Figure 2.7: Timeline of publication of manuscript, according to publication type and number of treatment courses described. Pandemic period ranges from the 1st of April 2009, to the end of the PHEIC on August 10, 2009

Geographical representation of publications: 39 countries reported treatment data. The most articles were published by the United States (n=25, reporting 2559 treatment courses), followed by China (n=16, reporting 14680 treatment courses) and Spain (n = 14, reporting 3072 treatment courses). Individual country level data describing the number of publications, number of treatment courses described, and the first date of patient enrolment in prospective research (where relevant) are shown in table 2.9.

Reporting on high risk populations: Sixty percent of prospective research included pregnant women (17% did not include pregnant women, and it was unclear in 23% of cases). Children under the age of 18 were included in 30% of the prospective research (not included 47% of the time, and it was unclear in 23% of articles). Elderly participants above the age of 65 were included in half of prospective articles (not included in 20% of articles and it was unclear in 30% of articles).

Reporting of drug effects: 23% (n=36) of all articles described if there had been adverse effects from treatment. In 42% of these cases, adverse effects or severe adverse events were noted. In articles that included treatment with an antiviral (where drug resistance is a possibility), 86% (n = 130) did not include information about resistance, or indicated that it was not assessed. 12% (n=18) of articles reported laboratory testing for antiviral resistance, and 3% (n=4) of articles indicated that there was clinical suspicion of resistance, but that testing was not undertaken.

Country reporting data	Number of treatment courses described in literature (by study enrolment type)			Total number of papers published	Date of first patient enrolment in prospective research
	Prospective	Retrospective	Total		
Argentina		517	517	2	
Australia	95	126	221	3	01/05/2009
Australia and New Zealand	67	390	457	2	01/06/2009
Austria	355	9	364	2	20/09/2009
Belgium		27	27	1	
Brazil		17	17	1	
Canada		98	98	4	
Chile	97		97	1	29/05/2009
China	384	14296	14680	16	01/06/2009
Colombia		26	26	1	
Denmark		84	84	2	
France	38	770	808	4	30/11/2009
Germany	39	79	118	5	05/10/2009
Holland		39	39	2	
Hong Kong	217	50	267	3	01/01/2009
India		813	813	7	
Iran		51	51	1	
Israel	262	449	711	2	12/07/2009
Italy		158	158	4	
Japan		670	670	6	
Korea		2451	2451	10	
Kuwait		196	196	1	
Lebanon		14	14	1	
Lithuania		69	69	1	
Malaysia		1306	1306	1	
Mexico		456	456	2	
Norway		63	63	1	
Oman		125	125	1	
Saudi Arabia	47	83	130	2	18/07/2009
Singapore	70	538	608	12	27/04/2009
South Africa	19		19	1	17/08/2009
South Korea		20	20	1	
Spain	2942	1156	4103	16	23/04/2009
Sweden		108	108	1	
Switzerland	78	11	89	2	01/10/2009
Taiwan	75	99	174	3	15/07/2009
Thailand	24		24	1	08/07/2009
Turkey	18	874	892	5	01/11/2009
UK	200	44	244	4	01/09/2009
USA	251	2308	2555	25	23/04/2009
Total	**5278**	**28599**	**33869**	**160**	

Table 2.9: Country level data demonstrating number of treatment courses described in the literature, and date of first enrolment in prospective research, where relevant

Findings from A(H1N1)pdm09 trial registrations 15 registration records are included in the review (figure 2.8). There were 10 interventional treatment trials and 5 observational studies (2 with treatment efficacy outcomes, and 3 with general acute clinical outcomes) planned during the pandemic. A total of eight different treatments were to be studied; oseltamivir, zanamivir, convalescent plasma, intravenous immunoglobulin, rosuvastatin, sirolimus (rapamune), Chinese herbs, and vitamin supplementation (vitamin A, C, E).

Of the fifteen studies, nine are reported as completed, four were terminated due to

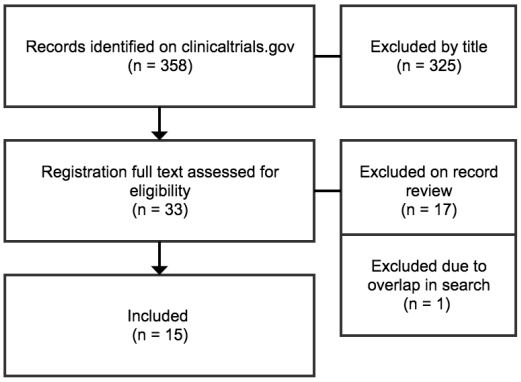
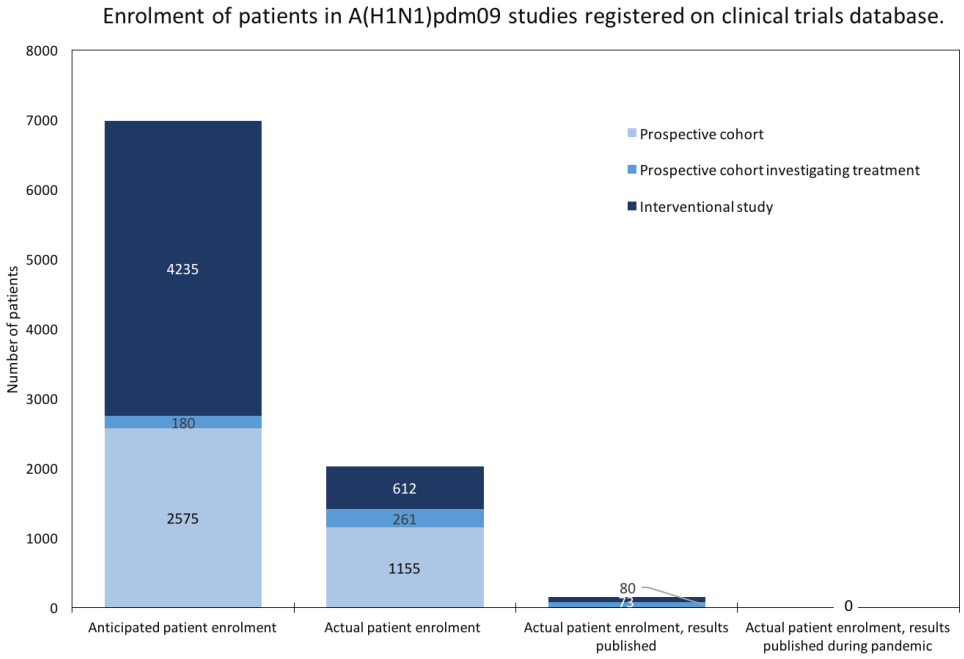


Figure 2.8: Prisma flow diagram describing screening, eligibility and inclusion for clinical trial registration records for studies aimed at enrolling A(H1N1)pdm09 patients.

the end of theA(H1N1)pdm09 pandemic or declining case numbers, and the status of two studies is not recorded. The anticipated and actual enrolment of patients into all studies is depicted in figure 2.9. Some study protocols excluded patients on the basis of young age (25%) and pregnancy (50%). Results are available in the literature for three of the completed studies (table insert, figure 2.9, representing 153 patients, and available on the clinical trials registry for an additional two of the terminated studies. A sub-group analysis of clinical trials that only included hospitalised or severe cases is provided in figure 2.10.



Registration number	Type of study	Treatment	H1N1 patients (n)*	First patient enrolment (month, year)*	Final patient enrolment (month, year)*	Date Published (month, year)
NCT01306773	Prospective cohort	Convalescent plasma	80	Sep-09	Jun-10	Jan-11
NCT01617317	Interventional	Hyper immune IV Immunoglobulin	35	Jan-10	Nov-11	Aug-13
NCT01620307	Interventional	Sirolimus	38	Winter-09	Spring -11	Feb-14

Figure 2.9: Anticipated versus actual enrolment of patients in clinical studies for A(H1N1)pdm09. Together, the studies planned to enrol 6837 patients. 2028 patients were actually enrolled (including an unknown proportion of patients enrolled in the post pandemic period). Of these, results are presently available in the literature for 153 patients. All results were published following the end of the pandemic. Table insert displays the enrolment number and publishing timeline for completed studies with results published. *where conflict existed between numbers in the clinical trial record and publication, publication numbers were used. Published literature is available at [100–102]

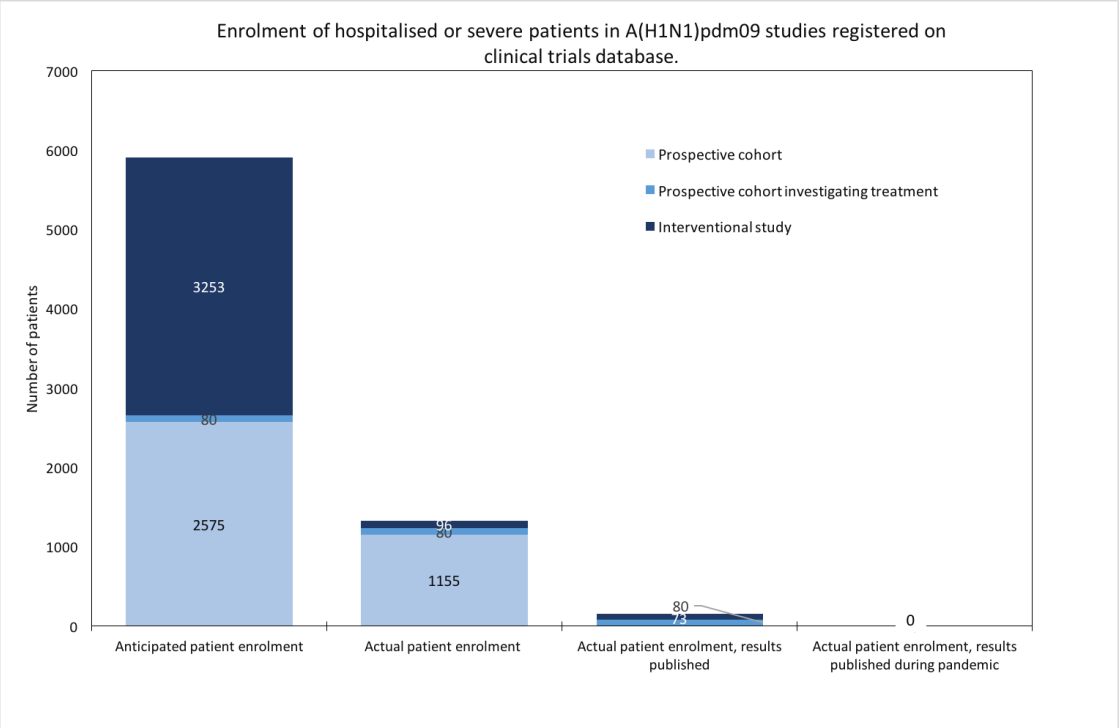


Figure 2.10: Enrolment metrics for studies enrolling 2009 H1N1 patients, when analysis is restricted to 'serious' and hospitalised patients. 10 records are eligible for inclusion. This includes 6 interventional trials and 4 prospective observational studies. Registered trials intended to assess the following treatments: convalescent plasma, IVIG, Chinese medicines, rosuvastatin, and rapamune. Together, the trials intended to enrol 5908 patients, 1331 patients were actually enrolled, results are publicly available for 153 patients, and for no patient were the trial results available before the end of the epidemic. Results are now available for three studies.

Findings from influenza trial registrations

18 influenza registration records were reviewed (figure 2.11). There were 16 interventional trials and 2 observational studies that were enrolling patients during the A(H1N1)pdm09 pandemic period. The treatments under investigation were oseltamivir, sambucol supplement, zanamivir, peramivir, amantadine, pomegranate supplement, nitazoxanide and favipiravir.

11 studies were completed, 4 were terminated early, the status of 2 are unknown, and 1 study has ongoing enrolment as of January 1, 2017. Results were available for 9 of 11 completed studies (figure 2.12), representing a total of 439 A(H1N1)pdm09 patients.

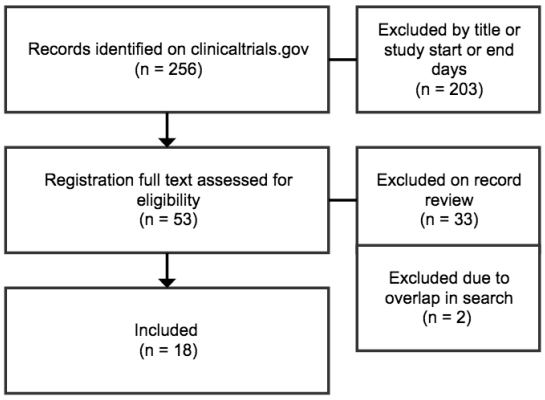


Figure 2.11: Prisma flow diagram describing screening, eligibility and inclusion for clinical trial registration records for studies aimed at enrolling influenza patients.

Registration number	Type of study	Treatment	Total patients* (n)	A(H1N1) pdm09 patients* (n)	First patient enrolment* (month, year)	Last patient enrolment* (month, year)	Date Published (month, year)
Enrolment commenced before pandemic							
NCT00298233	Phase 2	Oseltamivir	326	72	Apr-07	Feb-10	May-13
NCT00391768	Phase 1/2	Oseltamivir	87	37	Jan-07	Apr-10	Mar-13
Enrolment commenced during pandemic							
NCT00949533	Phase 3	Oseltamivir	37	unknown	Aug-09	Oct-10	Apr-16(Unp)
NCT00957996	Phase 3	Peramivir	127	94	Oct-09	Oct-10	Aug-13
NCT01199744	Prospective cohort	Zanamivir	1575	unknown	Nov-09	Apr-10	Mar-11(Unp)
NCT01014988	Phase 2	Zanamivir	130	92	Nov-09	Sep-11	Feb-14
NCT01052961	Phase 4	Oseltamivir	155	34	Jan-10	Jun-12	Dec-13
NCT01050257	Phase 3	Oseltamivir	118	unknown	Jan-10	Sep-12	Aug-13(Unp)
NCT01068912	Phase 2	Favipiravir	530	110	Feb-10	May-12	Feb-14(Unp)

Figure 2.12: Enrolment number and publishing timeline for completed studies where results are published in the literature (date followed by reference), or on the clinical trials registration site (date followed by unp). *where conflict existed between numbers in the clinical trial record and publication, publication numbers were used.[103–107]

2.3.4 Discussion

There is a consistent criticism that the patient centered research response to disease outbreaks is fractured and delayed [62, 90, 108, 109]. However, there has been little quantitative examination of these assumed insufficiencies. This paper demonstrates that despite over 33 000 treatment courses being described in the literature for hospitalised patients with influenza A(H1N1)pdm09 during the pandemic, fewer than

600 received treatment (or placebo) as participants in a registered interventional clinical trial with results available in the peer reviewed literature. None of these results were available during the timeframe of the epidemic. This constitutes a significant failure to collect useful data. As a consequence, treatment strategies for severe influenza continue to be based on low quality data[110, 111].

The detailed findings of this review help direct an improved research response:

Reinforce the importance of patient centred research

We found that the majority of descriptions of treatment courses were contained in retrospective observational studies or case series, without the data quality or design standards required to make valid conclusions about efficacy and safety. Few prospective studies were launched, despite both the inevitability of an influenza pandemic occurring at some point, and known priorities for evidence generation. For example, for approved NAIs, completed trials at the onset of the pandemic had only been conducted for mild seasonal influenza in healthy adults[112]. The safety and efficacy findings from this population cannot be extrapolated to more severe disease, or pandemic strain illnesses, or to high risk populations. Antiviral resistance to NAIs was known by the time of the pandemic and oseltamivir resistance for A(H1N1)pdm09 in June 2009[113]. Combination therapy trials should have been pre-planned (and should be for future pandemics). In addition, the known pragmatic limitations of these drugs (e.g. unavailability of intravenous formulation of osteltamivir) justified assessment of a broader range of agents in trials. The evidence vacuum was much greater for other treatments. As an example, at the time the peramivir EUA was issued in response to the pandemic, only 33 patients (all adults) had received the FDA authorised dosage [84, 114].

Where there was relative success, was in enrolling A(H1N1)pdm09 patients in

ongoing or seasonal influenza studies, who accounted for 439 of the 582 patients enrolled in a trial. Based on these findings, platform trials should be adopted as a way to expedite outbreak research. In this approach, multiple treatments (or multiple influenza strains) are evaluated under an overarching protocol and regulatory framework, improving efficiency [115]. Sleeper protocol research may also provide a solution. These pre-prepared and pre-approved protocols either lay dormant, waiting for cases of pandemic strain influenza, or in a modified format are used for seasonal influenza. This allows assessment of the logistics and feasibility of the protocol and potentially provides comparison data. An example of this type of protocol now exists for severe acute respiratory infections, including influenza (NCT02498587). This method may be limited by difficulties in 'awakening' the protocol if there are long inter-epidemic periods[116].

Where clinical trials are not undertaken, patient registries should exist to collected standardised information on patients receiving therapy. Given the volume of treatment use outside of a trial setting, these registries can be powerful. While 'practice based evidence' [117] has limitations and requires careful interpretation, large observational databases are preferable for examining treatment effects for pandemic influenza when compared to the present practice of retrospectively seeking individual patient level data from the published literature[66].

Launch patient centred research faster

We found that from the time A(H1N1)pdm09 was first detected, it was over three months before prospective data collection began, and eight months before the first interventional trial began recruitment (December 2009). While these delays compare well to research conducted in the UK[118], and even recent evaluations of delays for other epidemic observational research[85], it remains too slow. Recent recommendations that were compiled following the EVD epidemic in west Africa

now suggest that research should be initiated, enacted and completed by the time an epidemic peaks[61]. While some commentary based pieces have begun to discuss the regulatory and operational hurdles that contribute most to these delays, there have been few structured analyses from research groups that have conducted clinical research. These are required to focus efforts on tractable measures to contract timelines.

Facilitate multi-centre collaboration

In addition to highlighting an inadequate volume and speed of research, the small median sample sizes of literature included in this review indicates a fractured research response. It is estimated that a sample size of 800 patients is required to power a randomized controlled trial of an NAI in hospitalised patients (depending on the outcome criteria)[98]. No prospective interventional or observational research included in this review enrolled that many patients. Beyond the generally accepted benefits of increased enrolment and external validity, multicentre research provides a number of specific benefits in epidemics, including that it can compensate for unexpected variations in epidemiology at the regional level (such as the sudden end to the EVD outbreak in Liberia that prematurely halted a clinical treatment trial[119]) or the temporary closure of health care facilities with nosocomial transmission (such as occurred during the SARS outbreak of 2003[120]). Increasingly, multi-centre research is facilitated by use of harmonized case definitions and case reporting forms (such as those produced by the International Severe Acute Respiratory and Emerging Infection Consortium(ISARIC)) and compliance to international data standards (such as those produced by the Clinical Data Interchange Standards Consortium(CDISC).

Improve timely and complete reporting of results

This paper reports long delays between clinical data capture and reporting it in the peer reviewed literature. These findings are consistent with other bibliographic analysis undertaken for disease outbreaks, including epidemiology reporting for SARS(where only 7% of articles were published within the timeframe of the epidemic) [63] and randomized controlled trials of A(H1N1)pdm09 vaccines (where 29% of clinical trial results were published almost a year following the end of the pandemic[64]). The present WHO standard applied to interventional clinical trials is that the main findings are to be submitted for publication within 12 months of study completion[127] and although no analogous guidelines exist for observational clinical data, there are analogous scientific and ethical imperatives for timely reporting.

While the ramifications of delayed reporting are well described for other research fields[121] there are specific concerns during epidemics. As one example, observational data must be accrued to design interventional trials (such as approximating the type and rate of outcomes). Emerging evidence can also prioritise trials so that the most promising continue recruitment when there are a declining number of cases late during an outbreak[122]. Furthermore, the discordance in making preliminary data and results available to regulatory or public health authorities behind closed doors, but not publicly, means that the data does not reach the communities affected or researchers.

Initiatives to minimise publication delay now include fast track review for manuscripts likely to have significant implications for clinical practice[123]. However, in not all circumstances are these initiatives linked to rapid publication and so their influence is unclear. There is also some support for near real time, or pre-publication online release of preliminary findings (exemplified by release of genomic sequencing data for EVD[124], and animal viral challenge data during the Zika epidemic[124]).

The risks of pre-publication release include ensuring that careful evaluation of the accuracy of data and interpretation of findings has occurred (normally managed through the peer review process) and that scientists receive recognition of their contributions (such as publication authorship or citations). Pre-approval approval of trial protocols and results free-review (where initial review excludes results and some discussion) may assist especially in timely reporting of negative results.

Limitations

The scope of this systematic review was narrowed due to the high volume of literature reporting clinical aspects of A(H1N1)pdm09. In particular, we focused only on the setting where most antivirals were used, in hospitalised patients [125]. We included a variety of publication types, including case series or observational studies where the purpose was not to investigate a treatment effect (but patients did receive one or more drugs). This method was used to provide an estimate of patients who may have been eligible for inclusion in a clinical trial because they presumably had no contraindications to drug use. It is important to note that this estimate of patient numbers does not represent the true number of hospitalised patients who were treated. It is provided only to compare to the number of patients who received treatment in clinical trials and we chose this comparison only due to the absence of accurate international epidemiological case data. In particular, we have under-estimated the true figure by excluding articles where treatment was not clearly defined and when pandemic strain influenza was not laboratory confirmed. Furthermore, we cannot rule out inadvertent inclusion of manuscripts reporting duplicate data. Likewise our estimates of patients enrolled in clinical trials is almost certainly an underestimation– much of the momentum toward compulsory registration of clinical trials[126, 127] occurred subsequent to the pandemic (although the concept was quite well established by this time) and so it is likely that other trials were planned, initiated, or even completed without

public knowledge. We restricted our observational data collection to that captured before the declaration of the end of the PHEIC, and while we recognise that research continued to occur during the second and third waves of the epidemic, differentiating this work from routine seasonal influenza research became difficult. Only English manuscripts were reviewed, and there was only one reviewer.

2.3.5 Conclusions

We demonstrate how use of treatments under compassionate care circumstances during the 2009 influenza pandemic was not matched with a commitment to capture high quality data on treatment use. Moreover, we show that the data that was collected on patients was incompletely reported and published after prolonged delay. We recommend use of multi-centre and platform trials, and greater standardisation of reporting of compassionate use as potential solutions to improve accumulation of treatment data during a pandemic.

2.4 Contribution of the chapter

In this chapter I demonstrate a disparity between vast accumulation of case descriptions of patient treatment, compared with a small volume of prospectively designed, registered clinical research during outbreaks. I demonstrate that at times, the opportunity is missed to reach meaningful sample sizes in research due to delays in initiating research, and I describe how findings are slow to become publicly available in the literature. While I discuss the implication of these findings in each section, it is also worth commenting on what these findings mean collectively about the value of research during epidemics. A framework produced by Ioannadis et al. [128] describes factors that contribute to false research findings in a scientific field and we found evidence for several of these in the outbreak literature:

- **"The likelihood of a study result being true is highest for well conducted randomized controlled trials, followed by underpowered early phase clinical trials, and lowest for exploratory epidemiological studies":** We describe that 4.3% of patients who had treatment data reported in section 2.2 and 0.2% of patients in section 2.3 did so in the circumstances of a clinical trial. Overwhelmingly, the data reported was contained in observational epidemiological work. Rarely were these hypothesis driven.

- **"The smaller the studies that are conducted, the less likely the findings are to be true":** We reported small median sample sizes (the median number of patients per manuscript in sectionin section 2.2 was 44) and section 2.3 was 71 and described how no prospective research in section 2.3 met the minimum sample size estimated for a powered clinical trial of a neuraminidase inhibitor.

- **"The greater the flexibility in designs, definitions, outcomes, and analytic modes in a scientific field, the less likely the research is to be true":** For both outbreaks we quantified a wide variety of research designs, ranging from retrospective case reports, through to randomized controlled clinical trials. An example of the heterogeneity in the definitions used, manifests in our inability to assess the number of children and elderly patients described in each paper. Because there was variance in how this data was described, any meta-analysis examining treatment effect would be unable to stratify by age unless individual patient data was available. An indicator of the heterogeneity of outcomes is found in our assessment of SARS clinical trials in section 2.2, where each of the seven trials published used different outcome measures to assess drug efficacy, and for six of the seven trials there was no single primary outcome stated.

- **"The hotter a scientific field (with more scientific teams involved),**

the less likely the research findings are to be true". The underlying rationale for this is that if many independent groups are assessing the same relationship, the number of false positive results becomes tangible, and that these are more likely to publish than non significant findings. Undoubtedly epidemics create a surge in scientific interest - we included 160 manuscripts in the pH1N1 review and 81 manuscripts in the SARS review. It would be interesting to compare these numbers to diseases causing similar numbers of death over the same time period.

There are several consequences to inaccurate data - notably confusion during the outbreak regarding treatment strategies and a continued lack of evidence based guidelines. It is also worth noting that because treatment of patients in new outbreaks is often based on treatment efficacy in analogous outbreaks, the consequences can reach to other outbreaks. Much of the criticism of the scientific output from these outbreaks has been framed in terms of the opportunity missed to collect higher quality data. What is often missed in this debate is frank discussion of the potential harms to patients by widespread acceptance of empiric therapy using treatments with limited evidence. For example, a systematic review of SARS treatment effects by Stockman et al. found that all human studies evaluating ribavirin or corticosteroids were either inconclusive in their findings, or indicated the possibility of harm[67]. In addition we suggest that our findings should provoke further debate regarding the ethics of using unlicensed agents under compassionate use circumstances compared with focussing efforts on facilitating and scaling research.

The methodological limitations of each systematic review are outlined in the discussion for that section, but there are several changes I would make if we were to complete the work again. The temptation when conducting a review is to increase the number of variables extracted from each manuscript. The risk of doing so for

bibliometric reviews is that the work becomes unmanageable because this type of review is by nature, deliberately much broader in inclusion criteria to more completely characterise research during a period (for example, we undertook data extraction for over 240 manuscripts for these reviews). Therefore we limited our data extraction to three indicators of reporting quality. However, it would have been valuable to detail other markers of quality that would indicate if pooled analysis of findings is possible without substantial bias. Furthermore, most manuscripts that we reviewed described drug efficacy, or advised treatment protocol in their discussion. This usually occurred without an *a priori* hypothesis, without an adequate sample size to have confidence in estimates, and without clearly defined comparison groups to reliably detect signals. Therefore, it would have been useful to determine how frequently findings were presented that were not supported by data.

2.4.1 Future research

This chapter was the first to be undertaken during this DPhil and so the results have been instrumental in directing further work. To begin with, it became clear from our data that a detailed breakdown of the factors that contributed to delays to initiating recruitment, and delays to publishing work after completion would be helpful to clearly identify opportunities for change. These are not available from reviews of the literature. Therefore, to further this objective, we created a work plan to track the regulatory and operational components of a clinical trial conducted during an EID outbreak. We provide an example of this operational level analysis in Chapter 3. In addition, we are now using the detailed bibliometric and epidemiologic data generated by this work in a new collaboration with the University of Lancaster, where we are exploring trial design options that are responsive to the unique circumstances of an outbreak including heterogeneity in clinical care between sites, or changing rate of outcomes, such as case fatality rate, as the outbreak progresses.

3

Implementation of a phase II clinical trial during an EVD outbreak

Contents

3.1 Preface to the chapter

This chapter focuses on clinical treatment trials conducted during the west Africa (2013-16) EVD epidemic and meets objectives 2 and 3 of the thesis: to conduct primary clinical research, and to develop recommendations that facilitate clinical research conduct in future outbreaks.

3.1.1 Context

Ebolavirus was first identified in 1976. Fewer than 3000 human cases had been recorded up until 2013, and these had occurred in small, sporadic outbreaks[21]. In December 2013, a two year-old child living in rural Guinea[129] was infected, and the subsequent outbreak soon crossed into Sierra Leone and Liberia. An outbreak of unprecedented magnitude had begun. When WHO acknowledged in August 2014 that the outbreak constituted a PHEIC, there were already 1,711 reported cases and 932 deaths[130]. By the end of the epidemic, 28,646 cases and 11,323 deaths had been reported,[131] but the true numbers are likely to be much higher. The epidemic had far reaching effects for the region, including enormous economic costs and significant strain on already stretched healthcare systems[132, 133].

The focus of global response efforts was to provide humanitarian assistance and medical care, and to interrupt chains of transmission[134]. But there were also calls from WHO, funding bodies and governments to urgently scale-up the conduct of scientific research[135–138] that would enhance knowledge and allow investigation of potential interventions. There were major hurdles to overcome, however, including logistical challenges,[62, 139] and ethical and societal considerations[140, 141] that could impact the ability to reach conclusions within the lifetime of the epidemic.

This chapter describes original research conducted during the outbreak and also

provides broader analysis of the contributions and shortcomings of the research response within the unusual context of the outbreak.

3.1.2 Objectives of the chapter

Section 3.2 describes a phase II clinical trial for an EVD experimental therapy conducted in Sierra Leone during the outbreak. The trial was conducted as part of the Rapid Assessment of Potential Interventions and Drugs for Ebola (RAPIDE) platform led by the University of Oxford. In this section of the thesis, the primary findings of the trial are presented. This work constitutes primary research conducted within an epidemic (objective 2 of the thesis).

Section 3.3 provides a basis for broader analysis of how the trial was achieved, and in particular, the adjustments that were made to trial design and implementation to produce a pragmatic trial (objective 3 of the thesis).

Section 3.4 discusses more broadly how the progress made during the EVD epidemic can inform preparation and conduct of trials in the future. The first focus is on strengthening pre-clinical development which is an often overlooked area for discussion for those involved in clinical research. The second is on small scale innovation that can be achieved by single research groups or networks who participate in clinical trials.

Publications

The work in this chapter has been submitted or published in the following manuscripts:

1. Dunning, J., Sahr, F., **Rojek, A.,** et al. (2016). Experimental treatment of Ebola virus disease with TKM-130803: a single-arm phase 2 clinical trial.

PLoS medicine, 13(4), e1001997.

2. **Rojek, A.** Dunning, J, Castle, L. et al. (2017) Regulatory and operational complexities to conduct of a clinical trial during the Ebola virus disease epidemic in west Africa. *Accepted to Clinical Infectious Diseases.*

3. **Rojek, A. M.,** & Horby, P. W. (2017). Offering patients more: how the west Africa Ebola outbreak can shape innovation in therapeutic research for emerging and epidemic infections. Phil. Trans. R. Soc. B, 372(1721), 20160294.

3.1.3 Author's contributions

Section 3.2 reports a large collaborative clinical trial in which I was a member of the primary research team, and was the field project manager in Sierra Leone. I contributed to the following activities: protocol development and drafting; preparation of materials for submission to regulatory authorities and human research ethics committees; development of case reporting forms; development of field standard operating procedures; development of staff training plans; induction, training and management of teams in the field; organisation and supervision of daily activities in the field; management of health or safety incidents in the field; data entry; patient consent and enrolment procedures; patient care; long term patient follow up; organisation and undertaking of community engagement activities in the field; maintenance of the investigator site file; site close out; building and maintenance of partnerships with other organisations on site; drafting of the primary manuscript; collaboration with external partners for additional laboratory analyses (not yet published). I was not involved in the design of the multi-stage approach, I did not perform laboratory analysis, and I did not perform the statistical analysis for the primary results or produce the associated figures. I contributed to drafting of the manuscript.

For section 3.3 I devised the manuscript, wrote the first and subsequent drafts and designed and produced all figures and tables and was responsible for submission.

For section 3.4 I devised the manuscript, wrote the majority of first and subsequent drafts, designed and produced all figures and tables, and was responsible for submission.

3.2 A phase II clinical trial for treatment of EVD with TKM-130803

3.2.1 Introduction

Ebola virus causes severe illness in humans and is frequently fatal, with a case fatality rate of approximately 70%[52]. There is no specific treatment. The severity of EVD infection in humans is closely correlated with viral load[142–145]. Therefore anti-viral agents are one focus of drug development. One of the lead experimental therapies that WHO prioritised for testing in humans during the west Africa epidemic was TKM-100802 (Tekmira Pharmaceuticals Corporation, British Columbia, Canada). The drug is a lipid nanoparticle (LNP) formulation of small interfering ribonucleic acids (siRNAs) targeting gene products that encode two viral proteins: L polymerase (Lpol) which is involved in transcription and replication of Zaire ebolavirus, and Viral Protein-35 (VP35) which is involved in suppression of the host immune response[146].

Prior to the west Africa outbreak, TKM-100802 had been evaluated in guinea pig, non-human primate (NHP), and human Phase 1 clinical trials[147]. Prior to the west Africa outbreak, the TKM-100802 Investigational New Drug application (IND) was placed on partial clinical hold by the US Food and Drug Administration, meaning TKM-100802 may be used in individuals with confirmed or suspected EVD but not administered to normal healthy subjects as part of a clinical trial. The

basis for the clinical hold was concern about cytokine release syndrome or infusion-related reactions. Cytokine release syndrome is a pro-inflammatory reaction that occurs when activated lymphocytes and/or myeloid cells release soluble immune mediators following administration of some drugs, usually monoclonal antibodies. TKM-100802 was administered to five patients with EVD medically evacuated to the US and Europe, and to one individual as PEP (personal communication Mark Kowalski, Tekmira Pharmaceuticals Corporation). Since the product was administered on a compassionate basis to these individuals and because the patients simultaneously received other experimental products, it was not possible to assess the efficacy or safety of TKM-100802 in the treatment of EVD[148, 149].

TKM-130803 is a new formulation of TKM-100802 designed to ensure specificity to the west African Makona variant of Zaire ebolavirus causing the west African outbreak. The siRNA component has been adapted by two nucleotide substitutions in the VP35 siRNA and a single nucleotide substitution in the L-polymerase siRNA. The siRNA drug component of TKM-130803 is termed siEbola-3 and is formulated with lipid excipients (LNP1 composition) to form LNPs. In rhesus monkeys infected with a lethal challenge of Makona variant EBOV, administration of siEbola-3 formulated with a different LNP (LNP2) and dosed at 0.5 mg/kg/d for 7 days resulted in 100% (3/3) survival when commenced 72 h post-inoculation, a point in the disease course where viral RNA levels up to 10^6 RNA copies/ml are detected by blood sampling[150].

This report describes the RAPIDE-TKM-130803 trial, an open-label, non-randomised, single-arm trial to generate early evidence of the effectiveness of TKM-130803 for EVD.

3.2.2 Methods

Trial setting

The trial was conducted between 11th March and 15th June 2015 at the Port Loko (Mathaska) Ebola Treatment Centre (ETC) in Sierra Leone.

Patient enrolment

The eligibility requirements for enrolment in the clinical trial was laboratory confirmed infection in a patient aged 16 or over. Patients were excluded if they were unable to comply with protocol requirements, jeopardised patient or staff safety, were designated by the treating clinician as for end of life care only, had no possibility of gaining IV access, had used another investigational product within the previous 30 days (or planned to), or if they had been transferred late from another facility and were recovering from their illness. There were additional criteria to receive TKM-130803 (those not complying could participate in the observational cohort); pregnant and breast-feeding women and children could not receive treatment initially. For pregnant women this was due to an absence of fertility and reproductive toxicity study data for TKM-130803 or its predecessor compounds and for children because the bio-distribution and pharmacokinetics were not known in this population. After the first fifteen patients had received TKM-130803, the possibility of enrolling these patient groups would be considered by the Independent Data Monitoring Committee (IDMC) following review of all available safety data. A patient could be enrolled any time within 48 hours of either first arriving at the ETC with a confirmed diagnosis, or being informed of an ebolavirus positive PCR result if this occurred whilst in the centre.

Study drug

TKM-130803 is a liquid (non-lyophilised) formulation. TKM-130803 was administered at a dose of 0.3mg/kg/day for seven days by intravenous infusion at a rate of 1.25 mL/min over two hours, for a total infused volume of 150mL. The dose of 0.3 mg/kg/day was selected as this was the maximum tolerated dose in a single ascending dose study in healthy adult volunteers. In NHPs infected with ebolavirus Kikwit in a fatal infection model, 100% survival was observed following administration of TKM-100802 at 0.5 mg/kg/day and 66% survival was observed following administration of TKM-100802 at 0.2 mg/kg/day (personal communication, Tekmira Pharmaceuticals Corporation). In the event of suspected drug-related toxicity or change in the patients' clinical condition, the dose of TKM-130803 could be reduced to a minimum of 0.24 mg/kg/day.

Non-trial treatment

ETC clinicians provided supportive care in accordance with documented treatment guidelines of the lead operating organisation (GOAL Global). This included routine malaria testing and treatment, empirical antibiotics, antihelminthics, antiemetics, anti-diarrhoeal therapy, pain relief, oral and intravenous fluid therapy, and electrolyte supplementation, as appropriate.

Primary outcome

The primary outcome was survival in patients who received TKM-130803 at a dose of 0.3 mg/kg/day in addition to standard care, assessed 14 days after admission, excluding subjects who died within 48 hours of admission.

Trial design

An open-label, non-randomised, single-arm trial with a concurrent observational study.

Design rationale

The general approach of the RAPIDE platform in evaluating potential treatments of EVD was to begin with a phase 2 single-arm trial to generate early evidence of effectiveness or ineffectiveness. If the initial phase 2 trial provided evidence of effectiveness (as assessed against a pre-determined survival probability threshold at 14 days after enrolment), the phase 2 result would require confirmation in a further follow-up study. Alternatively, if the initial phase 2 trial demonstrated futility, the IND could be abandoned to investigate the next most promising agent. This may be especially important during the final phases of an outbreak in the event there are more agents available for testing than there are patients. If the findings of the phase 2 trial were indeterminate, a randomized control trial would be planned. A more detailed explanation of this multistage approach is provided elsewhere[151].

The evaluation of TKM-130803 was however constrained by the fact that only 100 courses of the drug were available, and that the incidence of new cases was falling. This led to a realisation that, should testing be indicated beyond phase 2, it might have to take place during a subsequent outbreak of EVD. Therefore, for the TKM-130803 trial, the single-arm phase 2 design did not allow early stopping in the case of evidence of effectiveness, because enrolling the full 100 patients would maximize the precision of the final estimate of effectiveness. A futility threshold remained to allow early stopping in the event of evidence of futility or harm[152]. To avoid early stopping due to enrolment of patients with very severe, late-stage EVD, who may not be expected to survive even with an effective anti-viral therapy, the

stopping rule was to be calculated after exclusion of patients receiving TKM-130803 who died within 48 hours of admission.

There were additional ethical considerations supporting use of a single-arm trial in the context of this outbreak. Randomisation to placebo was considered unacceptable to some (including, importantly, representatives from the most affected countries) given the high CFR and the doubt that clinical equipoise existed given the efficacy data in NHP models and the consistent and safe use in humans medically evacuated to high income settings[153, 154]. There were concerns regarding practical implementation of randomisation, especially in communities where engagement was difficult early during the outbreak[153, 155, 156].

The potential risk of infusion reactions meant that TKM-130803 was infused over a minimum two-hour period during which clinical monitoring took place. The intensity of required clinical monitoring and the challenges of care delivery within the ETC limited the number of participants who could safely receive TKM-130803 infusions concurrently. Therefore, the maximum number of patients receiving TKM-130803 on a single day was capped. Each day, the clinical trial lead physician decided the maximum number of beds available for patients to receive TKM-130803 (TKM-beds). If on any given day the number of patients eligible and consenting for inclusion in the trial exceeded the number of available TKM-beds, patients were to be randomly allocated to receive either TKM-130803 with standard care (as part of the interventional cohort) or standard care alone (as part of the observational cohort). This process is depicted in figure 3.1. Random allocation was to be conducted using R (R Core Team (2014)).

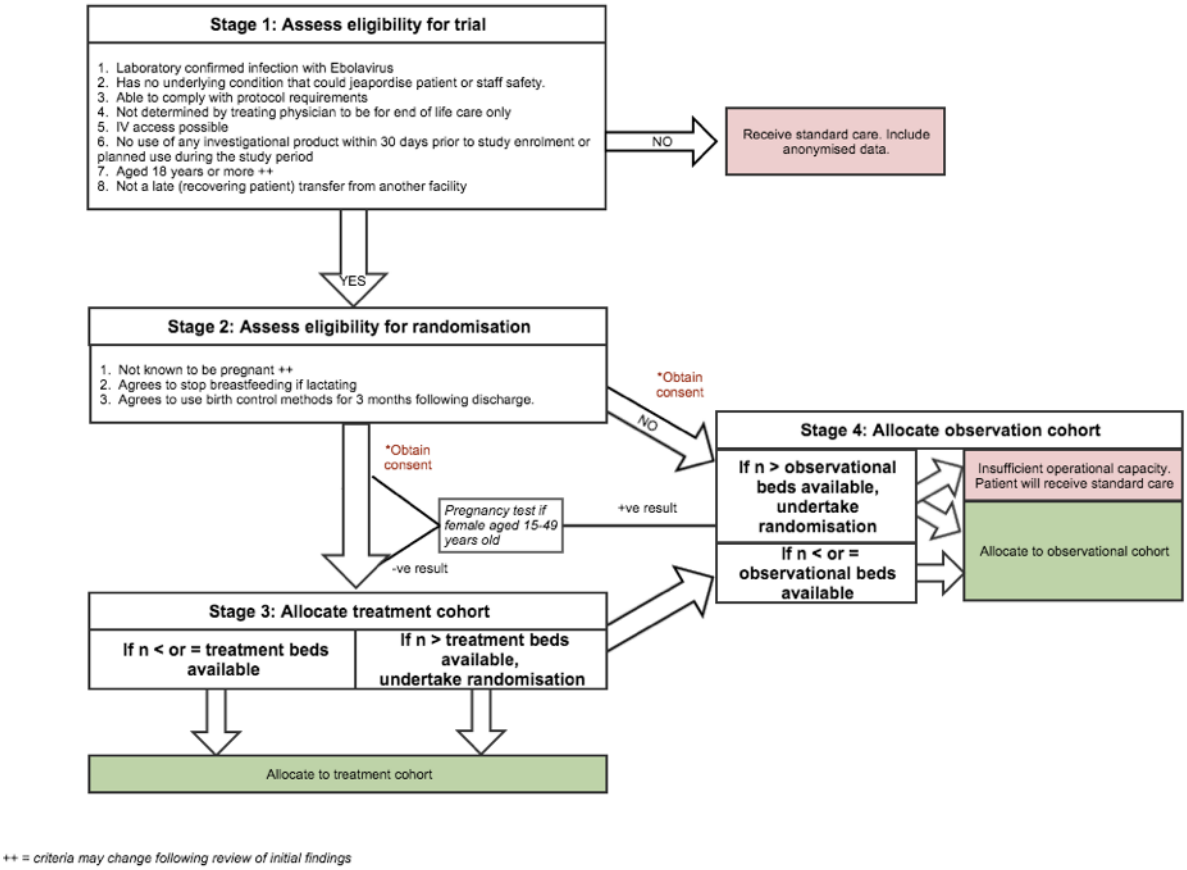


Figure 3.1: Enrolment process for TKM-130803 trial. Details inclusion and exclusion criteria, and procedures for operational randomisation.

Statistical analysis plan

For the purposes of determining futility, the effectiveness of TKM-130803 was judged in terms of the probability that a patient allocated to receive TKM-130803 would survive to day 14 after admission, after excluding patients who died within 48 hours of admission. If the survival probability (p) was >0.55 then TKM-130803 would be regarded as 'promising', otherwise it would be regarded as 'not promising'. The power of the study (1-beta) to detect that TKM-130803 was promising was 0.827 if the true success rate p was equal to 0.70, and 0.973 if p was equal to 0.75. If the trial ever reached a point at which significant evidence (at the one-sided 2.5% level) that p >0.55 could not be found, then continuation of the trial would be considered futile, and it would be stopped. The choice of 0.55 as the target

for p was made following an analysis of individual level data from the 2013-16 outbreak on 1820 adult patients with PCR confirmed ebolavirus infection (personal communication, Annick Antierens, Médecins Sans Frontières). Ebolavirus PCR cycle threshold values and viral load data were not available for this historic cohort. The properties of the design were calculated exactly, based on the independent Bernoulli distributions of each patient outcome.

The data management centre was informed every time a patient was enrolled and after 14 days whether that patient did, or did not, survive. Every time that a day 14 report was received, the number of patients who had survived to day 14 was plotted against the number who had been entered into the trial, and the plotted point was compared with a futility boundary. Enrolment to the trial would be stopped if the futility boundary was reached. When the trial was completed, a point estimate and a 95% confidence interval for p was computed using the method of Jovic and Whitehead[157]. Provided that all 100 patients received TKM-130803 without the futility boundary being reached, the formal conclusion of the trial would be that TKM-130803 was promising, and this would happen with probability ≤ 0.025 if in fact the success rate p was ≥ 0.55.

Safety assessments

Safety assessments of patients included monitoring of vital signs (pulse rate, blood pressure, respiratory rate, temperature, and level of consciousness), symptoms, and the occurrence of Serious Adverse Reactions (SARs) and Suspected Unexpected Serious Adverse Reactions (SUSARs). Clinical assessments were undertaken at the following time points: pre-infusion, during the infusion (between 30-90 minutes), at the end of infusion, and at 1, 2, 4, and 8 hours after the end of infusion, as well as at additional time points if indicated by the patients clinical condition. Trial staff observed patients directly throughout the entire infusion period. Trial assessments

and observations were in addition to routine clinical assessments performed by the ETC's clinicians.

To assess the feasibility and safety of dosing with TKM-130803 in an ETC in Sierra Leone, data on the first four enrolled patients were assessed by the IDMC prior to opening enrolment to additional patients. These patients (termed the safety cohort) were recruited sequentially, with each patient to receive at least three doses before dosing of the next patient started. If a patient died prior to receiving three doses, recruitment of the next patient could occur earlier. The safety cohort could be expanded following advice from the Independent Data Monitoring Committee (IDMC), although this was not required.

Laboratory methods

Diagnostic Ebolavirus PCR RNA extraction

In a flexible film isolator, viral RNA was extracted from EDTA-whole blood either (1) from 80 µL of plasma using the EZ1 Virus Mini kit v2.0 (Qiagen Inc., USA) in conjunction with the EZ1 platform (Qiagen Inc., USA), or (2) manually from 50 µL of plasma using the QIAamp Viral RNA kit (Qiagen Inc., USA). Intact MS2 phage was included in all extractions as an exogenous internal control for the downstream RT-PCR step. Extracts were resuspended in 60 µL AVE buffer (Qiagen Inc., USA) and diagnostic EBOV RT-PCR was carried out immediately. Residual extracts were frozen pending RT-qPCR.

Diagnostic RT-PCR analysis

Qualitative RT-PCR for detection of Zaire ebolavirus (EBOV) was performed using duplex RT-PCR with primers/probes directed against the Zaire EBOV nucleoprotein (FAM channel) and the MS2 genome (Alx532 channel; in-house assay) using TaqMan

Fast Virus 1-Step master mix (Applied Biosystems, CA, USA) on a SmartCycler II platform[158]. Assays were conducted with the following cycling conditions: 50°C for 5 minutes (1 cycle); 95°C for 20 seconds (1 cycle); 95°C for 3 seconds and 60°C for 30 seconds (45 cycles). A single fluorescence read was taken at the end of each 60°C step. Samples with Ct>40 and a positive internal control were interpreted as Ebola negative. Samples with Ctleq40 with or without a positive internal control were interpreted as Ebola positive.

Viral Load Determination by RT-qPCR

Viral loads were estimated by determining the levels of nucleoprotein containing RNA per ml of plasma based on a previously described assay[158]. RNA extracts were diluted 1:5 in 100ng/µL yeast RNA dilution buffer (Ambion) for genome quantification in triplicate by 1-step RT-qPCR. Briefly, diluted RNA extracts were mixed with Taqman Fast Virus 1-step 4X Mastermix (Applied Biosystems), primers NP1-F (TCTGACATGGATTACCACAAGATC) and NP1-R (GGATGACTCTTTGCCGAACAATC) and the NP1-probe (6-FAM-AGGTCTGTCCGTTCAA-MGB). One-step RT-qPCR was performed on a Lightcycler 96 (Roche) beginning with reverse transcription at 50°C for 5', followed by heat denaturation at 95°C for 20 s and 50 cycles of 95°C for 3 s and 60°C for 30 s. The genome copy number was interpolated from a standard curve generated by serial dilution of a plasmid containing the NP1 amplicon, and was calculated per ml of plasma. The limit of detection for diluted RNA extracts was 1 x 103 copies. For samples falling below the limit of detection, genome quantification was repeated using undiluted RNA.

Biochemistry and haematology

Biochemistry and haematology testing was introduced for all patients at the ETC only mid-way through the trial, due to constraints beyond the control of the study team. Haematology and blood chemistry assays were performed using

the Horiba ABX Micro ES 60 Haematology Analyser (HORIBA Ltd., Kyoto, Japan) and the Fuji DRI-CHEM NX500i platform (Fujifilm Corporation, Tokyo, Japan) respectively, according to the manufacturer's instructions. Coagulation tests [Activated Partial Thromboplastin Time (APTT and APTT Citrate) and Prothrombin Time (PT and PT Citrate)] were carried out using a Hemochron Signature Elite Whole Blood Microcoagulation System (International Technidyne Corporation, Edison, USA), according to the manufacturer's instructions. All patients were tested for malaria using the SD Bioline Malaria Ag P.f test (Standard Diagnostics, Inc., Gyeonggi-do, Republic of Korea).

Ethics

The trial was approved by the Sierra Leone Ethics and Scientific Review Committee and the University of Oxford Tropical Research Ethics Committee. Approval to conduct the trial and import the trial drug was granted by the Pharmacy Board of Sierra Leone (PBSL). The Committee for Medicinal Products for Human Use of the European Medicines Agency was asked for an opinion on the use of TKM-130803 in humans with EVD, and was of the view that conducting a clinical trial of TKM-130803 in the context of the Ebola outbreak was acceptable. The UK Department for International Development and GOAL Global granted approval for the trial to be conducted at the Port Loko ETC. The IDMC reviewed data on a sequential basis and reviewed any reported adverse events or other safety concerns. The trial is registered with the Pan African Clinical Trials Registry (PACTR201501000997429). The trial was conducted in compliance with the International Conference on Harmonisation Guidance on Good Clinical Practice (GCP), and PBSL conducted a GCP compliance inspection during the trial. Written informed consent was obtained for all participants, including those enrolled in the observational cohort.

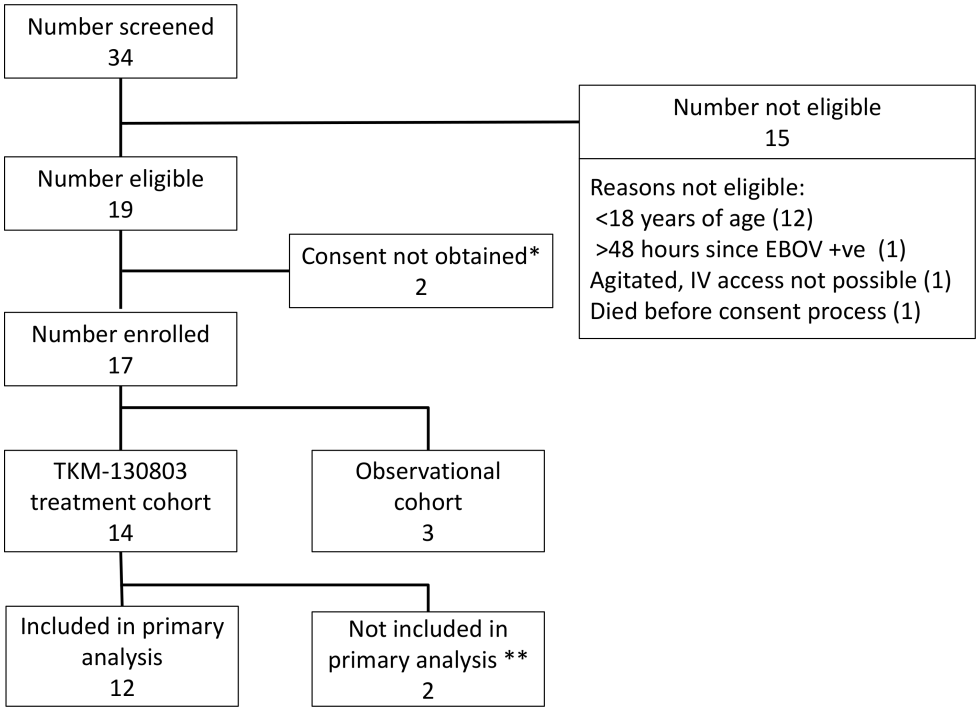


Figure 3.2: Patient screening and enrolment. *One patient did not give consent. One patient was not competent to give consent, and a suitable proxy to provide consent could not be identified within inclusion time limits. **Two patients who died within 48 h of admission were excluded from the primary outcome analysis, as specified in the protocol.

3.2.3 Results

Trial patients

Thirty-four patients with confirmed EVD were admitted to the ETC during the three-month recruitment period and 17 patients were enrolled (figure 3.2). Fourteen subjects were enrolled into the TKM-130803 cohort and three were enrolled into the observational cohort. The three observational cohort patients were recruited during the initial safety cohort phase. TKM-bed capacity was never exceeded following the initial safety cohort phase; randomisation of patients for operational reasons was therefore not required after that initial phase.

Baseline characteristics

The median age of the 17 subjects was 36 years (range 20-85 years) and the median number of days from illness onset to admission was two days (range 0-4 days) for the TKM-130803 recipients and five days (range 4-6 days) for the observational patients (table 3.3). Baseline values for vital signs and blood results were determined from the first set of post-enrolment collections in observational cohort patients, and from the collections taken immediately before the first infusion in the TKM-130803 cohort. At baseline, the geometric mean Ebola virus load in all 17 participants was $1.56\text{x}10^9$ RNA copies/ml plasma (95% CI $5.75\text{x}10^8$, $4.23\text{x}10^9$). The pre-treatment geometric mean Ebola virus load in the 14 TKM-130803 recipients was $2.24\text{x}10^9$ RNA copies/mL plasma (95% CI $7.52\text{x}10^8$, $6 \cdot 66\text{x}10^9$). The median time from admission to the ETC to receiving the study drug was 23 hours. Diarrhoea and or vomiting was reported or observed at baseline in 11/17 patients and there was evidence of bleeding at baseline in 5/17 patients. Three patients had a positive malaria rapid test on admission and all were treated with artesunate/amodiaquine. In six subjects in whom renal function could be determined on admission, three had evidence of renal failure (serum creatinine $\geq$ 3 fold the upper limit of normal). All five subjects in whom hepatic function could be determined on admission had results consistent with liver injury (AST $\geq$ 3 fold the upper limit of normal). Three of four subjects in whom coagulation studies were performed on admission had abnormal coagulation profiles.

Study drug received and outcomes

The 14 subjects enrolled into the TKM-130803 interventional cohort received between one and seven infusions of TKM-130803 (table 3.4). Of these 14 subjects, three survived to Day 14 and were discharged from the ETC, and 11 died. Two subjects died within 48 hours of admission and were excluded from the primary outcome

Characteristic	TKM-130803 Cohort															Observation Cohort			
	Patient ID														Summary	Patient ID			Summary
	001	004	005	007	020	021	022	025	027†	028	030†	031	032	034		002	003	006	
Age group (years)	30–39	20–29	30–39	20–29	30–39	20–29	≥70	30–39	30–39	30–39	60–69	50–59	40–49	40–49	35.5 (20–85)	40–49	30–39	40–49	40 (35–40)
Sex: male	Y	Y	Y	N	N	Y	Y	N	N	N	Y	Y	Y	Y	9 (64%)	N	Y	N	1 (33%)
Days since onset	4	2	4	3	1	1	2	2	1	1	3	0	NK	1	2 (0–4)	NK	4	6	5 (4–6)
Hours to TKM-130803/observation	20.5	65.4	41.2	43.2	21.2	23.5	38.4	21.9	23.3	22.8	18.5	18.5	30.7	16.2	23.05 (16.2–65.4)	46.2	21.7	28.5	28.5 (21.7–46.2)
Temperature (°C)	37.4	36.6	36.6	36.9	36.9	37.1	37.5	38.3	40.8	37.6	38.2	39.0	38.9	37.2	37.45 (36.6–40.8)	38.8	38.4	37.1	38.4 (37.1–38.8)
Weight (kg)	57	54	41	49	57	50	54	70	88	48	45	63	54	59	54 (41–88)	73	51	50	51 (50–73)
Heart rate (/min)	66	70	94	60	70	59	76	90	120	NK	120	84	77	83	77 (59–120)	80	102	72	80 (72–102)
Respiratory rate (/min)	16	38	22	20	18	20	24	18	NK	NK	40	22	NK	NK	21 (16–40)	32	27	22	27 (22–32)
Mean arterial pressure (mm Hg)	98.5	86	115	65	87	87	153	89	78.5	NK	70.5	73	100	93	87 (65–153)	106	119.5	80	106 (80–119.5)
Fever	Y	Y	Y	Y	Y	N	Y	Y	N	Y	Y	N	Y	Y	11 (79%)	Y	Y	Y	3 (100%)
Headache	Y	Y	Y	N	Y	Y	N	N	Y	N	Y	N	Y	N	8 (57%)	Y	N	N	1 (33%)
Fatigue/general weakness	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	N	10 (71%)	Y	Y	Y	3 (100%)
Joint or muscle pain/aches	Y	Y	Y	N	Y	N	Y	Y	Y	N	NK	N	N	N	7 (54%)	Y	Y	N	2 (67%)
Hiccoughs	N	N	Y	N	N	N	N	Y	Y	N	N	N	N	Y	4 (29%)	Y	N	N	1 (33%)
Loss of appetite/anorexia	Y	Y	Y	Y	N	Y	N	Y	Y	Y	Y	N	Y	Y	11 (79%)	N	N	Y	1 (33%)
Nausea	Y	N	Y	N	Y	N	N	Y	N	N	Y	N	Y	Y	7 (50%)	N	N	N	0 (0%)
Vomiting	Y	N	Y	N	Y	N	N	Y	N	N	Y	N	N	Y	6 (43%)	N	N	N	0 (0%)
Difficulty swallowing	N	N	N	N	N	N	N	Y	N	N	NK	N	N	N	1 (8%)	N	N	N	0 (0%)
Diarrhoea	Y	Y	Y	N	Y	N	N	Y	Y	Y	Y	N	Y	N	9 (64%)	N	Y	N	1 (33%)
Breathing difficulty	N	N	N	N	N	N	N	N	Y	N	Y	N	N	N	2 (14%)	Y	Y	N	2 (67%)
Cough	Y	N	N	N	NK	N	N	NK	Y	NK	Y	NK	NK	NK	3 (38%)	N	NK	N	0 (0%)
Chest pain	N	N	N	N	NK	N	N	Y	NK	NK	N	NK	NK	Y	2 (22%)	N	N	N	0 (0%)
Abdominal pain	N	N	N	N	Y	N	N	Y	N	N	Y	N	N	Y	4 (29%)	N	N	N	0 (0%)
Bleeding	N	Y	N	N	N	N	N	Y	N	N	Y	Y	N	N	4 (29%)	Y	N	N	1 (33%)
Died	N	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	11 (79%)	Y	Y	N	2 (67%)

Figure 3.3: Baseline demographic and clinical features of trial population. Summary data given as median (range) or number positive (percent).+ excluded since patient died within 48 hours of admission. N is no, NK is not known, Y is yes

analysis (and the on-going futility plot). Two patients died on 15th June 2015 at which point enrolment to the trial was stopped since the futility boundary had been reached, with survival in only three of the twelve subjects eligible for inclusion in the primary outcome analysis (figure 3.5). All deaths were considered to be consistent with severe EVD. In participants who died, viral loads where high at admission and remained high over time (figure 3.6); correspondingly, EBOV PCR cycle threshold (Ct) values were low at admission and remained low over time (Ct values are inversely proportional to viral load). Serial viral load and Ct data were available for two patients in the observational cohort; viral load steadily decreased in the survivor, whereas viral load increased in the patient who died. The final point estimate of the probability that a patient receiving TKM-130803 who survives for 48 hours will subsequently survive to Day 14 was 0.27 (95% CI 0.06, 0.58). Two of the three subjects in the observational cohort died.

Adverse events

A total of 56 infusions of TKM-130803 were administered. Adverse reactions consistent with an acute cytokine release syndrome were not seen during or following any of the infusions, and none of the infusions required termination or slowing of the infusion rate (figure 3.7). As such, the infusions of TKM-130803 were well tolerated. One subject was observed to have worsening tachypnoea in the 48 hours following the second TKM-130803 infusion. New onset or worsening of additional symptoms or signs that might indicate cytokine release syndrome (tachycardia, flushing, headache, hypotension, chills, nausea or vomiting) were not observed. The event was reviewed by the Trial Operations Group and was reported as a SAR because of the temporal relationship with the administration of the study drug, but it was also felt the event was compatible with progression of EVD.

Patient ID	Cohort	Day of Onset	DOA	DOA +1	DOA+2	DOA +3	DOA+4	DOA +5	DOA+6	DOA +7	DOA +8	Outcome
203-001	TKM	-4	EVD+	Dose1*	Dose2	Dose3*	Dose4	Dose5	Dose6	Dose7		Alive & Discharged DOA +15
203-002	OBS	Day of onset unknown	EVD+			Died						Died
203-003	OBS	-4	EVD+			Died						Died
203-004	TKM	-2		EVD+		Dose1 Died						Died
203-005	TKM	-4		EVD+	Dose1	Dose2	Dose3 Died					Died
203-006	OBS	-6	EVD+									Alive & Discharged DOA +9
203-007	TKM	-3	EVD+		Dose1	Dose2	Dose3	Dose4	Dose5	Dose6	Dose7	Alive & Discharged DOA +13
203-020	TKM	-1	EVD+	Dose1	Dose2	Dose3	Dose4	Died				Died
203-021	TKM	-1	EVD+	Dose1	Dose2	Dose3	Dose4	Died				Died
203-022	TKM	-2		EVD+	Dose1	Dose2	Died					Died
203-025	TKM	-2	EVD+	Dose1	Dose2 Died							Died
203-027 †	TKM	-1	EVD+	Dose1	Died							Died
203-028	TKM	-1	EVD+	Dose1	Dose2*	Dose3	Dose4	Dose5	Dose6	Dose7		Alive & Discharged DOA +11
203-030 †	TKM	-3	EVD+	Dose1*	Died							Died
203-031	TKM	0		EVD + Dose 1	Dose2	Dose3	Dose4	Dose5	Dose6	Dose7 Died		Died
203-032	TKM	Day of onset unknown		EVD + Dose 1	Dose2	Dose3	Dose4	Dose5	Dose6	Dose7 Died		Died
203-034	TKM	-1	EVD+	Dose1	Dose2	Dose3 Died						Died

Figure 3.4: Timelines, TKM-130803 doses received, and outcomes. OBS is observational cohort, TKM is TKM-130803 cohort. + is excluded from final analysis since patient died within 48 hours of admission, Day of onset is first reported day of onset of symptoms with EVD. DOA is date of admission, EVD+ is the day on which patient received EVD RT-PCR positive result.
$*$ is under-dosing event due to loss of study drug volume during additional unanticipated line priming: 203-001 dose 1 was 0.28mg/kg, 203-001 dose 3 was 0.28mg/kg, 203-030 dose 1 was 0.24mg/kg.

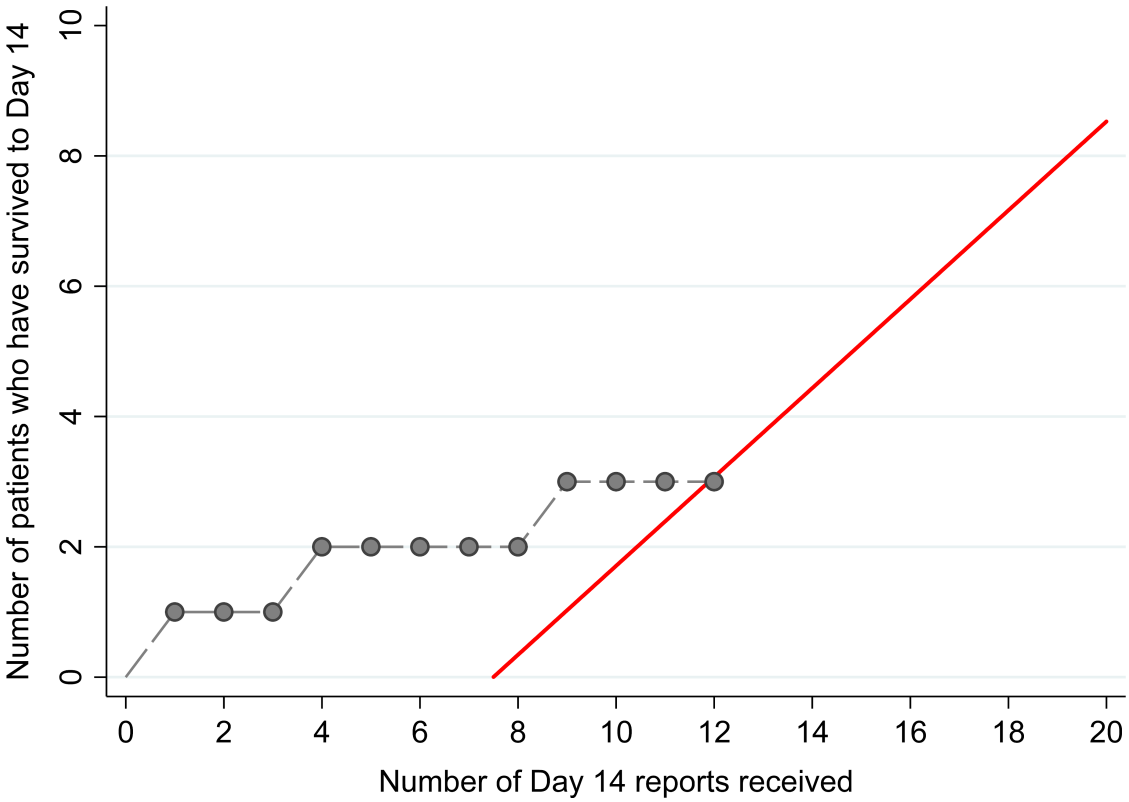


Figure 3.5: Survival plot with futility boundary for TKM-130803 recipients. The red line denotes the futility boundary. The points and dashed line denote the number of survivors at day 14 plotted against the number of day 14 reports.

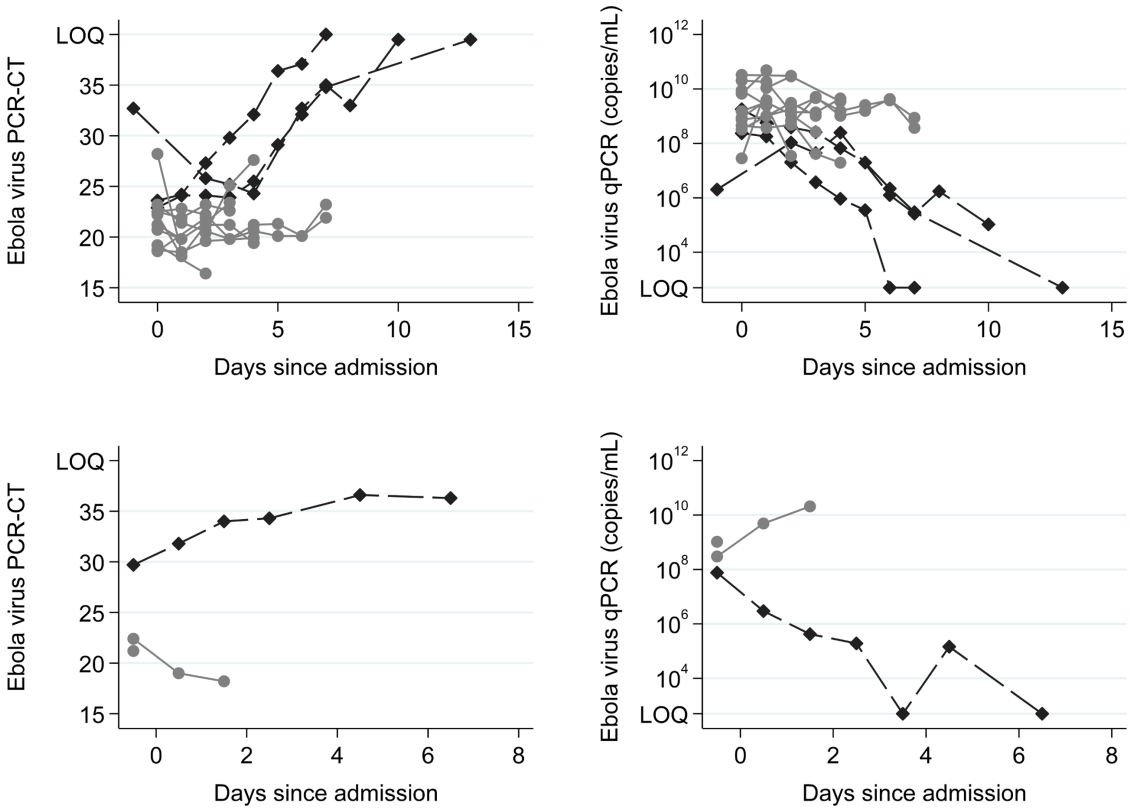


Figure 3.6: Ebola virus RT-PCR cycle threshold values and RNA copies/ml over time. Top row: TKM-130803 recipients. Bottom row: Observational patients. RT-PCR Ct upper limit of quantitation (LOQ) = 40. RT-qPCR lower limit of quantitation is 1,000 genome copies. The Ebola virus RT-qPCR quantification is expressed as the number of genome copies/millilitre of plasma. Black diamonds denote results for survivors. Grey circles denote results for non-survivors.

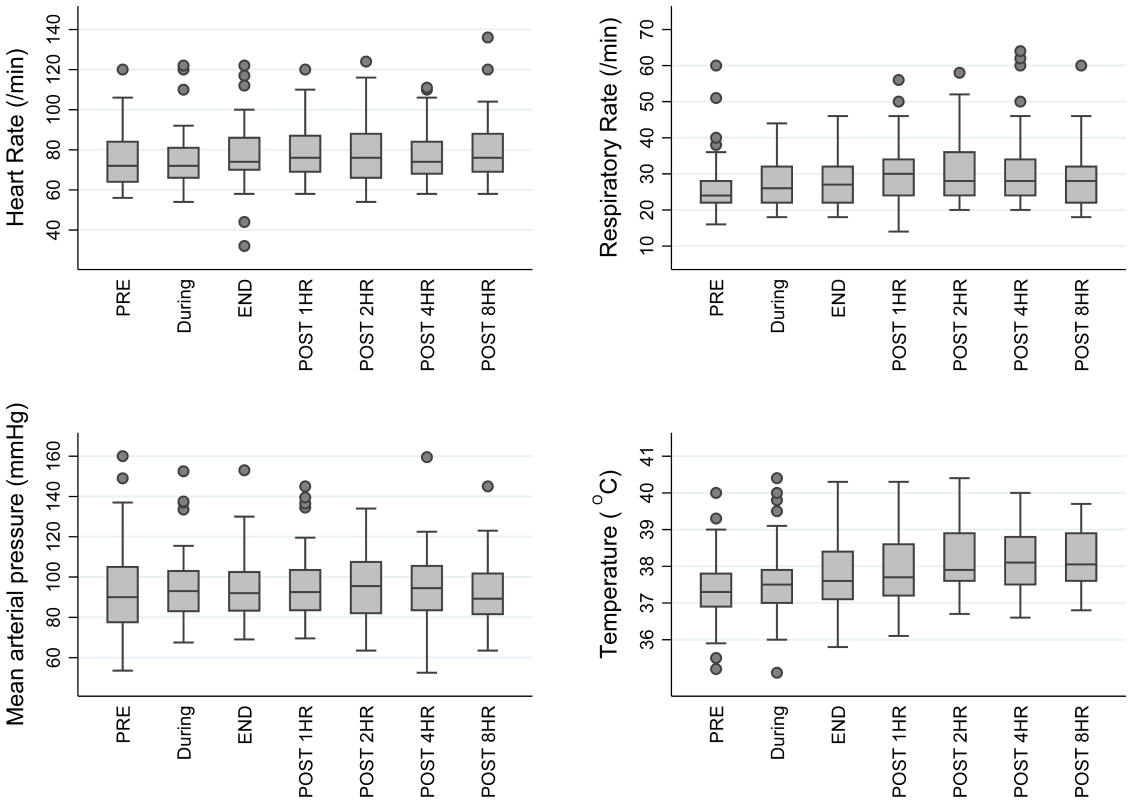


Figure 3.7: Box and whisker plot of vital signs in TKM-130803 recipients, before, during, and after TKM-130803 infusions. Heart rate, respiratory rate, mean arterial blood pressure, and tympanic temperature in patients administered TKM-130803 at the following time points: immediately prior to TKM-130803 infusion (PRE), during the infusion, immediately at the end of the infusion (END), and at 1, 2, 4, and 8 h after the end of the infusion. The middle line shows the median value, the box shows the interquartile range, and the whiskers spread from the lower to the upper adjacent values. Outside values, that is, observations that are larger/smaller than the upper/lower adjacent values, are shown as circles.

3.2.4 Discussion

Our trial has shown that the administration of TKM-130803 at a dose of 0.3mg/kg/day in adults with predominantly severe EVD and high viral loads does not improve survival when compared to historic survival rates. This result contrasts with protective efficacy of various formulations of this product observed in NHPs challenged with a lethal dose of EBOV[147, 150]. There are a number of potential reasons for the difference between the NHP studies and our results. In the animal challenge studies of siEbola-3 the first infusion was administered between 30 minutes and three days after a lethal viral challenge, with day three roughly corresponding to the first day of clinical illness and viral RNA detection (ranging from just detectable to 106 genomic equivalents/mL) in serum in the NHP challenge model used (1000 plaque-forming units administered intramuscularly)[159, 160]. In our trial, the mean number of days from reported illness onset to first infusion was two days (range 0-4 days), although there is uncertainty about the reliability of the onset dates reported by participants. This is shorter than the time from onset to admission in several large analyses of patients with EVD in west Africa, which was five to six days[142, 144, 161–163]. Nevertheless, assuming a mean incubation period of ten days, our patients were still presenting approximately 12 days after exposure. As such, we were administering the study drug to patients later in the infection and disease course than in the NHP models.

All of the 14 patients who received TKM-130803 had $\geq$ 1 x 10^8 RNA copies/mL plasma prior to their first infusion. Although data are limited, this level has been associated in other studies with a fatal outcome in >90% of patients[142, 143]. Of the eleven TKM-130803 recipients that died, nine had $\geq$ 1 x 10^9 RNA copies/ml prior to their first infusion, and seven presented with symptoms or signs reported by other studies to be associated with a high probability of death (haemorrhagic signs, hiccough, tachypnoea)[142, 144, 161, 162]. Therefore, the failure of TKM-130803 to

achieve a survival rate exceeding 0.55 may have arisen from an insufficient anti-viral effect in the face of high viral loads and existing organ injury in subjects presenting with advanced disease. Although data on viral load in our historic patient dataset are not available, it seems likely that the severity of patients recruited into this trial was greater than the average of the historic dataset. As such, in this patient group a target survival rate exceeding 0.55 is likely to have been too high to detect a small or moderate beneficial effect. Nevertheless, the survival probability of 0.27 (95% CI 0.06, 0.58) that we estimated is not encouraging. Whilst an RCT could potentially determine if a survival probability of 0.27 in the experimental treatment arm represents a survival improvement compared to concurrent controls of similar severity, a sample size of around 90 subjects in each arm would be required to have 80% power to detect a difference in survival of 0.10 versus 0.27. There were insufficient patients to have conducted such a study, with only one additional patient with EVD admitted to the treatment centre following closure of the trial. At the location and time our study was conducted there was also no possibility to determine effectiveness in less severe cases, since less severe cases were not presenting to the ETC.

The optimal dose of TKM-100802 in NHP studies was 0.5 mg/kg/day for seven days, whereas we administered a dose of 0.3 mg/kg/day based on observed tolerability data in healthy adult volunteers and an assumption that the pharmacokinetics of TKM-100802/TKM-130803 (which share the same LNP composition) in NHPs, healthy human volunteers, and patients with EVD is similar[164]. We did not have sufficient product or patients to conduct dose ranging or dose comparative studies. We do not know if the dosing regimen used in this trial resulted in adequate drug concentrations, and whether higher doses may have conceivably resulted in a therapeutic benefit.

Fifty-six separate TKM-130803 infusions were administered and the patients were monitored closely for adverse events. Contrary to our expectations based on healthy volunteers and medically evacuated EVD patients given TKM-100802, the infusions

were well tolerated and no obvious cytokine release reactions were observed. Only one SAR, worsening tachypnoea, was reported. This patient had severe EVD as evidenced by a high viral load, a coagulation disorder, and bloody diarrhoea on admission. Since tachypnoea was present prior to the first TKM-130803 infusion, and because tachypnoea is common in severe EVD, possibly related to metabolic acidosis or pulmonary oedema secondary to vascular leakage [165], the SAR may not have been causally related to drug administration[145, 149, 166–169]. Overall, TKM-130803 was well tolerated in this study and the clinical progression of all of the 12 patients who died in the TKM-130803 cohort was consistent with severe EVD with sustained high viral loads.

3.2.5 Conclusions

The RAPIDE studies were set up to rapidly triage potential therapies for EVD, to eliminate agents that are not effective and allow resources to be concentrated on agents with greater promise. The single-arm design with a pre-specified futility boundary and sequential analysis was able to rapidly identify a low probability of survival and cease recruitment - thus minimising harm to patients and risks to healthcare workers, and improving the probability that on-going trials of other interventions could recruit sufficient EVD patients to reach a conclusion.

In summary, administration of TKM-130803 at a dose of 0.3 mg/kg/day to adult patients with EVD and predominantly high levels of viral RNA in blood was well tolerated but did not demonstrate improved survival when compared to historic controls. Further work is needed to assess if the lack of observed effectiveness is generalizable to other patient subgroups in other treatment settings. Additionally, the potential influence of dose requires further investigation.

3.3 Overcoming regulatory and operational complexities for a clinical treatment trial during an EVD epidemic

3.3.1 Introduction

Several reports have evaluated the scientific response to the EVD outbreak in west Africa. These have lauded the clinical trials conducted in the most affected countries as 'ground-breaking'[61] successes in overcoming operational challenges and commended the involved researchers for their immense effort[61], but also criticised the absence of definitive findings, and in particular the continued lack of licensed therapies[61, 170]. They advise that improving the ability to conduct analogous trials rapidly is one of the highest priorities in preparing for future epidemics[61, 171]. What has been missing in these reports is clear explanation for the discrepancy between the rightfully ambitious research agenda and the limited scientific output to date. Understanding these factors is critical to improvement.

While the scientific findings from the clinical treatment trials have mostly been published[165], there are very few public records[139] that analyse the practicalities of how this research was undertaken within the context of the outbreak and the specific barriers to a swifter, larger research response. Here, we report the time-line to trial initiation for one clinical trial and discuss implementation at a field level.

3.3.2 Methods

Two RAPIDE clinical treatment trials enrolled patients with laboratory-confirmed EVD[119, 172], including the trial discussed here – a phase II clinical trial of the small interfering RNA lipid nanoparticle product TKM-130803. The results of the trial are presented in section 3.2.

Data sources: For this work, the investigator site file, internal team communications and reports, project management records, regulatory records, and meeting minutes were reviewed by the primary author (AR) to aid recollection of the sequence of events. Individual investigators were contacted where additional verification was required. Epidemiological information was extracted from publicly available WHO data, and admission records at the research site were provided by the clinical care partner, GOAL Global.

Data interpretation: Where study timelines are produced, the term 'delay' refers to the time period between initiating and completing an activity. All reporting of 'days' refers to the number of calendar days. Where we report the number of potential patients that could be enrolled during a period, this is estimated based on the proxy indicator of adult EVD admissions to the GOAL ETC during that period, as these represent patients who would be screened for eligibility.

3.3.3 Results

The RAPIDE-TKM-130803 trial opened for enrolment on the 11 March 2015 and closed to enrolment on the 15 June 2015 after meeting a pre-specified statistical boundary. A total of 34 patients were screened for enrolment, and 17 were enrolled. Figure 3.8 contextualises trial recruitment within the broader circumstances of the outbreak.

Timeline to trial initiation

Funding for the RAPIDE platform was awarded from the Wellcome Trust on the 19th of September, 2014. The RAPIDE-TKM-130803 trial was the second trial to be prioritised within the platform, after a phase II trial of the experimental

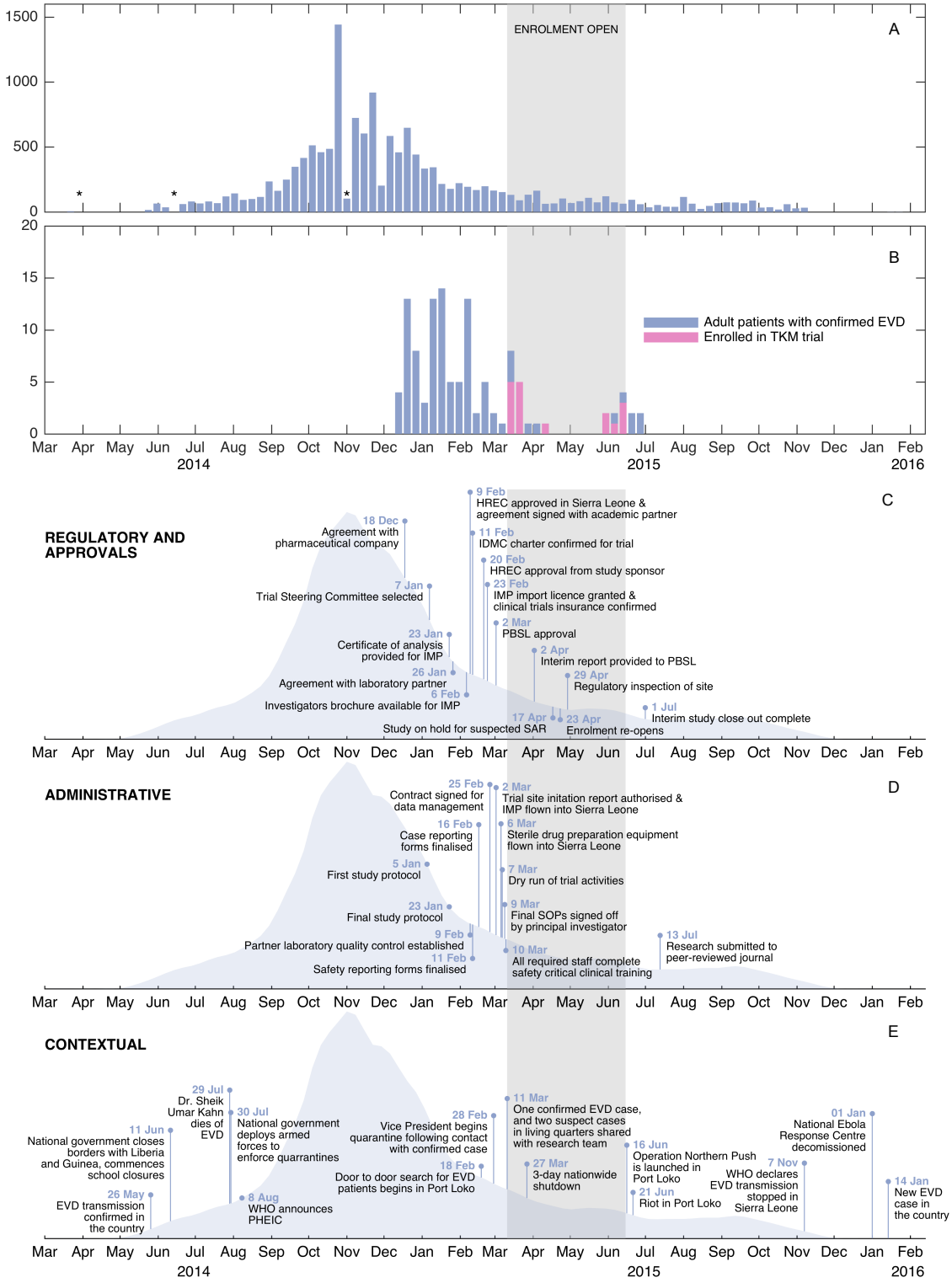


Figure 3.8: Timeline of RAPIDE TKM-130803 trial. Panel A: Epidemiological curve for Sierra Leone, constructed using publicly available information from WHO[131]. Data are aggregated per calendar week. Panel B: Timeline of admissions for adult patients with laboratory confirmed EVD admitted to GOAL ETC (unpublished data). Data are aggregated per calendar week. Panel C: Milestones to trial clearances and agreements. Panel D: TKM administrative milestones. Panel E: External events affecting Port Loko operating site. Data sourced from the United Nations[173], WHO[174], and personal records. *underestimation due to correction in total case count by WHO.

treatment brincidofovir that opened to enrolment on the 1st of January 2015 [7] and therefore the majority of regulatory and operational procedures did not begin until after this time. Table 3.1 outlines the timeline for activities relevant to regulatory approval to open the trial to enrolment. The longest delays were in reaching agreement on research partnerships with the laboratory and site partners (each 39 days). Specifically, the issues were with post-trial accessibility of TKM-130803 if it was effective, and legal liabilities. These, and the time taken to formalise the research protocol (18 days) were the most likely to affect possible enrolment of patients. While research protocol drafting was a relatively faster process compared to partnership agreement, it took place earlier during the outbreak when the disease was spreading more rapidly - hence its disproportionate effect on enrolment.

Preparation phase

Because clinical trials had not been conducted for EVD before, the suitability of Ebola Treatment Centres (ETCs) to support research was unknown. Beginning in October 2014, the trials investigators began site visits at ETCs across the three most affected countries. Site assessment parameters focused on the scope of routine clinical treatment and the extent to which treatment was following a written protocol, to ensure consistent quality of care provision for patients despite high staff turnover during the outbreak. Evidence of adherence to appropriate infection prevention and control procedures was required for short-listing. The essential site infrastructure needs were minimal – good accessibility by road, stable electricity supply for cold storage, ability to secure documents, and room for the research team. Projections for future site admissions were also considered because some centres were earmarked for early closure in the event of falling case numbers.

The site selected for the RAPIDE-TKM-130803 trial was the GOAL Ebola Treatment Unit in Port Loko, Sierra Leone. The first members of the TKM-

Regulatory or administrative milestone	Time taken			New EVD cases during delay period (n)	
	Start date (dd/mm)	Final date (dd/mm)	Duration (days)	Sierra Leone^	GOAL ETC (Adults)
Research agreements with partners					
Laboratory (Public Health England)	26/01/2015	06/03/2015	39	948-1279	28
Academic (College of Medicine and Allied Health Sciences)	26/01/2015	19/02/2015	24	585-961	23
Site (GOAL Global)	26/01/2015	06/03/2015	39	948-1279	28
Pharmaceutical (Tekmira Pharmaceuticals)		18/12/2014	N/A	N/A	
Human Research Ethics Committee (HREC) Approvals					
Prepare submission (Sierra Leone HREC)	23/01/2015	27/01/2015	4	<178	3
Review process (Sierra Leone HREC)	27/01/2015	10/02/2015	14	222-594	19
Prepare submission (trial sponsor HREC)	23/01/2015	29/01/2015	6	<400	6
Review process (trial sponsor HREC)	29/01/2015	20/02/2015	22	363-783	23
Prepare submission (WHO for opinion)		20/02/2015	N/A	N/A	
Protocol development					
Drafting	05/01/2015	23/01/2015	18	560-1072	28
IMP management					
Drug release	19/02/2015	09/03/2015	18	165-516	5
Import license	16/02/2015	23/02/2015	7	<367	5
Shipment of IMP	27/02/2015	02/03/2015	3	<165	3
Data Management					
Database design, set-up and testing	23/02/2015	16/03/2015	21	318-648	10
Case reporting form development	05/02/2015	10/03/2015	33	532-879	14
Other Required Documents					
Study insurance	03/02/2015	23/02/2015	20	363-783	12
European Medicines Agency opinion	06/02/2015	10/02/2015	4	<194	13

Table 3.1: Time taken to meet key regulatory requirements for the TKM-130803 clinical trial, and the implications of these delays on possible enrolment. National figures according to WHO weekly report.

130803 research team were deployed to Port Loko on the 11 February 2015 and the first patient was enrolled on 11 March 2015.

Management of the investigational agent

Due to limited commercial flight availability TKM-130803 was transported in a temperature-controlled hold on a routine United Kingdom Royal Air Force flight. Initially, only 14 treatment courses were shipped to minimize losses if there was a disruption to chain of custody or cold-chain. Travel within Sierra Leone was

via road using a dedicated 4 wheel drive vehicle. The temperature-sensitive drug was packed in shipment boxes that contained electronic temperature monitors (TempTale® 4 USB, Sensitech Inc, USA).

Once on-site, TKM-130803 was stored in a temperature-controlled, locked vaccine refrigerator with an automatic switch-over to a back-up generator in the event of power failure. Both integrated and free-standing digital recording thermometers were used and monitored daily. There were no significant temperature excursions recorded during the trial.

TKM-130803 was supplied as an aqueous dispersion that required dilution before administration. There were no aseptic drug preparation facilities available on-site. Dedicated trial pharmacists were deployed, and they were trained in use of a portable positive pressure aseptic preparation unit, designed for field use (The Posi-Dome™ Basic, Banthrax Corporation, Dayton, Ohio, USA).

Patient enrolment

The trial team were notified of all ebolavirus positive PCR results in the ETC by telephone. There was a 48-hour window for consent and enrolment, to maximise the potential efficacy of the antiviral and to prevent inadvertent enrolment of patients with convalescent phase EVD. Typically, four clinicians were required to enter the ETC's High Risk Zone (HRZ, where patients with suspected or confirmed EVD were located) to gain consent – a pair of English and Krio speaking trial clinicians, an interpreter fluent in local languages (Mende or Temne as required), and an independent witness supplied by the clinical team. All participants in the trial were illiterate and so required independent verification of consent. For two participants, proxy consent was required as they were too unwell to be competent for decision making. Only written consent was considered appropriate for proxy consent, which

created an unintended obstacle because next of kin could be quarantined (n=1) or also admitted in an ETC (n=1). If they could not be accessed by the trial team, this relation offered verbal assent, and then the next appropriate relation was approached for written consent. In one case, difficulty finding an appropriate next of kin, followed by extended travel time to meet the research team meant that a patient became ineligible for enrolment.

Patient monitoring

TKM-130803 was provided as a two-hour infusion via peripheral intravenous line, once daily for a 7-day period. Patients were monitored continuously during each infusion and then a minimum of 5 regular observations were taken for an eight-hour period following the infusion. Each set of observations required two trial clinicians to enter the HRZ. Phlebotomy was performed once or twice daily by trial clinicians, according to the trial protocol, and it was combined with phlebotomy requested by ETC clinicians where possible (to reduce the number of venepunctures). We prioritised laboratory analysis for clinically relevant samples and some trial specific assays (such as pharmacokinetics). All clinical work relevant to the trial was undertaken by trial employed clinicians but ETC clinicians remained responsible for overall patient care. The standard operating procedures used for collection of clinical data were consistent with the practices of the ETC clinical teams and all trial-obtained clinical and laboratory results were provided to them to prevent duplication of work.

Significant human resource was required to monitor participants. Clinical information was captured in the HRZ where extensive personal protective equipment (PPE) had to be worn. To prevent heat exhaustion, team members were restricted to entries lasting no more than 45-60 minutes, up to a maximum of three times per day (depending on ambient temperature and humidity). To maintain safety, all

staff worked in pairs. An example of the scheduling for a single patient is displayed in figure 3.9. In total, there were at least 592 individual research member entries into the HRZ for participant enrolment and monitoring during the trial. The actual number of entries is likely much higher, but the trial team did not permanently record unscheduled or additional entries for training purposes, administrative duties, or unscheduled patient care (e.g. replacing a peripheral venous cannula). There were no PPE breaches, percutaneous sharps injuries, or "man-down" episodes involving trial staff in the HRZ. There were no known instances of heat strain or heat exhaustion requiring medical management. On two occasions team members requested to cancel a planned entry due to feeling unwell.

DAILY HIGH RISK ZONE TIMETABLE

Drug start times:
Patient 1: 0800
Patient 2: N/A
Patient 3: N/A

1. Please check with team leads for up to date schedule before donning.
2. Please provide medical handover to team leads on HRZ exit.
3. The spotter must remain in place and log entry times on central command board.
4. Please notify the team leads if you feel unwell or do not wish to make an entry.
5. Donning starts 20minutes prior to entry
6. Maximum duration of entry is 45-60 minutes depending on time of day.
7. No more than 2-3 entries per day, with sufficient rest between.

HRZ entry time (HH:MM)	HRZ team members	Spotter	Tasks, in order of priority	Equipment to take in
0740 - 0840	A + B	E	Pre infusion clinical observations, start infusion, phlebotomy	Case report form, phlebotomy form, phlebotomy kit
0840 - 0940	C + D	G	Observations at 0900, observe infusion	New batteries if required
0940 - 1020	E + F	H	Observations at 1000, complete infusion, post infusion phlebotomy	Phlebotomy form, phlebotomy kit
1050 - 1110	G + H	A	Observations at 1100, photograph data from yesterday	Digital camera (in LRZ with spotter)
1150 - 1210	A + B	C	Observations at 1200	
1350 - 1430	C + D	E	Observations at 1400, consent new patient	Consent form, pregnancy kit
1750 - 1830	E + F	B	Observations at 1800, phlebotomy on new patient	Phlebotomy form, phlebotomy kit

Low risk zone tasks today (and delegation): Prescription writing (A), Data entry (B + C), Making lunch for teams who will be in HRZ during hours (D+E), Phlebotomy forms (G). Day off today: (I+J)

Figure 3.9: Example of the daily run-sheet housed in the LRZ (low-risk zone), demonstrating the human resources required to safely monitor one patient receiving TKM-130803 per day. A minimum of 10 clinical staff were required to monitor one patient through their treatment course. 592 individual team member entries were required to monitor all patients in the trial. The term *clinical observations* refers to assessment of pulse rate, blood pressure, respiratory rate, temperature, level of consciousness, signs and symptoms of EVD, SARS and SUSARS

Collecting patient data

Clinical data was written onto paper forms at the bedside. Writing was difficult while wearing PPE so these forms were simplified and used large fonts and large spaces for writing. Clinical data was entered by one staff member and checked by another in order to minimize error in the challenging conditions within the patient treatment area.

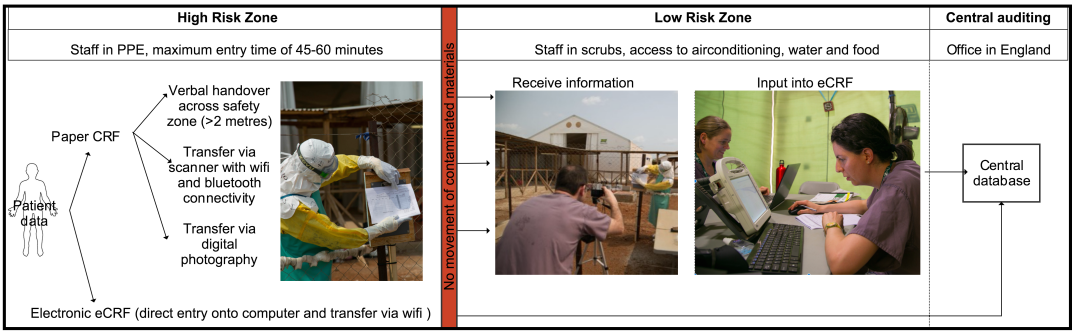


Figure 3.10: Innovations in data transmission from high risk zone

While this original source data would normally be filed for trial related monitoring and accessible for regulatory inspections, the infection prevention and control requirements for EVD prevented their transport out of the HRZ. Verbal handover of clinical information was not entertained due to the potential for inaccuracies in conveying information. Flat-bed scanning of completed paper documents (with wireless and cable connections between the HRZ and LRZ (low risk zone: air conditioned administrative area, where PPE is not required)) was trialled but found to be too slow, and frequent technical faults were encountered due to heat, humidity, and possibly environmental chlorine exposure. Instead, documents were placed on a stationary post in the HRZ and photographed with a simple digital camera in the LRZ (see figure 3.10). Trial staff in the LRZ entered information from printed copies of the photographs onto an electronic CRF (eCRF) (Infermed MACRO, Elsevier, UK) stored on a portable computer designed to withstand field use (Toughbook CF-H2, Panasonic). Because near real-time data reporting was required for the data safety monitoring board and to reduce the risk of losing data due to local hardware failure, the eCRFs were synchronized with the trial's secure server (located in the UK) at least once per 24-hour period using internet provided by the Emergency Telecommunications Cluster (etcluster.org).

Community engagement

Specific community engagement concerns in Port Loko included recent attacks on ambulances and burial teams[175] and perceptions of the outbreak as a supernatural event[176].

A multifaceted public engagement approach was led by a trial investigator (an experienced senior clinician from Sierra Leone) (table 3.2. The trial was presented to local chiefs and community leaders at an Ebola task force meeting and some of these representatives subsequently visited the research group. Affected communities received information about the trial through the existing community engagement infrastructure of GOAL Global. ETC staff, some of whom were members of affected communities, attended Krio and English language question and answer sessions and were provided with plain language summaries of the trial and the research team promoted an 'open door' policy for staff who had further questions. Other organisations involved in the regional EVD response received briefings through the District Ebola Response Committee meetings.

Priority group	Method of engagement
Local chiefs and community leaders	• Meetings with the local non-partisan government representative (e.g. the Paramount Chief) • Presentations to chiefdom representatives at the Ebola Task Force meeting.
Organisations involved in local Ebola response	• Presentation and regular attendance at District Ebola Response Committee • Meeting with government ministers and advisors in capital cities. • Presentation at MSF and GOAL staff meetings
Ebola treatment centre staff*	• Joint meetings conducted with senior ETC staff (national and international) • Repeated presentations in the ETC to give access to staff on different shifts • Question and answer sessions led by a trial doctor in Krio language • Promotion of an "open door" policy for staff with questions or concerns • Availability of plain language trial written summaries and contact numbers in staff common areas • A rapid, reactive response, in collaboration with senior ETC staff, when conspiracy theories or misconceptions arise
Local community	• Direct community engagement was managed by NGO partners who worked frequently in local villages and towns • Local radio presentations and press interviews, in collaboration with NGO partners
International community	• Press statements at commencement and conclusion of trials • Representation at media events, scientific meetings and public engagement events

Table 3.2: Priority community engagement groups and activities

Staff safety

The strategy used to prevent infection of trial staff included PPE training and continuous monitoring of PPE use; a strict 'no touch' and social distancing policy; health surveillance, including the immediate reporting of symptoms that could represent EVD; and body temperature monitoring when needed. A senior physician was responsible for the health and safety of team members on site. They were supported from the UK by a 24-hour on call rota that included a team coordinator and a duty doctor (both senior physicians), and an experienced health and safety officer.

Standard operating procedures were developed for heat exposure, snake or scorpion envenomation, motor vehicle collisions, a deteriorating security situation (such as civil unrest), potential EVD exposure (e.g. needle-stick injury), and medical illness (particularly management of illness that could represent EVD). Several of these protocols were utilised to manage incidents. For psychological support RAPIDE field staff received pre- and post-departure briefings, access to an independent counselling service if needed, as well as a formal group debrief.

3.3.4 Conclusions

RAPIDE-TKM-130803 was one of only a small number of clinical treatment trials conducted during the EVD epidemic. It was operationally successful in that it was completed to a pre-defined statistical threshold, in a manner compliant with international regulatory frameworks for research and while maintaining the safety of the research team. The practical perspective of how this was achieved is relevant to the ongoing evaluations of science conducted during the epidemic, especially when these reports are meant to inform future policy and preparedness efforts.

In particular, while this, and other clinical trials were successful in fast-tracking to recruitment compared with trials conducted in other settings[85, 118], the new target recommended by the Academy of Medical Sciences – that clinical trials are launched before the epidemic peak[61]- remains infeasible. There are several long-standing and broadly accepted agreements on how to achieve this, including adoption of data-standards, preparation of pre-approved protocols and case reporting forms, and improved global collaboration between researchers[90]. However, on the basis of the findings in this section, and section 3.2, we are in a position to suggest further improvements to practice. These are outlined in section 3.4.

3.4 Innovations to accelerate clinical research during outbreaks

3.4.1 Part 1: Preclinical development for EVD therapeutics

A stocktake of achievements

Various therapeutic agents were under pre-clinical development for EVD prior to the outbreak. Table 3.3 outlines the most promising candidate therapies and their development status at the beginning of 2014. This work was no doubt facilitated by national security concerns - EVD is one of few diseases in the highest priority category for national security and public health threat determined by the National Institute of Allergy and Infectious Diseases (NIAID) biodefense research disease program[177] and all of the most developed candidate drugs were advanced with funding from military agencies. As an example, the US Defense Threat Reduction Agency (DTRA) allocated 300 million USD for medical countermeasures for viral haemorrhagic fevers in the decade before the outbreak[178].

However, the pipeline was far from robust and pre-clinical progress had stalled for some agents despite promising preliminary data. For example, a registered phase I human clinical trial of the antiviral AVI-7537 – which would have been the first human clinical trial of an EVD therapeutic – was terminated in 2012 prior to enrolment due to government funding restraints[179]. Likewise, despite non-human primate trials demonstrating 100% efficacy for TKM-100802 and ZMapp[150, 180], these results had not yet been published at the time the west Africa outbreak started, and phase I human trial results were also not publicly available.

Pre-clinical progress accelerated as the epidemic progressed. In 2014, at least 70 million USD was spent on EVD drug development[181]. Some of this was pipeline

progression on existing candidates, including a number of new animal studies. A surge in compound library screening studies also attempted to identify existing FDA approved agents for re-purposing. However, the yield from this activity was low within the time-frame of the epidemic, with the exception of brincidofovir (which progressed to a clinical trial).

Candidate drug	Initial target	Year of first patent filing	Disbursed or committed public funding (USD)	Source	Pipeline development at start of outbreak
Brincidofovir	Smallpox and DNA viruses (CMV)	2004	36.1 million	US NIH [182]	Other applications: smallpox- unknown status. EVD: none
			CRADA, value unknown	USAMRIID	
			53 million	BARDA	
			5 million	NIH	
Favipiravir	Influenza	1998	138.5 million	DTRA	Other applications: Pandemic influenza-licensed for stockpiling (Japan)[183]. EVD: 100% survival in mouse model [184].
TKM-Ebola	Target specific to EVD, but delivered using technology applicable to other diseases	2005	44 million	US DOD	Other applications: none. EVD: Up to 100% survival in NHP model [150]
			26 million as part of a consortium	NIH	
BCX4430	Hepatitis C, Yellow Fever	2005*	20 million	NIAID	Other applications: Yellow fever- 100% survival in hamster model [185] EVD: none
AVI-7537	Target specific to EVD, closely related to precursor drug for Marburg disease	2010	28 million	DTRA	Other applications: close relative in phase I trials for Marburg virus[186]. EVD: support terminated by US Government[187]
			11 million	US defense appropriations earmark	
			<80 million	US DOD	
JK-05	Unknown. Bio-defense drug	2010^	unknown	PLA, China	Other applications: unknown. EVD: unknown, no human trials.
ZMapp (and precursors ZMab and MB-003)	EVD	2008	189 000	US army	Other applications: none. EVD: 100% survival in NHP model [180]
			24.9 million	NIAID	
			5.2 million	DTRA	
			2.6 million	DTRA	
			CRADA, amount unknown[1]	PHC	

Table 3.3: Financing of promising candidate EVD therapeutics prior to 2013 – 2016 west Africa outbreak. Data courtesy of Open Society Foundation (2014,unpublished report: Overview of intellectual property claims and public research support for candidate Ebola vaccines and therapies). CRADA: Cooperative Research and Development Agreement; US: United States of America; DTRA: Defense Threat Reduction Agency, US; USAMRIID: The United States Army Medical Research Institute for Infectious Diseases; NIAID: National Institute of Allergy and Infectious Diseases, US; DOD: Department of Defense; NIH: National Institute of Health, US; BARDA: Biomedical Advanced Research and Development Authority, US; PLA: Peoples Liberation Army (China); PHC: Public Health Canada; CMV, cytomegalovirus. *with some uncertainty (multiple components), ^with some uncertainty (insufficient information)

Improving practice: support new models for R & D

The lack of an available treatment for EVD, or at least a drug that was ready to go straight into patients enrolled in a clinical trial, at the beginning of the epidemic was a significant failure. An efficient, robust drug development pipeline that brings promising agents to human clinical trials before an outbreak should be viewed as an integral component of global health preparedness. There is clear economic sense for governments in doing so. As an example, most of the estimated 54 billion USD in costs caused by the SARS outbreak were carried by the public sector [41]. However, pre-clinical drug development for rare and poverty-related diseases is characterized by market failures with private sector R & D priorities being based on profit prospects from patent monopolies and large margins rather than health needs [188, 189]. While useful for rewarding companies for developing medicines for wealthy health systems and markets, these incentive mechanisms are ineffective when there is a low profit-prospect (either small patient numbers or low purchasing power of the affected population) or an unpredictable market (such as in the case of an epidemic). Unfortunately, increasingly seen as a tool to promote economic growth, public research and development (R & D) funding for pharmaceuticals has followed private sector priorities and is made contingent to partnerships with the industry, and disproportionately targets issues relevant to high income countries [189]. However, given the high cost of not being able to control an outbreak in a timely way, there is a case to be made for increased public investment to secure a robust preclinical candidates pipeline, ready to be tested in patients in case of an outbreak.

Aimed at remediating the failure of the current R & D model to prioritize health needs and deliver affordable products, a variety of reports have been released that suggest ways to strengthen global financing for R & D for neglected and epidemic diseases and ensure affordable access [41, 188, 189]. A recurrent suggestion is to

better lever the existing public sector funding already spent on drug development, in particular by ensuring that the resulting products are affordable and accessible[188]. For example, during the 2014 financial year, it is estimated that 90% (64 million USD) of EVD drug research funding was provided by the public sector[181], but it is unclear to what extent product accessibility and affordability are negotiated as a condition of this funding. There is also general agreement that alternative incentive mechanisms are required to replace drug patenting, but there is less consensus on which mechanisms to promote. Common suggestions include market push and pull mechanisms such as increased public research funding or advanced purchase agreements, or more innovative mechanisms such as the use of prizes (for progress or completion) and other ways that uncouple paying for R & D and product sales ("delinkage"). Not-for-profit Product Development Partnerships (PDPs) involving public and private sectors such as the Medicines for Malaria Venture (MMV, www.mmv.org) or the Drugs for Neglected Diseases initiative (DNDi, www.dndi.org) have demonstrated their effectiveness for specific neglected disease niches.

Improving practice: emphasise collaboration and harmonization

Furthermore it is important that for these incentive mechanisms to be efficient, that they do not occur in silos. It is clear that the research response to EVD was slowed by 'insufficient collaboration and transparency'[41]. There was wasted effort from duplication of screening of compounds that had already been deemed unlikely drug candidates in other studies. Furthermore, WHO were receiving 'almost daily' proposals for potential EVD treatments where there were already data indicating many of these agents were ineffective against the virus[190]. This problem is compounded by proprietary data held by commercial companies, and some secrecy around research funded through military channels. The development of collaborative frameworks that can pool R & D funding toward priority health goals, mobilize the appropriate public and private partners, and ensure affordability

and accessibility for the affected population are desperately needed.

A possible solution exists in adopting the new collaborative models developed for vaccine development. For example, the Coalition for Epidemic Preparedness Innovations (CEPI, www.cepi.net) provides one such initiative, aimed at approaching gaps in the vaccine pipeline for emerging diseases, focussing on diseases where market failure currently exists. This initiative is significant for several reasons. Firstly, the diversity of representation in the founders (including the Gates Foundation, India's Department of Biotechnology, and the World Economic Forum) and likewise in the organisation's board, should help prevent unnecessary duplication of effort by producing a unified strategic direction, with secure funding. Collaboration between different sectors should also improve the quality of horizon scanning, so that efforts and financing are directed toward the most likely threats. The end-to-end leadership possible in such a large consortium may have significant impact in providing a platform for rapid acceleration of vaccine development through all stages of the pipeline when a novel threat is encountered. It is arguable that such a model could be adopted for pre-clinical drug development given the vast similarities in the problem being addressed. It is also worth considering to what extent aspects of this ambitious approach can be used for supportive care strategies, and how elements can be adopted to clinical trial networks.

3.4.2 Part 2: Clinical trials of EVD therapeutics

A stocktake of achievements

Despite outbreaks occurring over a period of four decades, no phase II or III clinical trials of an EVD therapeutic had been conducted before 2015.

During the 2013-16 epidemic, promising experimental therapies were first adminis-

tered under a compassionate basis to healthcare workers who were being treated in high income settings[191]. Viewed by some as a further manifestation of the unethical discrepancies in care being provided to those affected in west Africa and others, these events fuelled a growing number of calls for experimental agents to be made available to patients in the most affected region. In September 2014, shortly following the announcement of the PHEIC, WHO convened an expert committee consultation to determine if and how therapeutic testing should progress. They affirmed the findings of an earlier ethics panel that there was a moral imperative to evaluate these treatments, within an umbrella of safe and ethical conduct, and under the condition that results were made available to further knowledge of the disease[146]. WHO also noted the extraordinary circumstances of the epidemic and sanctioned 'innovative methods of rapid assessment' to help identify safe and effective countermeasures quickly[243]. Shortly after, several funding agencies committed to funding therapeutic trials. Again, these were predominantly from the public sector and included the Wellcome Trust, National Institutes of Health, and the European Commission.

Subsequently, patients began receiving experimental therapies in the most affected regions. The first use of an experimental agent was provision of convalescent blood in December 2014, led by the staff at the 34 Military hospital in Freetown, Sierra Leone [192]. The first registered therapeutic clinical trial (favipiravir) did not begin until the 17th of December 2014, by which time, over 18 600 cases had already been reported to WHO [193]. Table 3.4 provides an overview of all registered trials that were registered during the outbreak. Several main approaches were taken. Investigation of convalescent blood and blood products (plasma) were the initial preference of WHO, in the context of clear safety data and scalability. Novel (TKM-130803) and repurposed (favipiravir) antivirals were also trialled and several monoclonal antibody cocktails were used (although only ZMapp in a clinical trial setting). Following the acute phase of the epidemic, a clinical trial of the

investigational therapy GS-5734 has recently (June 2016) been announced to assess EBOV eradication in the semen of survivors (clinical trial identifier NCT02818582). Trials of other repurposed agents (including azithromycin, sunitinib, erlotonib, atorvastatin, irbesartan and amiodarone) were planned but did not recruit patients and assessment of other agents (including amiodarone, atorvastatin + irbesartan (+/- clomiphene), lamivudine) provided insufficient details to WHO to make an assessment of efficacy[194]. Almost all of the registered clinical trials were conducted in partnerships between investigators in the most affected countries and international experts. Unusually, in many cases these trials were also conducted with non-governmental organisation (NGO) partners that were providing patient care, but who would not normally participate in drug development trials[146].

Experimental therapy	Trial design	Research question (PICO model)	Declared status (as of September 2017)	Result
ZMapp	Open label RCT with adaptive trial design	Intervention: 50mg/kg ZMapp, i.v, every three days; total of three doses Comparison: optimised care alone (including favipiravir in Guinea) Outcome: Day 28 survival.	Registered as PACTR201503001065306 NCT02363322 Completed	No statistically conclusive benefit
TKM-130803	Open label, single arm. MSA	Intervention: 0.3mg/kg of TKM-130803, i.v., once daily. Total of 7 doses. Comparison: historical controls Outcome: Day 14 survival.	Registered as PACTR201501000997429 Completed	No overall survival benefit
Favipiravir	Open label, single arm	Intervention: 6000mg (day 0) and 2400mg (day 1-9), po, daily of favipiravir. Total of 10 doses. Comparison: historical controls Outcome: Day 14 survival.	Registered as NCT02329054 Completed	No overall survival benefit
Convalescent plasma (CP)	Open label, single arm	Intervention: 400-500mL of CP from two donors. Administered as two consecutive (200-250mL) transfusions. One treatment cycle in total. Comparison: historical controls Outcome: Day 14 survival.	Registered as NCT02342171 Completed	No overall survival benefit
Convalescent plasma	Open label, single arm	Intervention:180-220mL of CP from two donors. Administered as two consecutive (90-110) infusions. Up to 3 treatment cycles, at least 48hrs apart. Comparison: none Outcome: EBOV viral load.	Registered as NCT02333578 Unknown	N/A
Convalescent plasma	Open label, single arm	Intervention: INTERCEPT plasma, dose not defined. Comparison: not defined. Outcome: 1 year survival.	Registered as NCT02295501 Open to enrolment	N/A
Convalescent plasma	Open label, random allocation	Intervention: Single transfusion of convalescent plasma, dose not defined Comparison: Ringer's Lactate solution Outcome: All-cause mortality at 14 days post treatment	Registered as ISRCTN13990511 Completed	N/A
Brincidofovir	Open label, single arm trial. MSA	Brincidofovir: 200 mg, po, initial dose, then 100 mg, po, twice weekly; total of 5 doses Comparison: historical controls Outcome: Day 14 survival.	Registered as PACTR201411000939962 Recruitment suspended	No statistical conclusion
Azithromycin Sunitinib Erlonitib Atorvastatin Irbesartan	Multi-arm RCT with adaptive trial design	Intervention: Azithromycin (1500 mg, po, daily for 5 days) vs Sunitinib (50 mg, po, daily for 7 days) and Erlonitib (150 mg, po, daily for 7 days) vs Atorvastatin (40 mg, po, daily until discharge) and irbesartan (150 mg, po, daily until discharge) Comparison: IV fluids and laboratory testing alone Outcome: Day 14 survival	Registered as NCT02380625 Unknown	N/A
Interferon-beta	Open label, single arm	Intervention: subcutaneous interferon-beta once daily for up to 10 days Comparison: not defined (safety and effectiveness study) Outcome: not defined	Registered as ISRCTN17414946 Completed	Inconclusive
Amiodarone	Open label, RCT	Intervention: amiodarone (20 mg/kg, i.v., on day 1,2,3 then 200 mg, po, three times daily, on day 4-10) Comparison: supportive care alone Outcome: Day 10 survival	Registered as NCT02307591 and PACTR201501001014425 Withdrawn	N/A

Table 3.4: Patient-based clinical trials of experimental therapeutics registered on clinical trial databases during the west Africa EVD outbreak. Where a dose of an intervention has been stated, it refers to the stated adult dose. Refer to trial protocols for weight adjustment. PICO is participant, intervention, comparison, outcome. RCT is randomised controlled trial. NA is not available.

Improving practice: explore, test, and implement a suite of trial design options

Early during their assessment of research priorities for the outbreak, WHO announced support for alternative trial designs. The reasons for this included concerns around the ethics and acceptability of randomisation in the setting of high mortality and community distrust – concerns voiced by some scientists and advocates from the most affected countries[140, 146]. Furthermore, it was hoped that the potentially smaller number of patients needed to reach statistical conclusions under certain conditions in these trial designs would help triage treatments faster[151]. As a result, single-arm and adaptive design trials commenced, followed at a later stage by randomized controlled trials. Although there was dispute amongst scientists as to the most appropriate trial designs in this setting, there was most certainly innovation in the approaches taken, and this needs to be used as springboard for further advances. Several areas of planning are possible to advance now, with the aim of having off the shelf, operationally tested trial designs available at the onset of cases.

Firstly, while modelling of epidemic threats is often undertaken for public health reasons, to date, very little work has used predictable elements of outbreak epidemiology to investigate the feasibility of different trial designs. In figure 3.11, using MERS-CoV as an example, we outline three likely epidemiological scenarios for emerging infections that even in simplistic form can help identify potential issues for trial design. For example, it is evident, but rarely made explicit, that for some emerging infectious diseases that result in a small number of sporadic cases with uncertain timing and location (figure 3.11, panel a), there can be no certainty that enough cases will accrue to complete a hypothesis driven randomised controlled trial. The issue of unpredictable numbers and difficulty in recruitment has been faced in other fields of clinical research, particularly research on rare inherited diseases and rare cancers, but it is also likely becoming an increasing problem in studies of uncommon phenotypes of antimicrobial resistance[195, 196]. However, with

infectious diseases there is always the possibility that a larger outbreak may cause a spike in case numbers (figure 3.11, panel b). For such a scenario adaptive Bayesian trial designs may be a solution, where, while numbers of cases are small, the initial focus is on estimating the treatment effect and safety rather than hypothesis testing, and drug registration may be possible with supplementary data from animal models. Such a trial could be designed so that in the event of a substantial outbreak the trial could switch to hypothesis testing whilst utilising the data already accrued. For larger outbreaks (figure 3.11, panel c), adaptive 'platform trials' may be an efficient option, which allow the testing of multiple agents simultaneously, even if there is no agreed best comparator group[197, 198].

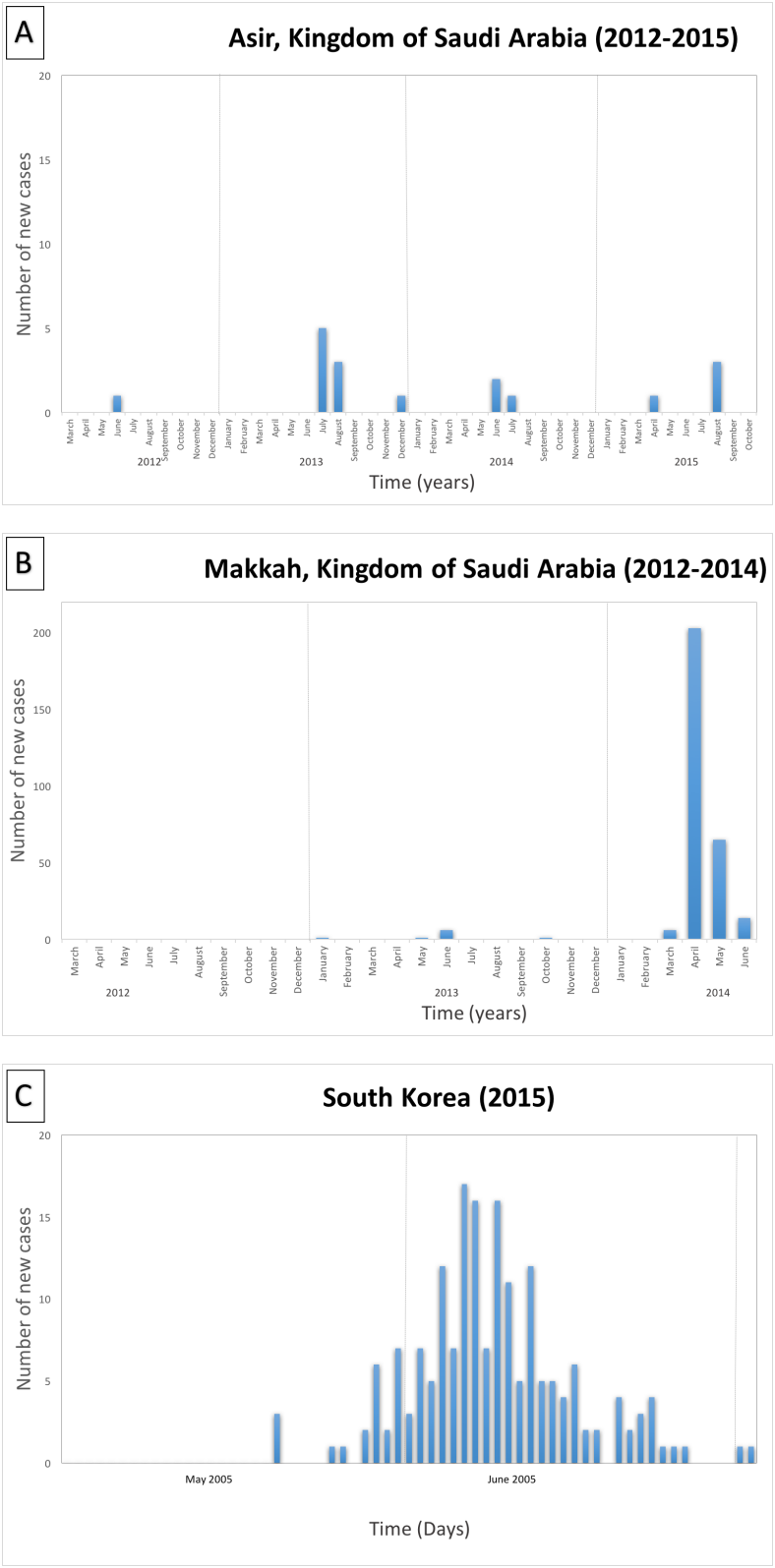


Figure 3.11: Possible epidemiological patterns for EEIDs, as demonstrated by MERS Co-V cases in different regions. In each of these, the possible number of patients eligible for recruitment and the time in which to enrol them differ markedly, despite the same causative pathogen: (a) small intermittent outbreaks over years, (b) small intermittent outbreaks followed by a larger outbreak and (c) propagated outbreak. Data adapted from https://public.tableau.com/profile/ian.m.mackay!/vizhome/MERS-CoV_0/.

Of course, not all elements of a trial design can be designed in advance. The most significant limitations include the evolving understanding of the natural history of an emerging disease (which may invalidate assumptions used to make estimates of effect), and that the research priorities of the affected populations (who should be prioritising trials) may differ depending on the location and circumstances of the outbreak.

Improving practice: between outbreaks accrue evidence in analogous infections

Now that the epidemic has concluded, there is a risk that the forward momentum of EVD research wanes. This is especially the case for patient centred research that necessarily relies on individuals with the disease to test hypotheses. It is also possible that a full scale trial may not be feasible if future outbreaks are small. As an example, the EVD outbreak in the Democratic Republic of Congo (DRC) in May of 2017 caused a total of eight cases[199]. A consideration for enabling progress is to advance knowledge in similar but more common infections and supplement this with smaller bridging studies that demonstrate that extrapolation to the emerging infectious disease is valid.

This approach has been suggested for rare antimicrobial resistance phenotypes[200]. Such an approach would for example be ideal for progressing the clinical development of drugs for avian influenza viruses, where proof of principle in seasonal influenza can then be bridged to rarer human cases of avian influenza infection. Between 3 and 5 million people are severely ill due to seasonal influenza epidemics each year[201]. Despite this, there are relatively few large therapeutic trials with a clinically significant primary outcome (death, ICU admission), and even fewer for high risk populations such as pregnant women. Given the significant similarities in disease pathogenesis, drug target, and at risk populations between seasonal and

pandemic influenza, this is a wasted opportunity to not only improve seasonal influenza care, but also to produce data driven statistical designs and endpoints for trials of novel influenza strains causing outbreaks, and extendible trial platforms.

This approach would be particularly suited to studies of host-directed therapies and trials of supportive care interventions, where common pathological pathways are targeted. For example, during the west Africa EVD outbreak, the experimental agent FX06 was provided on a compassionate care basis to two patients medically evacuated to high income settings (although not used in a clinical trial)[145]. FX06 is a host directed therapy aimed at reduction of vascular instability and capillary leak. The drug had demonstrated encouraging results in a mouse model of dengue virus induced shock syndrome (DVISS)[145] and it was based on the purported resemblance of the vascular leak in this syndrome with that of EVD that patients were provided the drug. While the extent of pathophysiological similarity between the two diseases is not well characterised, progression of the drug to clinical trials for DVISS or other diseases causing vascular damage will provide valuable information, for example regarding pharmacokinetics, for better clinical trials during an outbreak.

There are a number of benefits to adopting this groundwork by analogy approach. Firstly, similarities in the dosing and toxicity profile of the drug between diseases may help produce effective trial protocols if and when emergency trials are implemented. In addition, operational capacity is enhanced in centres recruiting patients during 'peace time' who can then respond more adequately during an outbreak. This is especially important if the opportunity is taken to conduct clinical trials in resource-poor settings where epidemic potential is high. In this scenario, capacity building enables local investigator leadership during an outbreak. However, there are limits to the extent that extrapolation is justified, safe and ethical and these require thorough examination each time this approach is considered.

Improving practice: consider supportive care when prioritising trials

Improvements in supportive care probably contributed to the gains in survival over time during the west Africa epidemic. However, the safety and efficacy of individual components remains unknown because no trials were conducted. In particular, there remains contention regarding optimal fluid resuscitation strategy, use of empiric antimicrobials, anti-diarrhoeals, non-steroidal anti-inflammatory agents and vitamin K[165]. Many of these questions are applicable to other viral haemorrhagic fevers at the least, but probably also to other emerging infectious diseases – because there is limited understanding of the pathophysiology of these conditions, and also due to the poor representation of patients from high risk countries in existing medical literature. There are several key benefits to prioritisation of supportive trial therapies. Firstly, significant gains in patient survival may be possible without the 'silver bullet' of an effective novel agent. Gains in sepsis survival over the last few decades provides an excellent exemplar given the lack of specific therapy for that syndrome despite a large volume of research. Secondly, these trials are not reliant on a costly and time-consuming drug development pathway to be completed and do not suffer the issues of post-trial pricing and accessibility to the same extent as novel therapies – it should be more operationally feasible to implement findings into limited resource settings.

However, given there is often a limited number of appropriate trial sites during an outbreak, crucial considerations include deciding what factors (such as predicted benefit of a drug, accessibility) should determine how supportive care trials are prioritised relative to novel drug trials, or under which circumstances simultaneous evaluation of supportive and interventional measures can occur.

Improving practice: triage clinical trials

That several clinical trials were launched during the timeframe of the EVD epidemic is undoubtedly a success. However, this, perhaps for the first time, led to the unintended consequence of multiple trials vying for enrolment during the final phases of the epidemic, as case numbers fell. Allegations of 'chaotic land grabs for sites and patients' surfaced[62], despite good collaboration between many research groups.

There are multiple consequences of too many clinical trials enrolling simultaneously. Most importantly a fractured research response can lead to inadequate sample size enrolment in each trial and an overall failure to identify drugs that improve patient survival, or that do not work. There is the risk of overwhelming regulatory authorities in the affected region who are required to deal with a surge in applications to conduct trials. For example, the number of applications to the national ethics committee in Guinea increased fourfold in 2014-5 compared with the previous year[202]. During the EVD epidemic there were very few senior, research experienced clinicians from the affected countries available to lead trials and many of these also had significant responsibility in the humanitarian response to the emergency. Subsequently, overlap of principal investigators between trials was substantial and they risked being placed in a compromised position due to the competing interests of different groups. For future outbreaks, it is worth considering triaging of clinical trials, and potential clinical trial locations. This is a decision that should be led by the most affected countries, but WHO are likely required to convene all appropriate stakeholders.

If appropriate prioritisation of therapeutic interventions and/or trial designs can occur in advance of an outbreak, there is another important benefit. Of the delays to clinical trials during the west Africa EVD epidemic, the weeks to months taken to develop and authorise clinical trial agreements were especially needless - it is

entirely feasible for components of these contracts to be produced in advance. These contracts delineate the legal and financial responsibilities of the trial partners and during the epidemic these were developed ad hoc, as trial site access was negotiated. However, the international epidemic diseases research consortia and networks that are likely to provide expertise during an epidemic are already known. Likewise, the non-governmental agencies that will be engaged in a research response in a given region are foreseeable. As mapping of medical countermeasure pipelines improves for epidemic diseases, the companies and research institutions involved with the most promising candidates can be engaged early and ownership issues including possible intellectual property clarified. As there are predictable areas of negotiation (e.g. post- trial drug pricing and access conditions, reflecting the respective public and private investments) for each of these stakeholders, pre-prepared contracts would be an excellent outcome of increased collaboration.

The most significant difficulty in generating 'peace time' protocols is ensuring that local governments and populations are adequately represented when the location of the next epidemic is not known. In the quest for a harmonized, rapid research response, it is important that globally agreed priorities do not discount the specific needs and agenda of local stakeholders. Early recognition of the most appropriate local representatives during an outbreak is therefore critical, but often can be hindered by poor research infrastructure or unclear government organization.

Improving practice: prepare for operational challenges

Practical difficulties, as opposed to scientific or trial design issues, are a major contributor to delayed and inadequate recruitment for clinical trials[203]. The additional operational complexities during outbreaks can be significant, and are perhaps underappreciated. Key constraints for EVD trials included stringent infection prevention and control requirements impairing ready access to patients

and the expanded breadth of logistical activities undertaken by research staff working in a resource limited environment that had limited research infrastructure[139]. The consequences can include a difficulty in scaling up recruitment without threatening staff or patient safety, or detracting from the immediate humanitarian priorities of the clinical team. In the TKM-130803 trial this logistical complexity also contributed to delays in scaling the research further to include other ETCs. At the time there was sufficient operational capacity to do so, case numbers were declining.

The most significant improvement to the operational feasibility of outbreak clinical research will occur when research is integrated into the overall outbreak response. When high quality clinical data are captured according to standard operating procedures and using agreed data standards, it minimizes duplication between clinical and research teams. The benefits are multi-fold – patients will be protected from additional examinations or interventions, the utility of clinicians is maximized, and multi-site research will be simpler to conduct. Training to strengthen the capacity of researchers from high risk countries so that they can build outbreak research onto their existing platforms will also help. Such an initiative is underway through the African Academy of Sciences endorsed Clinical Research During Outbreaks (CREDO) course. Technological and human factors advancements that will automate or simplify the collection of clinical data has particular potential for infectious diseases that require PPE to be worn.

Improving practice: learn from other disciplines

There is no doubting that epidemics are a challenging context in which to conduct research, but that just means we must adapt, not abandon, our research approach. Other fields have demonstrated the feasibility of conducting patient centred research in austere environments.

One example is the field of pre-hospital medicine. Here, inclusion of research is relatively new and early attempts were plagued by the complexities of obtaining patient consent and collecting high quality data within the short time on scene[204, 205]. Furthermore, assessments of the barriers to implementing research suggested that pre-hospital providers have felt that research was not their responsibility, was unethical given the severity of patient presentations and was infeasible given time constraints[205, 206]. However, recent leadership by various organisations to develop capacity has led to publications of randomized controlled trials of prehospital cooling (induction of hypothermia) for patients with cardiac arrest[207, 208], blood products for major traumatic haemorrhage[209], and alternative treatment strategies[210, 211], and analgesia[212]. Increasingly too, this field is investigating alternative trial designs as an approach to dealing with a small and specialised patient population[213].

Likewise, innovations from military medical research in Iraq and Afghanistan have led to advances in resuscitation strategies (such as optimisation of blood product transfusion ratios, early use of tourniquets, and administration of tranexamic acid for major haemorrhage[214–217]) that are now widely adopted in civilian settings. Barriers to research in this setting are again analogous to outbreaks. These have included feasibility in a chaotic working environments, difficulties obtaining informed consent from critically ill patients, delays to receiving approvals for research that rendered research infeasible within the time frame of a scientist's deployment, and intense scrutiny regarding the ethics of research in such a setting[218]. In spite of these, research in this field is prolific - as an example, the Joint Combat Casualty Research Team of the United States Military had undertaken over 100 research projects in the first decade following the September 11 attacks[218].

The question is, why have these other fields have been so successful in overcoming some of the barriers that EEID researchers have stumbled on? It appears that both pre-hospital and military medicine have adopted a strong culture of structured

evaluation of processes and outcomes (such as regular audit, clinical governance, and morbidity and mortality meetings) that some indicate are superior to that seen in hospital practice [218]. Under this paradigm, research studies become a natural extension to systems that seek quality improvement. Encouragingly, some humanitarian providers are including quantitative evaluation of their activities and embedding operational research into their projects[59, 219, 220].

3.4.3 Conclusions

Strategic efforts for epidemic prevention and control must take a more innovative approach to securing the R & D pipeline for promising treatments and accelerating the conduct of rapid, flexible clinical trials. Potential solutions include the creation of ambitious, multi-sector initiatives for preclinical development and improved use of inter-epidemic periods to progress clinical trials through analogous diseases, conduct methodological work on trial design, and triage trials in preparation for the next outbreak.

3.5 Contribution of the chapter

The three sections in this chapter apply one of the core recommendations of chapter 1 - that we should seek opportunities to reduce barriers to the conduct of high quality evidence generation during outbreaks, especially clinical trials.

Section 3.2 reports a clinical treatment trial with a finding of no treatment efficacy. However, alongside work conducted by other groups, it demonstrates that is possible to conduct clinical trials to international standards during an outbreak. In this section, the efforts made to reduce barriers to undertaking the trial and reaching a statistical conclusion are best exemplified by the adaptations made to the trial design.

In summary, we were one of the few groups that met a pre-specified statistical boundary for completion. This was facilitated by selection of a single-arm study as part of a multistage approach (MSA), where the statistical design minimized the number of cases that received an ineffective therapy (as was the case here), or could more quickly roll out a therapy identified as effective in the single-arm phase when compared to an RCT[151]. Likewise, if a randomised control trial was initiated in a subsequent phase of the MSA, sequential analysis was planned to identify efficacy or harm as soon as possible. We were not alone in recognising the need to identify findings quickly, and other clinical trials employed forms of interim analysis and or the use of early stopping rules[221].

I also discussed the selection of a single-arm study on pragmatic and ethical grounds. Compared to other trial design adaptations, this remains less widely adopted as a strategy amongst research groups. Certainly randomised trials were initiated and found to be practically feasible by other groups later during the outbreak, and this remains a valid, high quality trial design. However, I argue that while rigorous debate regarding the most appropriate selection of trial designs is important, if it is allowed to become a preoccupation of debate, it distracts from the more critical issue that very few patients had any opportunity to receive any experimental agent in a registered, ethically sound clinical trial irrespective of the nuances of analysis method, and that all researchers involved have a shared intent to address this.

We included one other significant adaptation to trial design made to enhance practical implementation. This was the inclusion of operational randomisation to manage a sudden surge in patient numbers. For the TKM-130803 trial we would not have been able to safely administer and monitor an unlimited number of patients given we were using an intravenous drug with a known potentially serious adverse event profile. While planning for surges seems counter-intuitive given the emphasis on selecting a trial design that requires a small sample size, there are several reasons that case numbers may surge even if the outbreak is waning. This

may include the infection of a cohort from a highly transmissible individual (so called super-spreader[222]) or highly transmissible event (such as a traditional burial ceremony[223]), or the redistribution of patients due to the sudden closure or temporary interruption of other facilities (as we experienced). Similar approaches were adopted by other clinical trial groups, such as the inclusion of a control cohort in a single-arm study of convalescent plasma in the event that there was insufficient compatible plasma available for infusion [139].

In section 3.3 I discuss how field level innovations helped remediate practical barriers to trial implementation. Several of my findings agree with an assessment of the field level challenges for a trial of favipiravir conducted in Guinea[224]. These authors also found benefit to harmonising trial SOPs and blood sampling procedures with clinician practice to reduce duplication, and required innovation to transfer data from the high risk zone. They also report similar difficulties to the TKM130803 trial such as seeking the most acceptable consent processes for special populations (unaccompanied minors), delays to consent processes that delayed administration of the first dose of the drug, and technical laboratory problems when adding additional analyses. However, there remain few published field level experiences published in the literature, hampering efforts to consolidate practical lessons learned.

The value of section 3.4 is its deliberate focus on innovation. There is a strong pre-existing agenda of advocacy for clinical research in outbreaks. There are therefore, generally agreed reforms that are essential to progress, such as strengthening national research capacity [225–227], use of pre-prepared protocols for clinical data capture [85, 228, 229] or improving commitment to data harmonization and data sharing initiatives [85, 229–231]. However, inertia exists in adopting some of these, given that they require a whole of system response with commitment from a myriad of stakeholders. My focus in the clinical component of this section was on more agile opportunities for change.

3.5.1 Future research

There are two further analyses underway of samples of the TKM-130803 trial. The first is pharmacokinetic analysis which is important given that the TKM-130803 trial was the first time that this formulation of the IND was used in a human population. The second analysis is of cytokine profiles of patients who received TKM-130803, compared with a control group of EVD patients (who did not receive an experimental therapeutic). Cytokine profiles are valuable given our limited understanding of the immune response to EVD, but especially because cytokine release syndrome is a known adverse effect of TKM-130803.

4

Validity of clinical characterisation of EVD in the published literature

Contents

4.1 Preface to the chapter

Chapter three described a clinical trial for EVD, and then provided suggestions to improve the conduct of clinical trials during outbreaks. This chapter focuses on strengthening the quality of observational data collected and reported during outbreaks. It contains the preliminary data from a quantitative systematic review of the clinical presentation of EVD in hospitalised patients, and then explores the

validity and usefulness of the data for informing clinical trial design.

4.1.1 Context

I, and others have published reviews detailing the new clinical insights gained from the west Africa epidemic (appendix 1). These new findings include improved recognition of high risk populations; greater awareness of the spectrum of clinical presentations (especially the predominance of gastrointestinal symptoms)[52, 53, 144, 148, 232–234]; improved awareness of the extent of multi-organ dysfunction[53, 235–237]; and publication of the most extensive haematology and biochemistry datasets to date[235]. In addition, much more is now known about survivor syndrome[238–242] and viral sequestration in immune privileged sites following acute infection[243–245]. These findings were usually published in small case series of patients. Therefore, while it has been possible to highlight novel presentations or previously unforeseen laboratory abnormalities, assessment of baseline risk has been difficult because the representativeness of these cohorts to the underlying population is unknown.

One prior systematic review of EVD has been published[246]. This work was submitted in early 2015 and includes only two datasets from the west Africa epidemic. There were a number of methodological limitations to this piece of work. The patient cohort was not well defined - it included patients with different viral strains, and did not stratify according to hospitalisation status. The inclusion and exclusion criteria for articles were incompletely defined, and there was no description of article quality. Pooled estimates were used without statistical exploration of heterogeneity, nevertheless the authors concluded that the most significant limitation of their work was the lack of uniformity, and incompleteness of the underlying data. Given that the number of patients during the west Africa epidemic eclipsed that of all previous outbreaks combined, and the volume of publications describing clinical data for EVD was unprecedented, there is an opportunity to produce more precise

estimates of clinical parameters, risk factors, and outcomes.

A theme of this thesis is that data accumulation in epidemics without specific intent to inform or influence patient care is limited in utility. The second aim of the systematic review is therefore to determine the extent to which the body of literature constitutes "actionable data"[117]. This is the extent to which the findings are accessible, objective, and sufficient to inform clinical, public health, and research decision making[117].

4.1.2 Objectives of the chapter

The objectives of the work in this chapter are:

1. Provide a systematic review and meta-analysis detailing the clinical presentation of patients hospitalised with EVD during the epidemic in West Africa, in order to describe the evolution of patient symptoms and clinical biomarkers during the course of illness; and identify clinical signs, vital signs and laboratory tests that identify patients at increased risk of death with greater confidence.

2. Determine the extent to which the included literature are sufficient to inform the conduct of clinical trials, assessed according to the criterion of representativeness, 'transparency', and compatibility.

3. Use these findings to suggest ways to improve the utility of observational data in disease outbreaks.

4.1.3 Author's contributions

The work contained in this chapter is part of a larger collaboration. I will be the first author on the systematic review manuscript. I co-designed the study with Alex Salam. I interviewed, trained and then co-managed (with Catrin Moore and Alex Salam) the team of research assistants. I designed and wrote the data collection database, standard operating procedures, the systematic review registration application, the statistical analysis plan, and the data dictionary and associated statistical code. I reviewed all manuscripts for short-list for full-text review. I undertook final article selection along with our team of research assistants and was the senior reviewer that resolved discrepancies in duplicate data extraction (assisted by Anna Carlqvist) and then undertook additional data extraction when required. I undertook the majority of data management and cleaning, but with assistance from Lyndsey Castle. I undertook the statistical analysis with support from the study statisticians, Kasia Stepniewska and Lang'O Odondi. I drafted the first version of the manuscript and produced all figures and tables. For the preliminary work contained in the contributions of the chapter, I was the principal investigator, supervising our medical student James Moran who assisted with literature searching.

The data contained in this chapter is preliminary and not yet finalised for publication.

4.2 Clinical features of EVD and risk factors for death: A systematic review and meta-analysis

4.2.1 Introduction

Alongside the remarkable public health response to the west Africa EVD epidemic there was a sustained effort to collect data on the natural history of the disease, laboratory correlates and predictors of outcome. The value of this data is in providing an evidence base for treatment of patients, developing and testing hypotheses regarding the pathophysiology of disease, and informing the development and testing of medical countermeasures.

While WHO reported large epidemiological datasets[53, 247], there has been no comprehensive synthesis of clinical data. The primary purpose of this work is to provide the first systematic review (and meta-analysis where appropriate) of the clinical presentation of patients hospitalised with EVD during the epidemic. The aim is to provide estimates for the evolution of patient symptoms and clinical biomarkers during the course of illness; and identify clinical signs, vital signs and laboratory tests that identify patients at increased risk of death with more precision than has otherwise been possible.

However, the west Africa EVD outbreak was a difficult and demanding environment in which to collect high quality data[248]. While it is expected that the data-set will be imperfect, it nonetheless constitutes the best available clinical data for EVD, and maximising data utility is critical. Therefore, the secondary purpose of this work is to determine the extent to which the included literature is adequate to meet the requirements of evidence based healthcare ('actionable' data). We focus on one aspect - utility in informing clinical treatment trial design, where observational data are frequently used to decide on trial outcomes, undertake power calculations,

refine eligibility criteria and decide on variables for stratification.

4.2.2 Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines[249]. The study was prospectively registered on the PROSPERO systematic review database (registration number CRD42017070150).

Data sources and searches

We conducted a systematic search of PubMed for articles published between the 1st of January 2014, to the 31st of May 2017, updating a search published previously (appendix 1). The search term was "Ebola".

For comparative epidemiological data, we used individual level patient data (IPD) available from WHO[250], where possible. Within this dataset, only information for patients who were hospitalised and with a known outcome were used. Where IPD data was not available, aggregate data from an alternative WHO source was used[53].

Study selection criteria

Articles that described hospitalised patients with confirmed EVD who were treated in one of the three most affected countries (Liberia, Sierra Leone, Guinea) during the epidemic in West Africa (2013-16) were eligible for inclusion.

Further, articles must have described one or more clinical aspects of disease (defined as a sign, symptom, examination finding, or laboratory investigation finding) and described acute patient outcomes (at a minimum, case fatality up to, or within

14 days of discharge from the treatment facility). Hospitalisation was defined as treatment in an Ebola Treatment Unit/Centre (ETC) or a pre-existing hospital. A post-hoc addition was made to this criterion; there were a number of sites that were originally designated as Ebola holding centres, but that admitted patients and provided supportive medical therapy (at times including intravenous fluids) and then were later re-termed as ETCs by national governments. Facilities that fell within this category were also included. Articles must have included all EVD patients in their facility for the defined period where sufficient data existed for reporting. Articles that report a subset of their cohort (usually on the basis of age or pregnancy status) were excluded as the cohort would not be representative of the underlying population. Articles that included additional enrolment criteria (usually clinical trials) were also excluded for this reason.

We excluded articles that described fewer than ten patients. Language was restricted to English and French.

Data extraction and quality assessment

Review of manuscript titles, abstracts and full text was undertaken in duplicate based on pre-agreed selection criteria. In cases of disagreement, the senior author determined eligibility (AR). For eligible articles, data were extracted in duplicate into an electronic data form, with each independent reviewer following the study data management standard operating procedure. Extraction of article details was performed by the following authors (AC, AE, AR, DK, EL, MB, RR). Discrepancies in duplicate data extraction were resolved by a third author (AC or AR). Quality assessment has so far, only been performed by one author (AR).

Where hospitalised and non-hospitalised patients were included in an article, we extracted only data on hospitalised patients.

We extracted study characteristics including author names, included sample size, selection criteria, dates of patient enrolment, and dates of publication.

We extracted patient information including demographics (age, gender), temporal characteristics of disease (such as time between symptom onset and admission), clinical manifestations of disease (based on the symptoms in the WHO case definition used during the outbreak[251]), vital signs (heart rate, respiratory rate, body temperature, oxygen saturation, level of consciousness, blood pressure), laboratory results (ebolavirus PCR result, malaria result, haematology, biochemistry, and coagulation profiles), predictors of mortality (adjusted relative risk, or hazard ratio), and mortality. Data extraction was stratified by survival status of the patient where that information was available. We recorded symptom data at three time points (on admission (defined as <24hrs.), during hospitalisation (subsequent days), or at any time (for when an article did not describe the time of data collection, or this could not be deduced). Vital sign and laboratory data were collected on admission only. We report findings of 'myalgia' and 'myalgia/arthralgia' together as myalgia +/- arthralgia.

For all variables, we extracted numerator and denominator data for dichotomous variables, and summary statistics for continuous variables (mean or median, and standard deviation, or interquartile range, or range). For predictors of survival we extracted unadjusted and adjusted odds or risk ratios with 95% confidence intervals. Data was reported as missing for each variable when it was not available in the manuscript. Manuscript authors have not yet been contacted for missing data.

Quality assessment

Quality assessment was initially undertaken using the Newcastle Ottawa scale[252] but the scale was found to be unsuitable for the dataset despite modification of the

criteria. Therefore, quality assessment was undertaken using an original scale. Data quality was assessed based on parameters identified in the literature as critical for outbreak evaluation[248]. These include that the source of data is defined; there is a clear temporal component to the data; and there is transparency regarding the proportion of cases for whom an outcome is known. Clinical interpretability was assessed based on whether each article provided an estimate of variables (age, pregnancy status, viral load, and time from symptom onset to patient admission) that were known or suspected co-variates of outcome at the time the west Africa outbreak commenced[253–255].

Treatment of duplicate data

Duplicate or overlapping datasets were first identified on the basis of similarity of authors, facility locations, periods of enrolment, included sample size, and/or statement of prior publication of data. Because some facility names and affiliated organizations changed throughout the outbreak, these methods alone were insufficient to identify duplicates. Therefore manuscripts were then manually mapped by a senior author (AR) familiar with ETC operations during the outbreak.

When duplicate or overlapping datasets occurred, they were ranked from that which included the most, to least number patients. The first ranked (largest dataset) was always used for reporting variables when data was available. However, because more detailed clinical description could be included in one or more lower ranked datasets, these were not excluded outright. Instead, a variable of interest was searched for in a step-wise manner down the ranking scale and then reported for the highest ranked article where it was contained. The ranking system is detailed in figure 4.1. We refer to the first ranked datasets collectively as the 'primary' dataset.

Statistical analysis

Descriptive statistics are presented as frequencies for categorical variables, means and standard deviations for normally distributed data, and median with range for other continuous variables. Relative risks were calculated from the number of events and participants in each group. All p values are calculated from two-tailed tests of statistical significance with a type I error rate of 5%.

Systematic review and meta-analysis Individual proportion or relative risk estimates are displayed graphically with forest plots, and then on summary forest plots when variables are clinically related. For meta-analysis of proportions the data were pooled using random effects meta-analysis because heterogeneity was substantial. This meta-analysis used a binomial specific method (stata program *metaprop*) that allowed for Freeman-Tukey arcsine transformation of data under conditions where normal approximation may fail[256]. For meta-analysis of relative risk the data were pooled using random effects meta-analysis due to substantial heterogeneity. Log transformation of the relative risk was used to provide a log-normal distribution for estimates, then exponentiated for graphical depiction. Heterogeneity for all meta-analysis was assessed using the Chi-squared test for assessment of heterogeneity and quantified with the I^2 statistic. Estimates of publication bias in meta-analysis (such as Egger's test) are not included due to their limited utility when there are a small number of publications included [257]. Systematic review and meta-analysis was only performed when three or more articles reported data for the variable of interest.

Actionable data analysis Three criterion were used to perform an actionable data analysis. These were: the representativeness of the data to the underlying population, the 'transparency' of reporting[117], and the ability for the data to

be quantatively synthesised (compatibility)[117].

We assessed *data representativeness* by comparison to WHO datasets, where data were available to do so. For symptom data, this was aggregate data from all patients reported during the first twelve months of the outbreak (no data stratified by hospitalisation status was available)[53]. There are no aggregated estimates available for vital sign or laboratory data. The comparative estimate for CFR was based on hospitalised patients reported over the entire outbreak[250].

We assessed *data transparency* by reporting whether anonymised IPD was available for an article, and whether publication of the same cohort in another manuscript was acknowledged when there was duplicate publication of patient data.

We assessed *data compatibility* for synthesis by comparing the consistency of data reporting for two key co-variates of outcome that could be used in meta-regression: patient age, and estimate of viral load.

We performed all analyses with Stata/MP version 15.0.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

4.2.3 Results

3653 articles were identified from electronic searches, and 34 articles were eligible for inclusion (figure 4.1)[142, 161–163, 166, 235, 236, 258–284]. Details of the included articles are provided in table 4.1. 16 of these articles represent the largest,

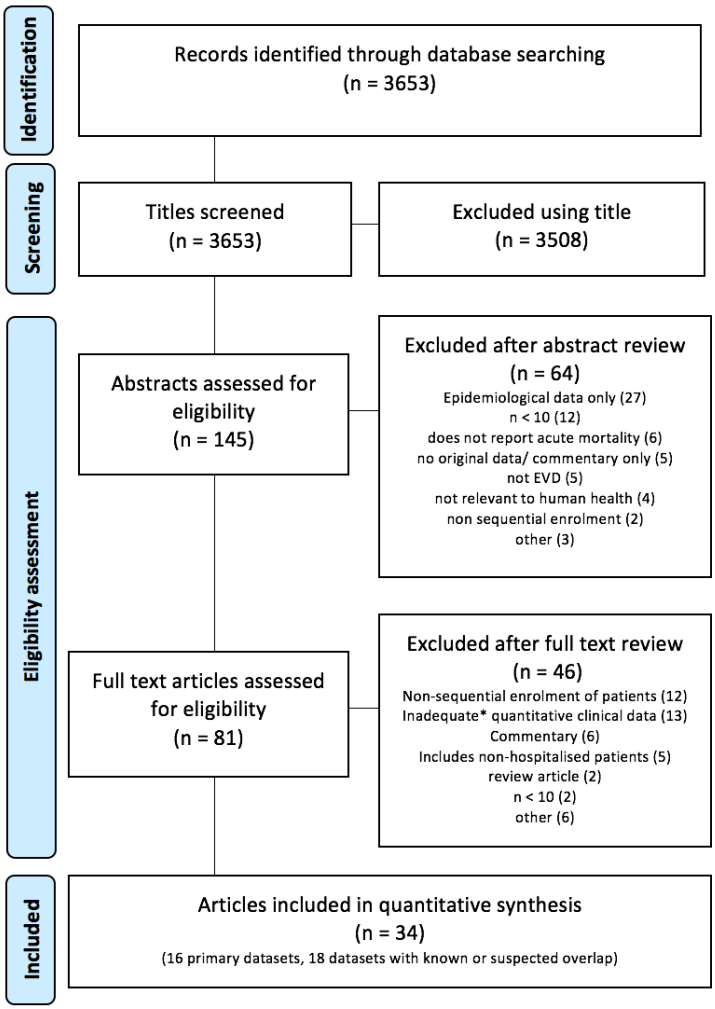


Figure 4.1: Summary of article selection process.

or only representation of patients (termed the primary dataset) and 18 articles duplicate, or overlap with this data and are only used when data are missing from the primary dataset. Details of the aggregate WHO clinical dataset that provides reference estimates is also shown[53].

The geographical distribution of the primary dataset is shown in figure 4.2. The total number of patients included in the primary dataset is 6168. For this dataset the median number of patients per article was 207 (IQR108-607).

	PubMed ID	Author	*n*	Data from >1 ETU?	Type	Name of ETU	Medical care provider	Date of first patient inclusion	Date of last patient inclusion	Date published	Is the article reporting duplicate data or overlapping data? (If yes, ranking in brackets)
Guinea	26625118	Faye *et al.*	699	Yes	Ret	Hospitals in Conakry area, undefined	Not applicable	01/03/2014	28/02/2015	01/12/2015	(1/4) Donka Hospital, (1/3) French Military Hospital, (1/3) Conakry Healthcare Worker ETU
	26134358	Bordes *et al.*	22	No	Pro	Conakry French Military ETU	French Military	xx/01/2015	xx/04/2015	02/07/2015	Yes (2/3)
	26338789	Cournac *et al.*	22	No	Pro	Conakry French Military ETU	French Military	xx/01/2015	xx/04/2015	03/09/2015	Yes (3/3)
	26541219	Cotte *et al.*	14	No	Pro	Conakry Healthcare Workers ETU	French Military	xx/01/2015	xx/03/2015	02/11/2015	Yes (2/3)
	26582958	Janvier *et al.*	14	No	Ret	Conakry Healthcare Workers ETU	Unknown	unknown	unknown	17/11/2015	Yes(3/3)
	25391486	Barry *et al.*	90	No	Pro	Donka National Hospital*	DDITD & MSF	25/03/2014	20/08/2014	23/10/2014	Yes (2/5)
	25770172	Barry *et al.*	89	No	Pro	Donka National Hospital	DDITD & MSF	xx/03/2014	xx/08/2014	13/03/2015	Yes (3/5)
	25992182	Qureshi *et al.*	70	No	Ret	Donka National Hospital	DDITD & MSF	25/03/2014	05/08/2014	xx/02/2015	Yes (4/5)
	25372658	Bah *et al.*	37	Yes	Ret	Donka National Hospital & Hopital de l'Amite Sino-Guineene	MSF	25/03/2014	26/04/2014	05/11/2014	Yes (5/5)
	27638946	Kerber *et al.*	1231	Yes	Ret	Hospitals in Gueckedo, undefined	Multiple	30/03/2014	30/03/2015	16/09/2016	No
	28352651	Vernet *et al.*	97	Yes	Pro	Macenta and Nzerekore ETU	IFRC France, ALIMA	29/11/2014	31/01/2015	23/03/2017	Yes (1/2)
	27928085	Loubet *et al.*	76	No	Ret	Nzerekore ETU	ALIMA	02/12/2014	23/02/2015	10/10/2016	Yes (2/2)
Sierra Leone	27806732	Ji *et al.*	285	No	Ret	Freetown China-SL Friendship Hospital* ^	Chinese Medical Team	01/10/2014	21/03/2015	03/11/2016	Yes (1/6)
	26523640	Li *et al.*	288	No	Ret	Freetown China-SL Friendship Hospital	Chinese Medical Team	01/10/2014	20/03/2015	30/10/2015	Yes (2/6)
	27317404	Xu *et al.*	139	No	Ret	Freetown China-SL Friendship Hospital	Chinese Medical Team	15/11/2014	18/01/2015	14/06/2016	Yes (3/6)
	26223324	Yan *et al.*	85	No	Ret	Freetown China-SL Friendship Hospital	Chinese Medical Team	01/10/2014	09/11/2014	30/07/2015	Yes (4/6)
	26398207	Zhang *et al.*	63	No	Mix	Freetown China-SL Friendship Hospital	Chinese Medical Team	01/10/2014	18/01/2015	23/09/2015	Yes (5/6)
	25995207	Qin *et al.*	61	No	Ret	Freetown China-SL Friendship Hospital	Chinese Medical Team	01/10/2014	14/11/2014	20/05/2015	Yes (6/6)
	26551684	Lanini *et al.*	84	No	Ret	Emergency NGO ETU, Freetown	Emergency	13/12/2014	20/04/2015	09/11/2015	No
	28151955	Hartley *et al.*	158	No	Ret	GOAL-Mathaska ETU	GOAL Global	14/12/2014	15/11/2015	02/02/2017	No
	28258817	Waxman *et al.*	254	Yes	Ret	Lunsar, Makeni, Kambia ETU	IMC	01/12/2014	15/10/2015	28/02/2017	No
	27268303	Haaskjold *et al.*	31	No	Ret	Moyamba ETU	Medicos del Mundo	19/12/2014	17/02/2015	15/09/2016	Yes (1/2)
	27334891	Arranz *et al.*	31	No	Ret	Moyamba ETU	Medicos del Mundo	xx/12/2014	xx/03/2015	22/06/2016	Yes (2/2)
	26812579	Crowe *et al.*	151	Yes	Ret	Bo District ETU*	MSF	12/09/2014	07/01/2015	xx/02/2016	Yes (1/2)
	26551677	de La Vega *et al.*	632	No	Ret	Kailahun ETU	MSF	01/07/2014	30/11/2014	09/11/2015	Yes (1/4)
	26002981	Fitzpatrick *et al.*	525	No	Ret	Kailahun ETU	MSF	26/06/2014	12/10/2014	22/05/2015	Yes (2/4)
	25565430	Dallatomasina *et al.*	489	No	Ret	Kailahun ETU	MSF	23/06/2014	05/10/2014	02/03/2015	Yes (3/4)
	28459838	Theocharopoulos *et al.*	249	Yes	Ret	Kailahun (n=210), Magburaka (n=118), and Bo (n=44)	MSF	12/09/2014	21/03/2015	01/05/2017	(4/4) Kailahun, (2/2) Bo
	26271406	Hunt *et al.*	118	No	Pro	Kerry Town	Save the Children	08/12/2014	09/01/2015	11/08/2015	No
	25353969	Schieffelin *et al.*	87	No	Ret	Kenema Government Hospital	SL government	25/05/2014	18/06/2014	29/10/2014	No
	25539447	Ansumana *et al.*	581	No	Ret	Hastings Police Training School	Unknown	20/09/2014	07/12/2014	24/12/2014	No
Liberia	25845607	Levine *et al.*	160	No	Ret	Bong County ETU	IMC	15/09/2014	04/01/2015	03/04/2015	No
	26735991	Gignoux *et al.*	381	No	Ret	Foya ETU	MSF	05/06/2014	24/10/2014	07/01/2016	No
	27531847	Rosenke *et al.*	1182	No	Ret	Eternal Love Winning Africa 3	MSF	xx/08/2014	xx/02/2015	15/08/2016	No
All	25539446	WHO *et al.*	5564	Yes	Ret	not applicable	not applicable	30/12/2013	24/11/2014	24/12/2014	Comparison data only

Table 4.1: Details of included articles. Abbreviations used: Ret = retrospective; Pro = Prospective; Mix = Mixed prospective and retrospective enrolment; SL = Sierra Leone; DDITD = Donka Department of Infectious and Tropical Diseases; xx/ = date is unknown. *Status of facility changed from holding unit to ETC; ^ETC known by various names.

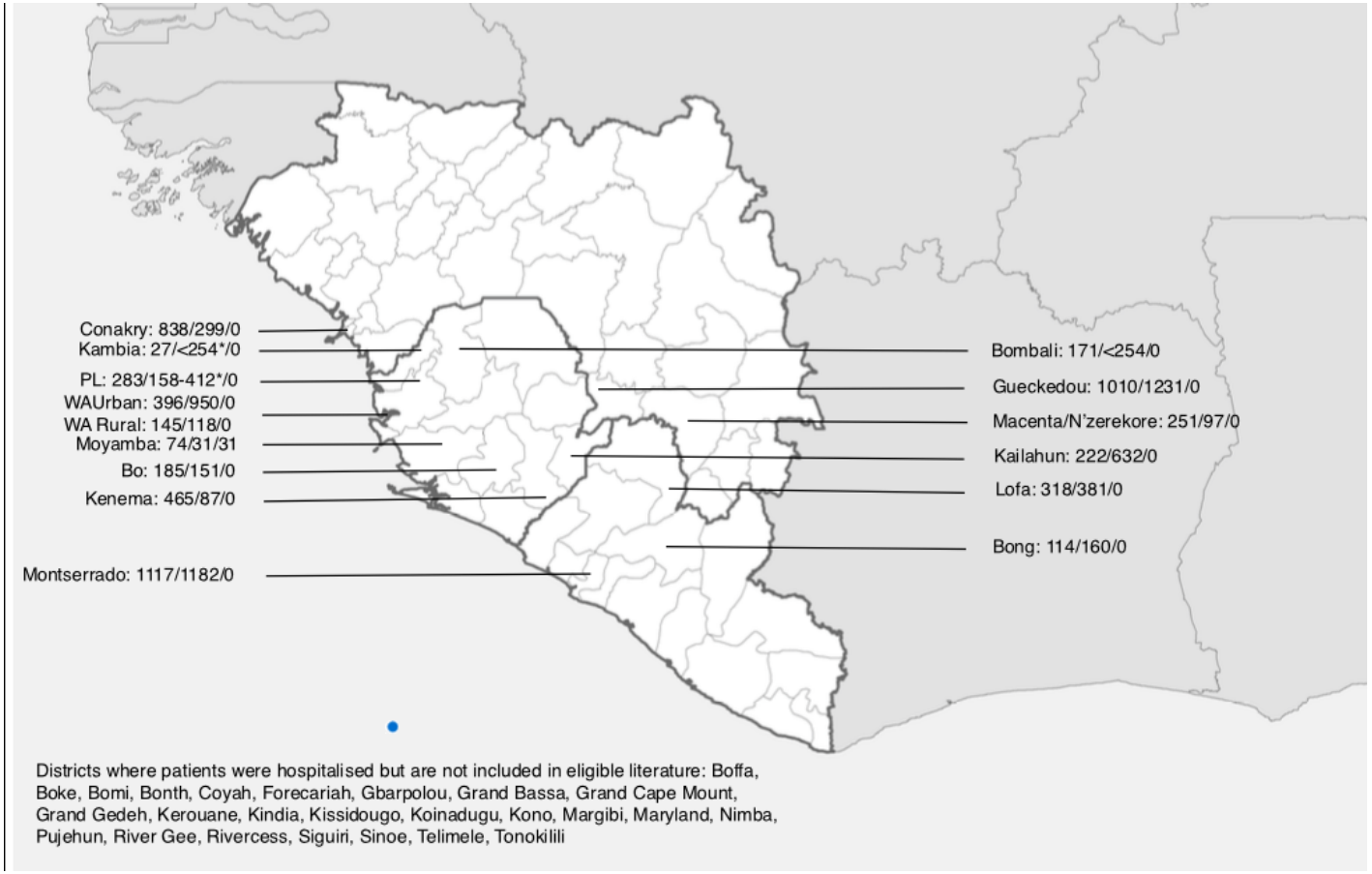


Figure 4.2: Map of most affected countries showing geographical distribution of included ETCs. The numbers represent *the number of hospitalised cases with known outcome[250] / the number of hospitalised cases in the included articles / the number of hospitalised cases with individual level patient data accessible* for each region. Map boundaries shown are administrative 2 boundaries for Sierra Leone and administrative 1 boundaries for Liberia and Guinea, consistent with WHO regional reporting during the outbreak. *Data were across two administrative regions.

Quality of evidence

Table 4.2 details the quality of included evidence. Articles reported the data source and time of data collection in 47%(16/34) of articles, the exact time-frame of the study in 79% (27/34) of articles, and provided specific detail of how cases were laboratory confirmed in 59% (20/34) of articles. Most (53%, 18/34) articles provided description of inclusion and exclusion on the basis of data completeness, and detailed the proportion of cases for whom the clinical outcome was known (97%, 33/34). However, fewer articles (35%, 12/34) detailed the extent of missing data for other variables, or described how this was managed statistically. Article reporting of confounders for estimates of mortality varied by category - 88%(30/34) reported age, 68%(23/34) time from symptom onset to hospitalisation, 59% (20/34) viral load (or Ct), and 9% (3/34) pregnancy.

Study details			Markers of data quality						Markers of clinical utility			
	PubMed ID	Author	Source of clinical data is defined S = source document defined T = time of data collection provided	Temporal indicators for data D = exact days M = closest month	Confirmed case is defined LC = laboratory confirmed A = details of assay	Inclusion and exclusion criteria clearly defined D = detailed C = 'all confirmed' CO = "confirmed and outcome known" CC = "confirmed and complete data"	Records proportion of cases where clinical outcome is unknown	Statement or data^ demonstrating completeness of other clinical data	Average age* of patients provided	States if any patients were pregnant NS = no statement	Average* viral load/Ct provided	Average* symptom onset to admission interval provided
Guinea	26625118	Faye *et al.*	T & S	D	LC	D	Yes	Yes	yes	NS	yes	yes
	26134358	Bordes *et al.*	T	M	No	C	Yes	No	yes	NS	yes	no
	26338789	Cournac *et al.*	T	M	LC	C	Yes	No	yes	NS	yes	yes
	26541219	Cotte *et al.*	No	M	No	C	Yes	No	yes	NS	no	yes*
	26582958	Janvier *et al.*	No	No	No	C	Yes	No	yes	NS	yes	yes
	25391486	Barry *et al.*	No	D	LC + A	C	Yes	No	yes	NS	no	yes
	25770172	Barry *et al.*	T &S	M	LC	C	Yes	No	yes	NS	no	yes
	25992182	Qureshi *et al.*	T &S	D	LC	O	Yes	No	yes	NS	no	yes
	25372658	Bah *et al.*	T &S	D	LC + A	C	Yes	No	yes	NS	yes	yes
	27638946	Kerber *et al.*	T &S	D	LC + A	D	Yes	Yes	yes	NS	yes	no
	28352651	Vernet *et al.*	S	D	LC + A	D	Yes	No	yes	yes	yes	yes
	27928085	Loubet *et al.*	T &S	D	LC	C	Yes	No	yes	NS	no	yes
Sierra Leone	27806732	Ji *et al.*	no	D	LC + A	C	Yes	No	yes	yes	yes	no
	26523640	Li *et al.*	T & S	D	LC + A	CO	Yes	Yes	yes	NS	yes	no
	27317404	Xi *et al.*	no	D	LC + A	C	Yes	No	yes	NS	yes	yes
	26223324	Yan *et al.*	T & S	D	LC + A	CO	Yes	No	yes	NS	yes	yes
	26398207	Zhang *et al.*	T & S	D	LC + A	CO	Yes	Yes	no	NS	no	no
	25995207	Qin *et al.*	no	D	No	CO	Yes	No	yes	NS	no	yes
	26551684	Lanini *et al.*	no	D	No	D	Yes	No	yes	NS	yes	no
	28151955	Hartley *et al.*	T & S	D	LC + A	C	Yes	Yes	no	NS	no	no
	28258817	Waxman *et al.*	T & S	D	LC + A	D	Yes	Yes	yes	NS	no	no
	27268303	Haaskjold *et al.*	no	D	LC + A	C	Yes	No	yes	NS	yes	yes
	27334891	Arranz *et al.*	T & S	M	LC	C	Yes	No	yes	NS	no	yes
	26812579	Crowe *et al.*	S	D	LC + A	D	Yes	Yes	yes	NS	yes	yes
	26551677	de La Vega *et al.*	no	D	LC + A	CC	Yes	Yes	yes	NS	yes	yes
	26002981	Fitzpatrick *et al.*	S	D	LC + A	CO	Yes	No	yes	NS	yes	yes
	25565430	Dallatomasina *et al.*	T & S	D	LC	C	Yes	Yes	yes	NS	no	yes
	28459838	Theocharopoulos *et al.*	T & S	D	LC + A	C	Yes	No	yes	NS	yes	yes
	26271406	Hunt *et al.*	S	D	LC + A	D	Yes	No	yes	NS	yes	yes
	25353969	Schieffelin *et al.*	S	D	LC + A	C	Yes	Yes	no	yes	yes	yes
	25539447	Ansumana *et al.*	no	D	no	C	no	no	no	NS	no	no
Liberia	25845607	Levine *et al.*	T & S	D	LC + A	C	Yes	no	yes	NS	yes	yes
	26735991	Gignoux *et al.*	T & S	D	LC + A	CC	Yes	yes	yes	NS	yes	yes
	27531847	Rosenke *et al.*	No	M	LC + A	CO	Yes	Yes	yes	NS	yes	no

Table 4.2: Quality of evidence in included articles. ^such as denominators for each variable.

Case fatality rate

The CFR in our analysis was 51% (CI 46% to 56%) however heterogeneity in the literature was substantial (I^2 = 92.8%, p <0.01). The forest plot for CFR is provided in figure 4.3. To explore heterogeneity a second meta-analysis was performed stratified by country (supplementary figure C.1), but there was no significant difference in estimates of CFR between countries (p = 0.83).

Symptoms of EVD infection

Symptoms on admission A summary meta-analysis displaying the risk of an EVD patient presenting with a symptom is provided in figure 4.4. In our analysis, the most common presenting symptoms were fever (76%, CI 66-85%), fatigue (71%, CI 64-74%), anorexia (64%, CI 51-76%), and headache (56%, CI 50-62%). The proportion of patients presenting with each symptom of EVD at admission are shown in figures 4.5, 4.6, 4.7 and 4.8. Overall there was high (defined as I^2 value of >75%[285]) heterogeneity for the following symptoms: haemorrhagic manifestations, hiccups, difficulty breathing, conjunctivitis, abdominal pain, myalgia (with or without arthralgia), vomiting, headache, anorexia and fever. There was low to moderate heterogeneity for all other presented symptoms based on I^2 estimate although the Chi square for heterogeneity was still significant for diarrhoea (p<0.01) and lethargy (p=0.01).

A summary meta-analysis displaying the relative risk of death in patients presenting with a symptom (compared to when the symptom is absent) is shown in 4.9. In our analysis, patients presenting with haemorrhage, hiccups, diarrhoea, breathing difficulty, conjunctivitis, and difficulty swallowing were more likely to die. Haemorrhage at presentation was the best predictor of mortality (RR1.8, CI 1.50-2.18). Heterogeneity was low to moderate for all estimates except for confusion

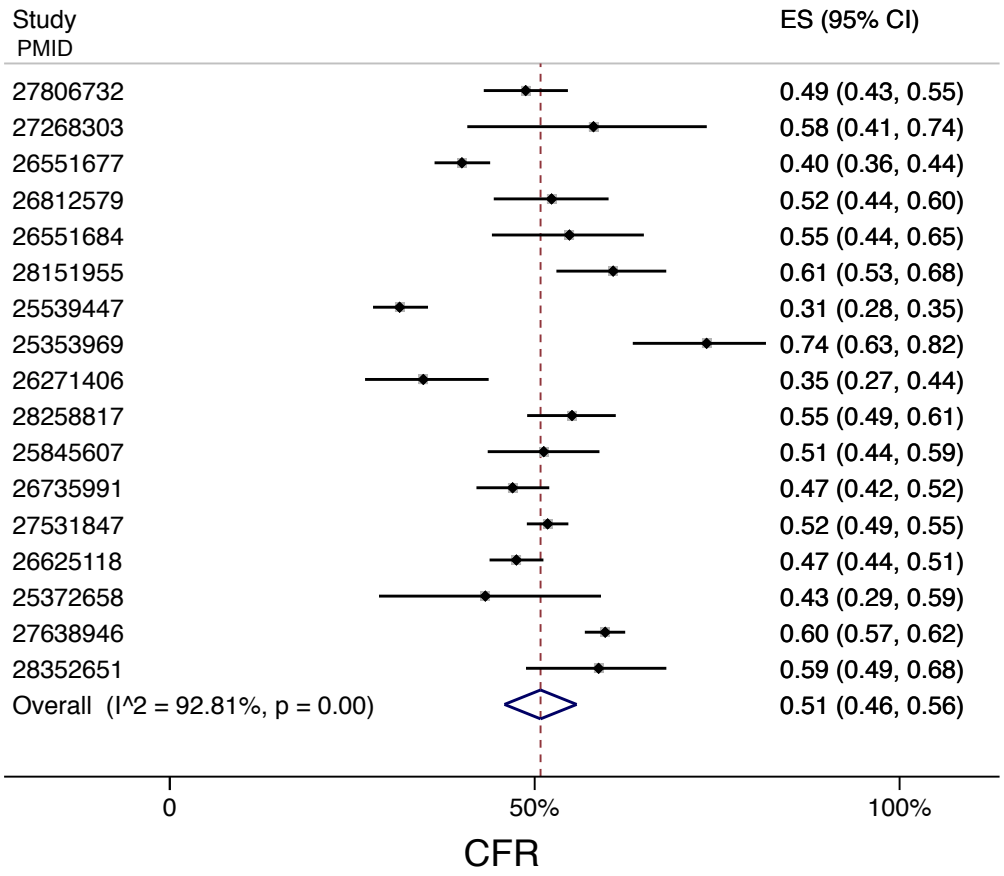


Figure 4.3: Meta-analysis for case fatality rate. Analysis includes the primary data-set only. ES is effect size.

and vomiting, although the Chi square test for heterogeneity was significant for headache (p=0.046). Forest plots for each symptom (where three or more articles presenting data) are available in supplementary figures C.2,C.3, C.4, and C.5.

Symptoms during hospitalisation Systematic review was infeasible for symptoms during hospitalisation. Only one article systematically reported patient symptoms in the period following admission[268], although sporadic reporting of some other symptoms occurred in a further two articles.

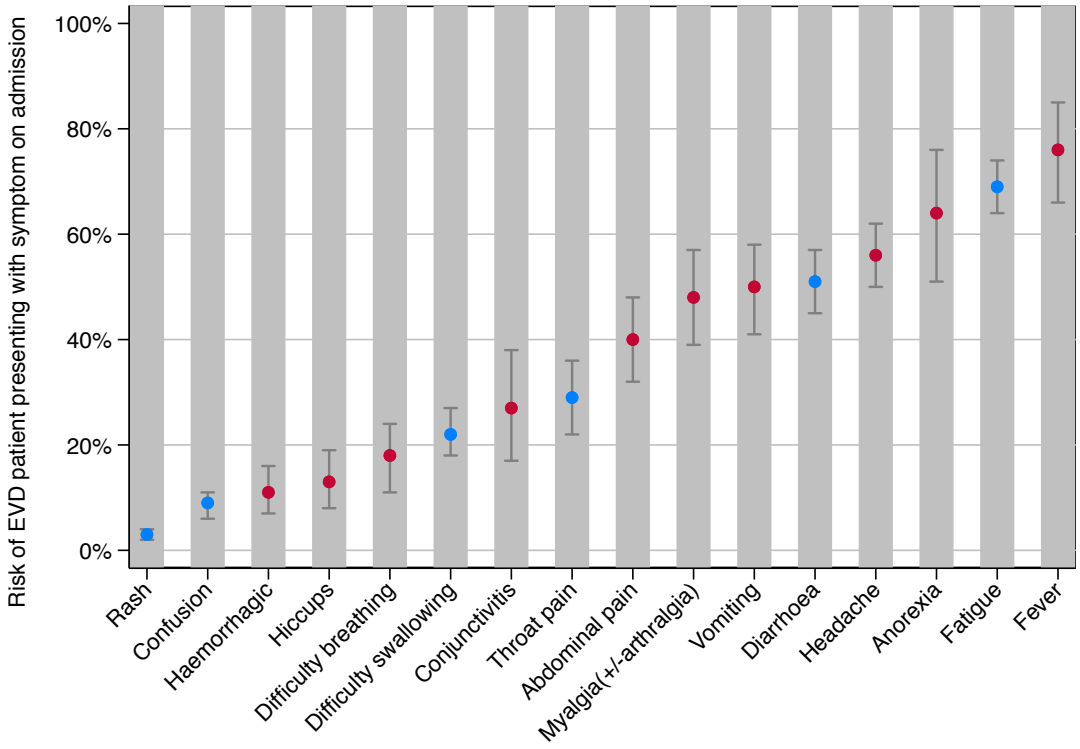


Figure 4.4: Summary meta-analysis displaying proportion of EVD patients presenting with symptom on admission. Blue indicates that there is low or moderate heterogeneity for the pooled estimate, red represents pooled estimates with high heterogeneity. All estimates are shown with their 95% confidence intervals.

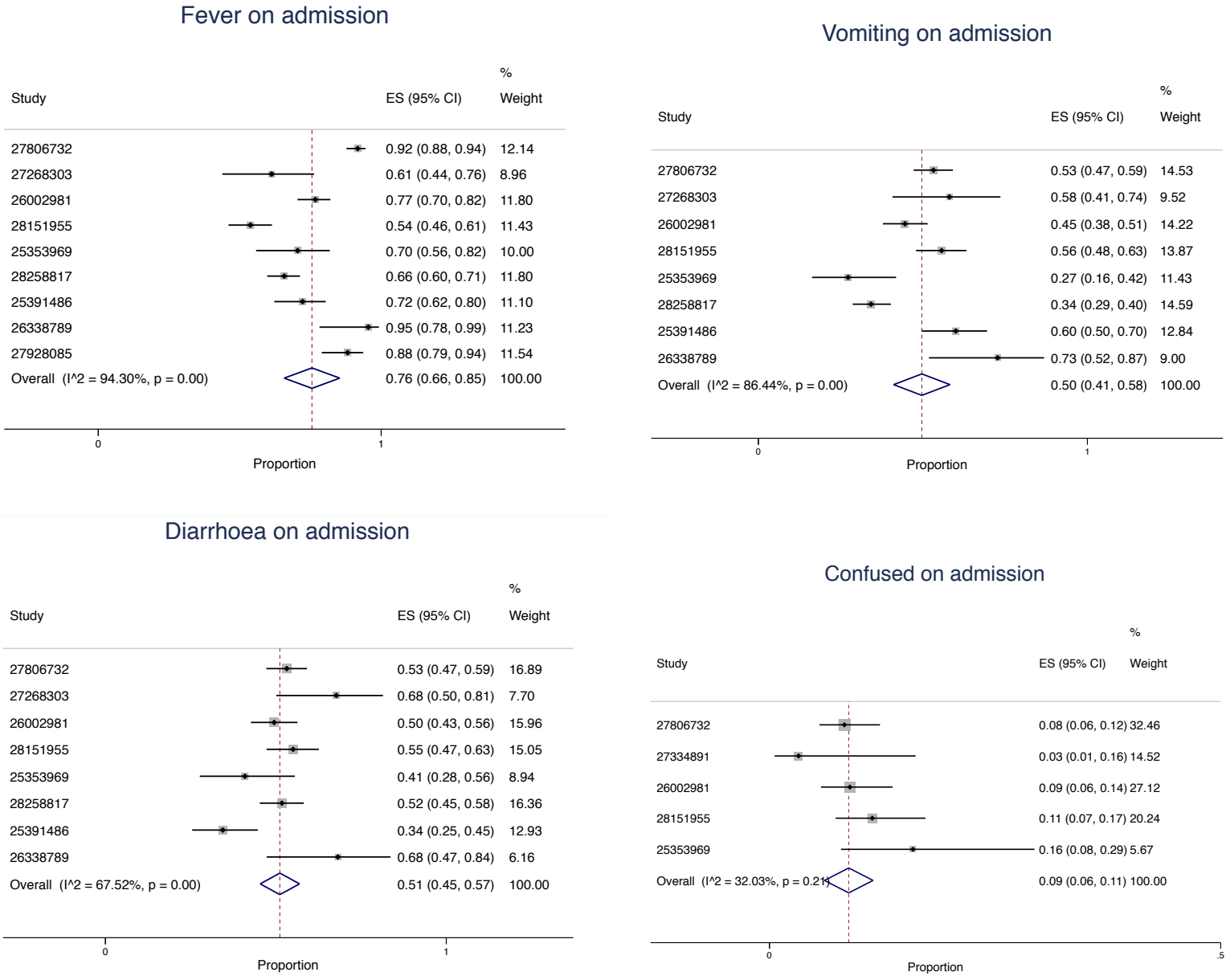
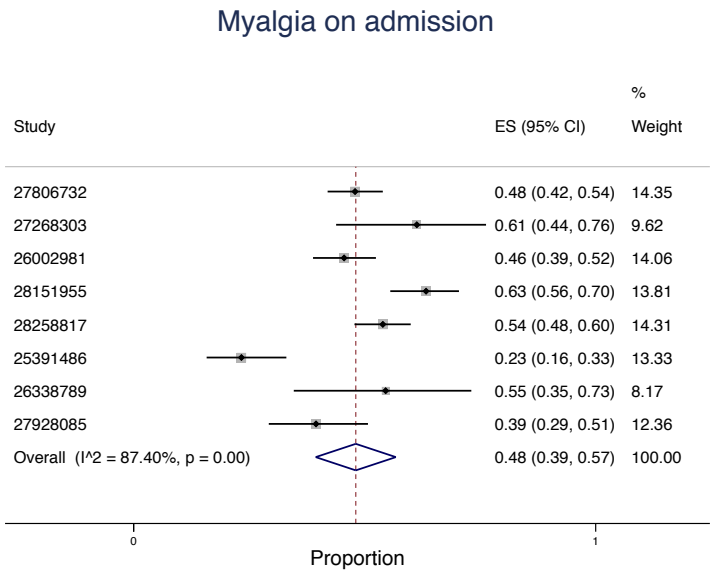
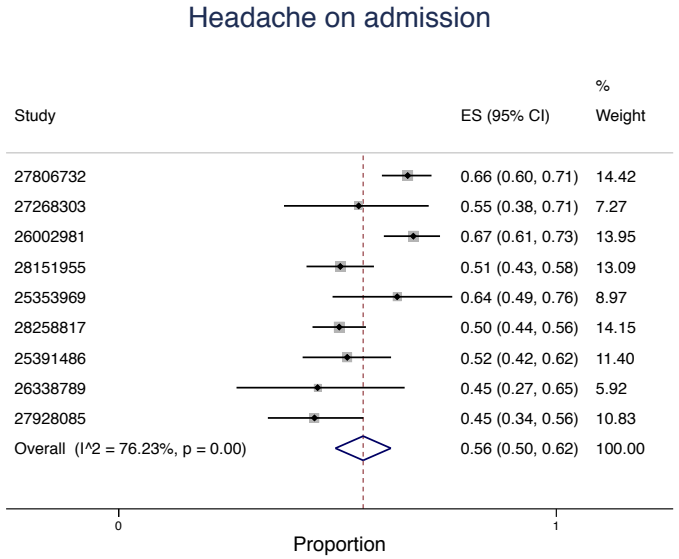
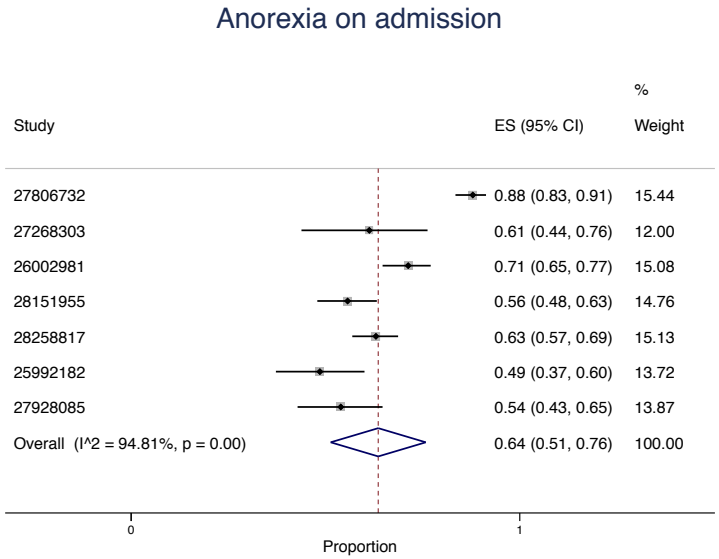


Figure 4.5: Proportion of EVD patients presenting with a symptom - panel A. ES refers to effect size







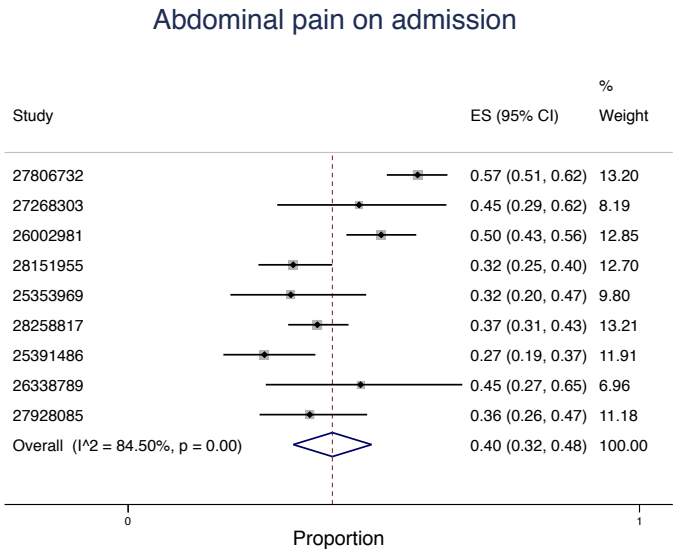


Figure 4.6: Proportion of EVD patients presenting with a symptom - panel B.

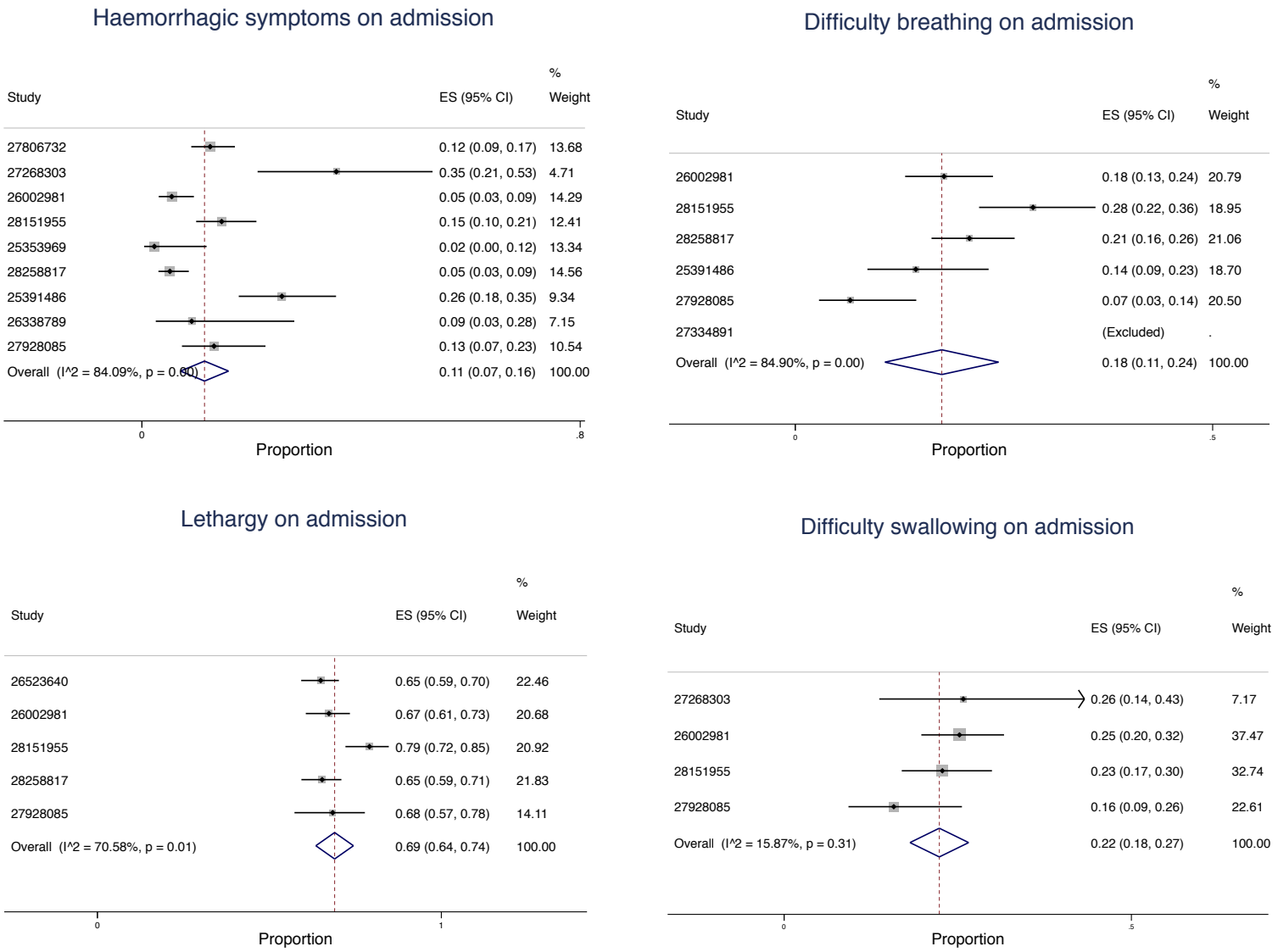


Figure 4.7: Proportion of EVD patients presenting with a symptom - panel C.

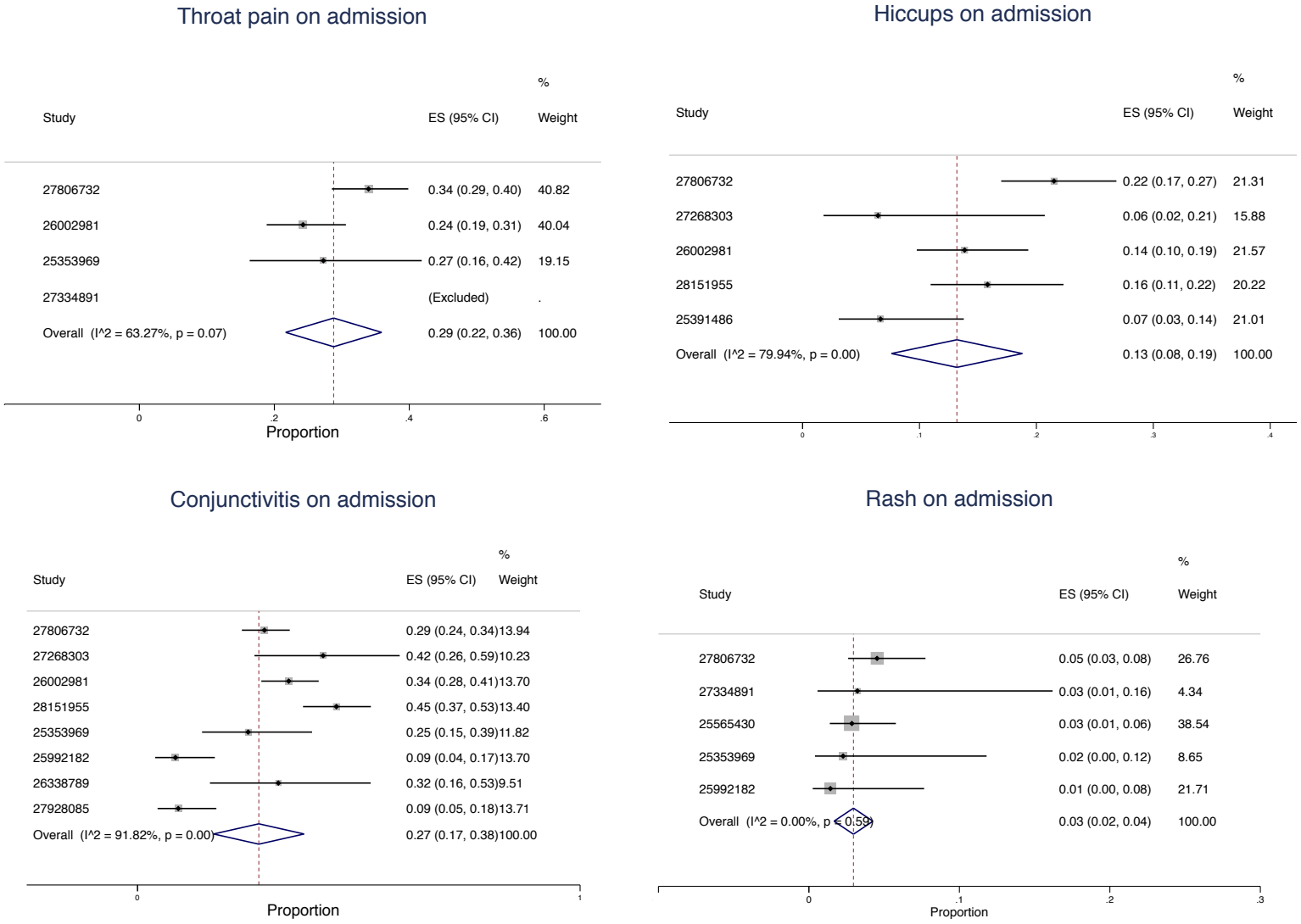


Figure 4.8: Proportion of EVD patients presenting with a symptom - panel D.

Vital signs in patients with EVD infection

Systematic review and meta-analysis of vital sign data was not possible, because only two articles systematically reported data[142, 275] with some detail in one further article[163].

Laboratory findings in patients with EVD infection

Systematic review and meta-analysis of laboratory data was not possible the variables could not be synthesised based on the variability of presentation amongst the few articles reporting data.

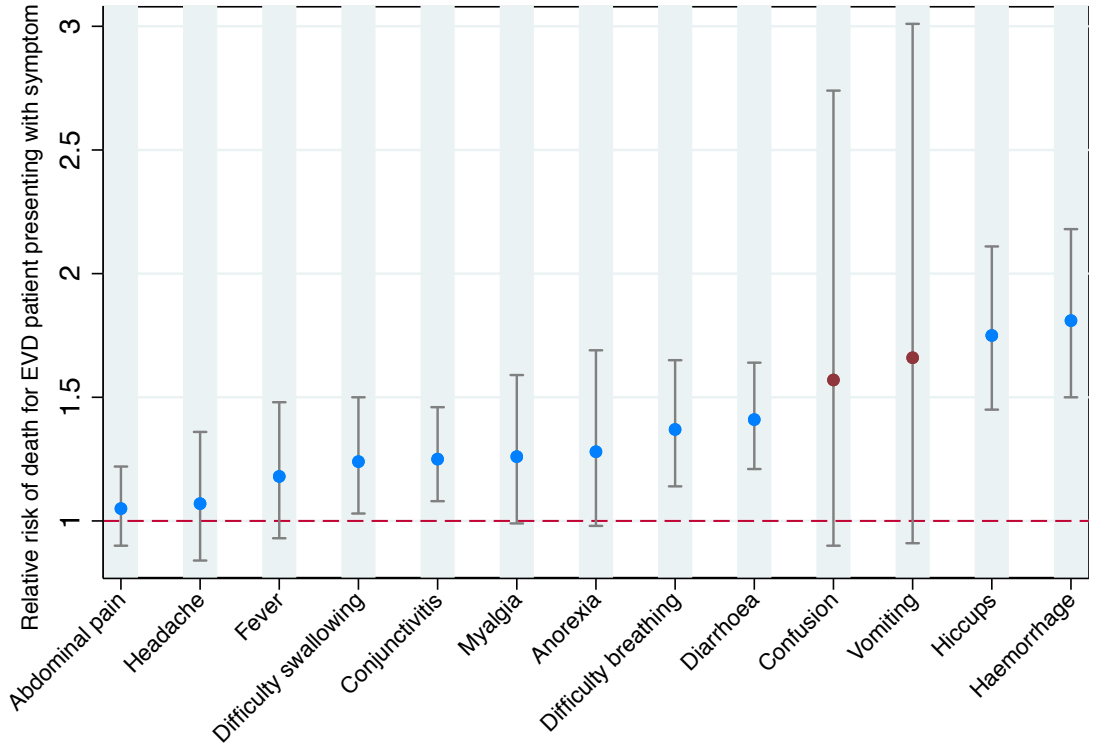


Figure 4.9: Meta-analysis of the relative risk of death in patients presenting with a symptom (compared to with symptom absence). Blue indicates that there is low or moderate heterogeneity for the pooled estimate, red represents pooled estimates with high heterogeneity. All estimates are shown with 95% confidence intervals.

Utility of the literature in informing clinical trials

Data representativeness: The funnel plot in figure 4.10 demonstrates the CFR reported in articles, compared to reference data from WHO (CFR from all hospitalised patients during the outbreak)[250]. Our estimate of CFR was 51%, the WHO estimate was 54.5%.

The graph in figure 4.11 demonstrates a comparison of our meta-analysis estimates for the proportion of patients presenting with a symptom compared with reference data (WHO data for all confirmed and probable patients presenting during the first year of the outbreak).

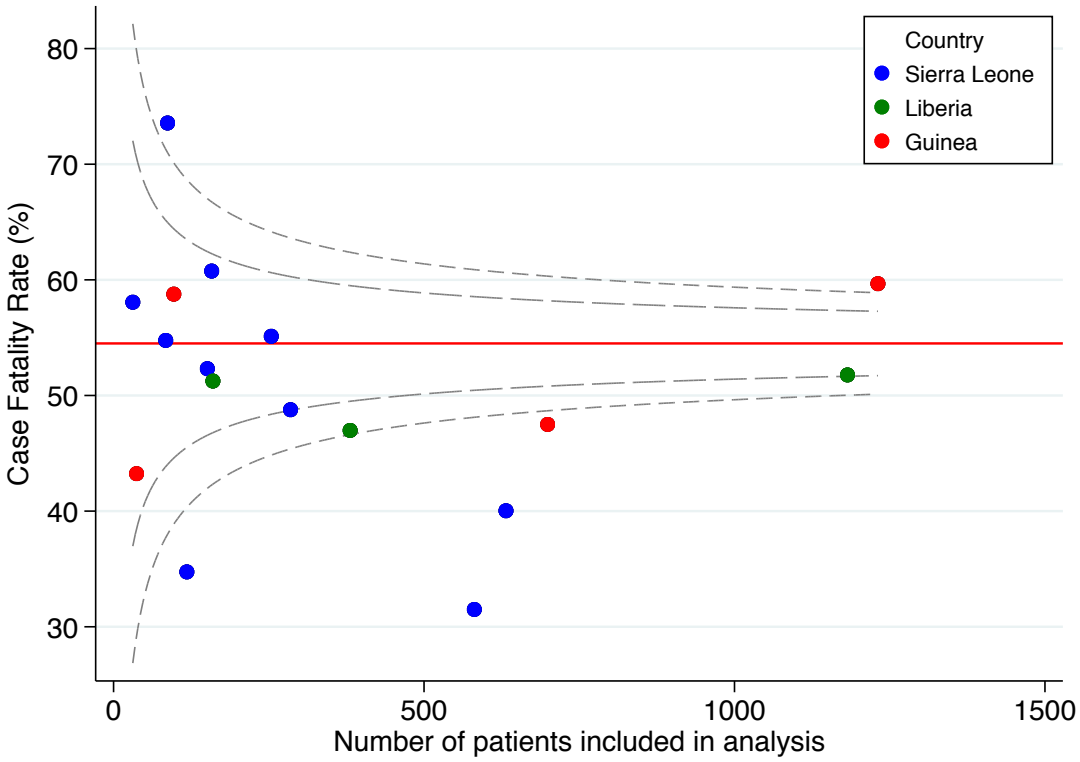


Figure 4.10: Funnel plot of CFR by country. The horizontal red line is a reference value that depicts the overall CFR for hospitalised patients during the outbreak, with 95% and 99.8% CIs

Figure 4.12 demonstrates a comparison of our meta-analysis estimates for the relative risk of death for a patient with presenting with a symptom at the time of admission, in comparison with reference data (as outlined above).

Data transparency: 85% (29/34) of articles made no statement on individual patient level data availability, and data was only available for 2/5 articles where a statement was made. 82% (28/34) of manuscripts were reporting patient data that were also reported in one or more other hospitalised cohorts (although this may be an underestimation, as we did not systematically extract data that did not meet our inclusion criteria. In 1/28 of these articles was there acknowledgement that some or all patients were otherwise published, however it is not possible to determine if authors were aware of other submissions. Details are provided in figure 4.13.

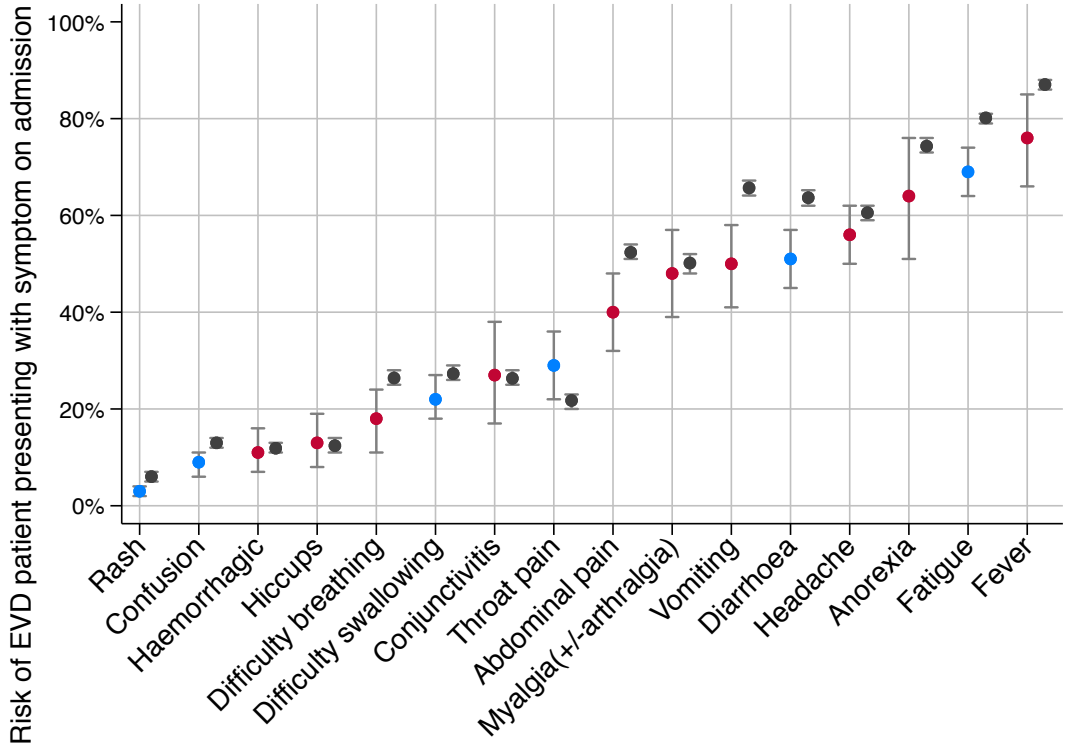


Figure 4.11: Meta-analysis of proportion of EVD patients presenting with a symptom compared with reference data. Summary meta-analysis displaying proportion of EVD patients presenting with symptom on admission. Blue represents a meta-analysis estimate low or moderate heterogeneity for the pooled estimate, red represents a meta-analysis estimate with high heterogeneity. Grey is WHO reference data. All estimates are shown with their 95% confidence intervals.

Data compatibility: Alternative methods of reporting of average age included mean (41%, 14/34 articles), median (47%, 16/34 articles), age categories (44%, 15/34 articles) and averages of sub-groups of data only (5%, 2/34 articles). Two articles did not report patient age. Alternative methods of describing the distribution of data included standard deviation (32%, 11/34), interquartile range (35%, 12/34), range (26%, 9/34), and 95% confidence interval (3%, 1/34). Figure 4.14 provides a representation of age reporting. The age categories selected for stratification by articles are shown in figure 4.15. Of the fifteen manuscripts presenting data according to age categories, the categories were the same for only two articles. Likewise for reporting of viral load, there was a relatively even split between reporting the mean or median (although this varied depending on the subgroup of

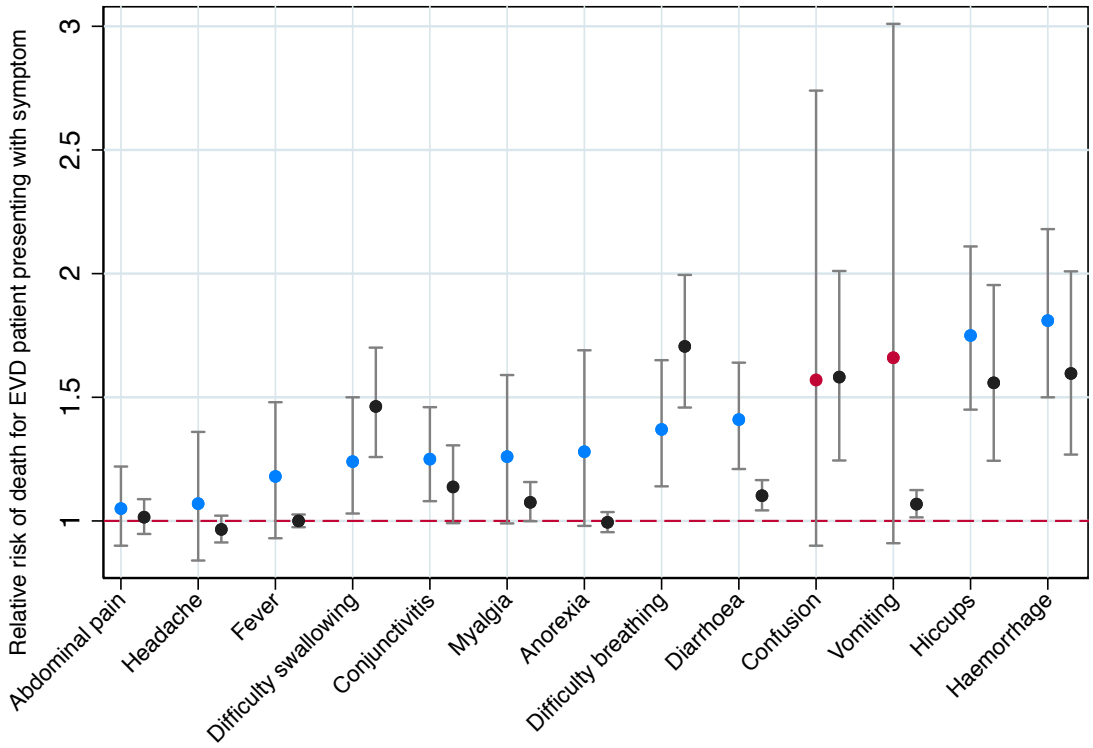


Figure 4.12: Meta-analysis of the relative risk of death in EVD patients presenting with a symptom on admission, compared with symptom absence, and showing comparison reference data. Blue represents a meta-analysis estimate low or moderate heterogeneity for the pooled estimate, red represents a meta-analysis estimate with high heterogeneity. Grey is WHO reference data. All estimates are shown with their 95% confidence intervals.

analysis). Between the fifteen manuscripts that stratified by viral load for analysis, ten different categorisation systems were used. A representation of the reporting of viral load is shown in figure 4.16.

Study details			Markers of transparency	
	PubMed ID	Author	Acccess to individual patient level data NS = no statement	Acknowledges that cohort is otherwise published n/a = only dataset no/c = no, and cites duplicate data without linking
Guinea	26625118	Faye *et al.*	No (1)	no
	26134358	Bordes *et al.*	NS	no
	26338789	Cournac *et al.*	NS	no
	26541219	Cotte *et al.*	NS	no
	26582958	Janvier *et al.*	NS	no
	25391486	Barry *et al.*	NS	no
	25770172	Barry *et al.*	NS	no
	25992182	Qureshi *et al.*	NS	no
	25372658	Bah *et al.*	NS	no
	27638946	Kerber *et al.*	NS	n/a
	28352651	Vernet *et al.*	NS	no
	27928085	Loubet *et al.*	NS	no
Sierra Leone	27806732	Ji *et al.*	NS	no/c
	26523640	Li *et al.*	NS	yes
	27317404	Xi *et al.*	NS	no/c
	26223324	Yan *et al.*	NS	no
	26398207	Zhang *et al.*	No (2)	no
	25995207	Qin *et al.*	NS	no
	26551684	Lanini *et al.*	NS	n/a
	28151955	Hartley *et al.*	No (2)	n/a
	28258817	Waxman *et al.*	NS	no**/c
	27268303	Haaskjold *et al.*	NS	no
	27334891	Arranz *et al.*	Yes (3)	no
	26812579	Crowe *et al.*	NS	no
	26551677	de La Vega *et al.*	NS	no
	26002981	Fitzpatrick *et al.*	NS	no
	25565430	Dallatomasina *et al.*	NS	no
	28459838	Theocharopoulos *et al.*	Yes (4)	no/c
	26271406	Hunt *et al.*	NS	n/a
	25353969	Schieffelin *et al.*	NS	n/a
	25539447	Ansumana *et al.*	NS	n/a
Liberia	25845607	Levine *et al.*	NS	no**
	26735991	Gignoux *et al.*	NS	n/a
	27531847	Rosenke *et al.*	NS	n/a

Figure 4.13: Indicators of data transparency for included articles. **Cohort is duplicated in another dataset that is not included in this review, as it did not meet eligibility.

PMID	n	Mean	SD	Med	IQR1	IQR3	Min	Max	Cat	Other
25353969	98	.	.	.	.	.	.	.	.	.
25372658	37	.	.	38	28	46	19	61	.	.
25391486	90	34.0	14	.	.	.	.	.	Y	.
25539447	n/a	.	.	.	.	.	.	.	.	.
25565430	489	.	.	28	17	40	0.3	80		.
25770172	89	.	.	32	25	44	.	.	.	.
25845607	160	.	.	33	.	.	.	.		(1)
25992182	69	34.0	14.1	.	.	.	.	.	Y	.
25995207	61	26.4	5.81	28	.	.	1.2	67	Y	.
26002981	525	.	.	27	16	40	.	.	.	.
26134358	22	33.0	9	.	.	.	.	.	.	.
26223324	108	27.4	13.6	.	.	.	.	.	Y	.
26271406	118	25.9	14.7	.	.	.	.	.	Y	.
26338789	22	32.9	9.2	.	.	.	.	.	.	.
26398207	63	.	.	.	.	.	.	.	Y	.
26523640	288	.	.	28	17	38	.	.	Y	.
26541219	14	.	.	31	27	33	.	.	.	.
26551677	632	26.9	.	25	.	.	0.3	75	Y	.
26551684	84	.	.	.	.	.	.	.	Y	.
26582958	14	30.0	7	.	.	.	.	.	.	.
26625118	699	31.0	.	.	20	42	.	.	.	.
26735991	n/a	.	.	.	.	.	.	.	Y	.
26812579	151	.	.	.	.	.	.	.	.	(2)
27268303	31	.	.	30	.	.	0.25	85	.	.
27317404	139	30.1	17.1	29	.	.	0.5	75	.	.
27334891	31	.	.	30	17	40	.	.	.	.
27531847	1110	29.8	15.6	.	.	.	0.01	83	Y	.
27638946	1225	.	.	30	19	45	.	.	.	.
27806732	285	29.2	16.4	28	19	38	0.08	80	Y	
27928085	76	.	.	28	18	48	.	.	Y	.
28151955	158	.	.	.	.	.	.	.	.	.
28258817	254	.	.	.	.	.	.	.	.	(2)
28352651	97	30.1	.	.	.	.	1	68	Y	.
28459838	248	.	.	28	17	38	.	.	Y	.

Figure 4.14: Mapping of reporting of patient age in included articles. N is the number of patients with age recorded; SD is standard deviation; Med is median; IQR1 is the first interquartile range; IQR3 is the third interquartile range; (1) reports the 95% confidence interval as the distribution; (2) reports ages of sub-groups only.

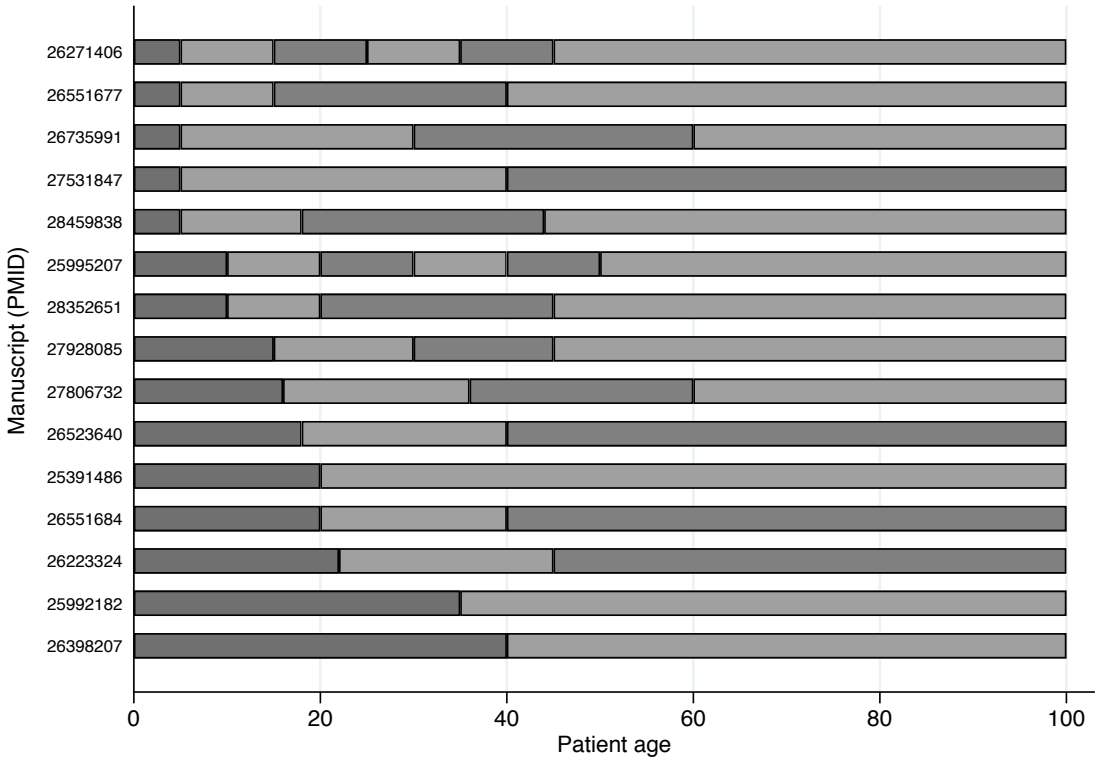


Figure 4.15: Age categories selected for stratification of results or analysis in included articles

rticle	Patients who survived									Patients who died									All patients								Other	
MID	n	mean	SD	Med	IQR1	IQR3	Min	Max	95%CI	n	mean	SD	Med	IQR1	IQR3	Min	Max	95%CI	n	mean	SD	Med	IQR1	IQR3	Min	Max	Unit	Categorisation of viral load
7806732	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	log copies/ml	VL = <10^6, >10^6
6523640	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	288	.	.	6.68	5.3	7.75	.	.	log copies/ml	VL = <10^6, >10^6
7317404	63	.	.	.	.	.	.	.	.	76	.	.	.	.	.	.	.	.	139	.	.	.	.	.	.	.	copies/ml	VL = <10^5, 10^5-10^7, >10^7
6223324	34	25.2	4.1	.	.	.	.	.	.	51	28.7	5.7	.	.	.	.	.	.	85	26.3	4.4	.	.	.	.	.	Ct	.
6398207	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	VL = <10^6, >10^6
5995207	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
7268303	13	.	.	26.5	.	.	22	36.5	.	17	.	.	20.5	.	.	15	23	.	30	.	.	22	.	.	15	36.5	Ct	.
7334891	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6551677	379	6.18	.	.	.	.	.	.	6.0-6.3	253	4.02	.	.	.	.	.	.	3.9-4.2	632	.	.	.	.	.	.	.	log GEQ/mL	Ct = <20, 20-30, >30
6002981	255	.	.	31	25	34	.	.	.	270	.	.	22	20	25	.	.	.	346	.	.	.	.	.	.	.	Ct	Ct = <25, ≥25
5565430	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
8459838	.	.	.	.	.	.	.	.	.	110	.	.	.	.	.	.	.	.	249	.	.	26	20	32	14	38	Ct	Ct = <22, ≥ 22
6812579	72	27.9	.	.	.	.	.	.	.	72	20.5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Ct	Ct= <24, ≥ 24
6551684	38	7.11	.	.	.	.	.	.	.	46	8.36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	log copies/ml	.
8151955	62	24.9	.	.	.	.	.	.	.	96	20.6	.	.	.	.	.	.	.	144	22	.	.	.	.	13.5	37.9	Ct	Ct= <20, ≥ 20
5539447	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5353969	.	.	.	.	.	.	.	.	.	45	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	copies/ml	VL = <10^5, 10^5-10^7, >10^7
6271406	73	23.4	4.3	.	.	.	.	.	.	39	18.3	2.7	.	.	.	.	.	.	112	21.4	4.5	.	.	.	.	.	Ct	Ct= <20, ≥ 20
8258817	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5845607	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6735991	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	381	.	.	19.4	17.1	22.8	.	.	Ct	.
7531847	570	28.4	4.3	.	.	.	.	.	.	612	25.9	3.8	.	.	.	.	.	.	1182	27	4.7	.	.	.	.	.	Ct	Ct = < 25, 25 - <30, 30 to <37
6625118	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	548	.	.	.	.	.	.	.	log copies/ml	log VL = <104.4, 104.4-105.2, ≥ 105.2
5391486	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5770172	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
5992182	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6541219	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6582958	.	.	.	.	.	.	.	.	.	5	.	.	19.5	.	.	.	.	.	14	.	.	.	.	.	.	.	Ct	Ct = <20, 20-30, >30
6134358	16	23	3.1	.	.	.	.	.	.	6	17.7	3.8	.	.	.	.	.	.	22	21.6	3.9	.	.	.	.	.	Ct	.
6338789	16	23	3.1	.	.	.	.	.	.	6	17.7	3.8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	Ct	.
5372658	21	8207	17189	1079	148	5059	.	.	.	16	68361	111340	1915	141	12998	.	.	.	.	.	.	.	.	.	.	.	copies/ml	VL <100,000, >100,000
7638946	479	.	.	23.2	20.4	26.5	.	.	.	719	.	.	18.1	16.2	20.8	.	.	.	.	.	.	.	.	.	.	.	Ct	.
7928085	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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Figure 4.16: Mapping of reporting ofviral load in included articles. N is the number of patients with age recorded; SD is standard deviation; Med is median; IQR1 is the first interquartile range; IQR3 is the third interquartile range; 95%CI is the 95% confidence interval.

4.2.4 Discussion

This work is the first systematic review to describe the clinical presentation of hospitalised patients, following the EVD epidemic in West Africa.

Findings of the systematic review and meta-analysis

While the published literature report relatively comprehensive data for patient symptoms on admission (and their prognostic utility), these data have been aggregated for most patients in the epidemic by WHO based on case reporting forms, and so estimates from the published literature do not provide a significant new insight. We examined the published literature to describe the evolution of patient symptoms and clinical biomarkers and identify clinical signs, vital signs and laboratory tests that identify patients at increased risk of death because this information is not available in the existing epidemiological datasets. However, we found there is inadequate information available in the literature to perform such analyses. In some ETCs, the lack of data reflects the clinical reality of working in a humanitarian setting, where at times, careful clinical observation was abandoned for pragmatic reasons. However, in many other ETCs, especially those reporting data for later during the outbreak, this data was expected to exist to some extent.

In general, meta-analysis indicated substantial heterogeneity between studies for analysis of the proportion of patients presenting with a symptom, and for analysis of CFR, although the reliability of statistical measures for heterogeneity is impacted by lack of data. We attempted to limit clinical diversity by only including articles that enrolled all patients in a sequential manner presenting for treatment. However, populations may have differed between studies due to ETCs using different admission procedures, ranging from acceptance of all patients presenting in the community, through to admission of patients with a confirmed ebolavirus PCR

result from a holding facility - which likely impacted the stage of patient disease at the time of admission. These admission patterns may have also caused a survival bias, where the sickest patients died before admission. Moreover, selection bias may have occurred because early during the outbreak some overwhelmed ETCs turned away patients and in other cases fears and rumours regarding ETCs may have altered admission patterns. Planned sub-group analysis by admission route was not possible due poor consistency of reporting. We elected not to perform sensitivity analysis and exclude outlying articles because there was no clinical rationale for doing so. Sub-group analysis for CFR stratified by time may be valuable, but is not possible because the distribution of date of admissions within each study is not known. Meta-regression was planned using co-variates of outcome (time between symptom onset and admission, patient age, pregnancy status, and viral load). However, progress has been slow in performing this analysis because inconsistent reporting of these variables has impaired data synthesis, as reported for age and viral load in the results section.

The reality is that even with meta-regression, significant heterogeneity in the data may remain. Under this scenario, which values should be used for decision making becomes unclear, and it may in-fact be that a summary effect measurement that incorporates heterogeneity through use of a random-effects model remains the most useful estimate in the absence of additional data, unless there is a clear reason that the estimates reported in one or more articles provides the most representative or relevant data.

Findings of assessment of utility for clinical trial design

Representativeness: By comparing to WHO estimates, the limitations of decision making based on the literature becomes evident. For example, our estimates of the proportion of patients presenting with vomiting (50%, CI 41-58%) and

diarrhoea (51%, CI 45-57%) underestimate WHO estimates (66%, CI 64-67%; and 64%, CI 62-65% respectively) and our comparisons for the relative risk of death when presenting with each of these symptoms overestimates when compared to WHO (diarrhoea: 1.4(CI 1.2-1.6) compared with 1.1(1.0-1.2); vomiting: 1.7(CI 0.9-3.0) compared with 1.1(CI 1.0-1.1)). Whether gastrointestinal manifestations are a common manifestation of EVD amongst all patients, or is relatively rarer but more discriminative of patients at risk of death is unclear, limiting the utility of this information for clinical triage. For a clinical trial, this uncertainty may create difficulty in ascertaining whether gastrointestinal symptoms in patients receiving treatment are due to underlying disease or represent adverse drug effects. The meta-analysis estimate of CFR is lower than for the WHO estimate in all hospitalised cases with known outcomes. This likely represents publication bias, where ETCs with high survival rates are more inclined to publish their findings. It may also be that articles represent patients treated later during the epidemic (when CFR improved) because more detailed clinical data could be collected when ETCs were less overwhelmed. While use of an external comparison for assessment of representativeness of the data is perhaps unusual in this context, given the substantive heterogeneity in the literature, identification of whether the articles included approximate the findings in the underlying population is otherwise unclear. The WHO data are not without limitations, and in particular for symptom data it includes data from non-hospitalised patients and probable cases.

Transparency: The lack of individual patient level data (IPD) availability represents a significant impediment to harmonisation of findings, especially given lack of consistency in reporting of summary measures. That some articles presented overlapping cohorts of patients with others only became evident following thorough mapping by an author experienced with the outbreak, and only one article declared prior publication. While authors of some manuscripts will not be aware of duplicate publication, and additional publications may not be planned until after the first

manuscript is submitted, the extent to which this occurred is concerning. The consequence is that those relying on the literature as an evidence base may see consensus in the literature where it does not exist. This indeed has occurred within the subset of literature included in this review, where some articles report agreement with the literature, while citing details of an overlapping patient cohort. While this is undoubtedly not best practice, a solution is difficult to foresee - for example, requesting additional statements of originality for all articles being published may be irrelevant for authors whom are not presenting duplicate data, and may not change practices.

Compatibility: The poorly standardized reporting of clinical measures found for key co-variates of outcome in this review, mean that comparison between studies is difficult for those reliant on the literature to inform evidence. It also impairs meta-analysis and specifically meta-regression. In comparison, there was near universal consistency in the reporting of symptoms on admission in this systematic review, no doubt facilitated by use of standardized case reporting forms. Increasingly, recognising the need to synthesise clinical data, standardised protocols are being produced for reporting the natural history of EEIDs, such as the WHO protocol for Zika virus infection[286] and the findings of this work support their prioritisation as an outbreak response activity, and their wider promotion and adoption.

Next steps

We have collected information on prognostic indicators identified by multivariate analysis in the included articles, but have not yet completed de-duplication. Confounders for patient outcomes will continue to be synthesised, where possible, in order to undertake meta-regression. We are interested in mapping the variables used in prognostic models in the literature to examine the consistency between

models, and consequently their utility for clinicians.

Implications for practice

These findings demonstrate that anonymised IPD level aggregated databases should be urgently developed for EVD, and other EEIDs. The aggregated epidemiological datasets curated by WHO are clearly valuable - confidence in estimates of the epidemiology of EVD, including the incubation period, series interval, and overall CFR have improved markedly and have been instrumental in directing public health control of EVD. An evidence base of this caliber is just as important for informing the clinical care of patients (including through clinical research), but does not exist. This is feasible given that sufficient data for publication of a clinical dataset exists for at least 6168 patients. Standards (such as the CDISC Ebola therapeutic standards) exist to facilitate synthesis of Ebola research data and could provide the foundation for observational data aggregation. Importantly, some clinical organisations (such as MSF) are already collaborating on these data alignment initiatives for EVD (Laura Merson, personal communication). On this basis, the Infectious Diseases Data Observatory has planned an EVD data sharing platform, with the draft research agenda including retrospective analysis of clinical data to describe the evolution of patient symptoms and clinical biomarkers, identify host, pathogen and healthcare factors that influence prognosis, and prioritise monitoring of clinical signs, vital signs and laboratory tests that identify patients at increased risk of death[287]. This approach has been used previously for aggregation of clinical trial data (wwarn.org) for malaria, and in this instance has allowed IPD pooled meta-analysis to inform treatment guidelines with greater statistical power than would otherwise be possible[288]. Centralised data repositories have also been suggested for clinical trials or other tropical diseases including visceral leishmaniasis[289], and more broadly for clinical data on rare diseases[117], and for emerging and resistant pathogens[290] where fractured data reporting also occurs. Criticisms of

IPD repositories - including that patient anonymity may be compromised[291, 292] and that they may exacerbate inequities in research when primary data is collected in low income countries [288] can be overcome by use of curated repositories that have clear requirements for access[291, 292].

Limitations

The validity of our comparison to WHO data may be questioned. We do not wish for our use of this data to be misconstrued as comparison to 'gold standard' data, but rather that it constitutes the best available comparison to approximate the representativeness of the data within the systematic review. There are a plethora of ways in which to present clinical data, however, in order to produce functional data extraction forms, we limited analysis to data available in standardised formats (mean, median, categorical data etc.). For this reason we did not extract all clinically meaningful data from the literature. Articles were diligently searched to avoid presentation of duplicate data, but the lack of transparency of data sources means that accidental inclusion of some duplicate data cannot be excluded. We limited language to English and French. We were only able to report on articles published before the end of May 2017, but recognise that case reports will continue to accrue in the medical literature for EVD.

4.2.5 Conclusions

This work summarises the clinical manifestations at presentation of 6168 patients hospitalised for EVD during the west Africa outbreak. However, meta-analysis was challenging due to lack of standardisation and transparency in reporting of clinical data. Given the volume of clinical data that does exist, profound gains in our knowledge regarding the natural history of EVD and factors influencing

patient mortality are possible if the utility of this data is better harnessed. The most appropriate method of achieving this is through a commitment to data-sharing and harmonization initiatives.

4.3 Contribution of the chapter

There was a missed opportunity to better understand the natural history of EVD. The clear need for more actionable observational data in this instance, should inform improved data collection and synthesis practices aimed at facilitating clinical research in future outbreaks (objective 3 of this thesis).

The WHO R& D blueprint for action to prevent epidemics highlights the major pathogens that pose a severe public health risk and for which there are insufficient countermeasures[42]. The focus of this blueprint is to identify gaps, prioritise investment, and advance pipelines for therapeutics (along with vaccines and diagnostics). Importantly, in order to improve the available treatments for these priority diseases, phase II & III clinical trials must be performed during outbreaks in order to assess safety and efficacy of INDs. However, I suggest on the basis of the findings of this chapter that the WHO blueprint risks failing to meet its objectives if not complemented by a substantive effort to obtain robust data on the clinical phenotype and natural history of these diseases.

The success of a clinical trial depends upon precise understanding of the clinical disease in the target population. In table 4.3, I describe the core minimal data needs to design a phase II or III clinical therapeutic trial - data that should be assembled for all WHO priority pathogens.

Beyond the findings of this chapter, there are clear indications that the observational data for other EEIDs is insufficient for the purpose of clinical trial design. For

Criterion	Explanation
Size of previous outbreaks	Serves as rudimentary estimate of the feasibility of sample size requirements. Clinical trial groups should prioritise the most efficient trial designs when a low number of cases is expected.
Temporal and geographical profile of previous outbreaks	This is required for logistical planning, to ensure that local teams are sufficiently trained in research practices (such as good clinical practice) and trial specific equipment are available.
There is an agreed case definition	Clinical characteristics of the disease are used to define enrolment criteria.
Factors associated with increased disease severity are known.	Stratification (or other statistical adjustment) on the basis of severity is often required when interpreting the clinical trial outcome.
The type and rate of clinical outcomes are well described	Clinical outcomes will function as a trial outcome measures. Understanding the natural course of illness will also help differentiate disease course from adverse events from treatment.
There is confidence in estimates of clinical outcomes	Heterogeneity in patient outcomes between or within outbreaks creates uncertainty for power calculations and will affect selection of a statistical design for a trial. Spurious heterogeneity may occur due to random error in small cohorts, or represent ascertainment, lead-time, measurement, or follow up bias. Real heterogeneity can occur due to improvements in care over an outbreak, pathogen evolution, or changes in host susceptibility and vulnerability but should be adjusted for.
Known or suspected co-variates of outcome are well described	Highlights possible confounders that will alter outcome independently of treatment and that will require adjustment for if unequally distributed between treatment and control arms.
The mean time from onset of symptoms to outcome is known	Allows for an estimation of the feasibility and logistics of medical intervention.
There are agreed standards of care for patient treatment	Determines if there is standardized supportive therapy to be adopted in all arms of a trial. This is especially important for multi-centre research
The performance characteristics of the favoured diagnostic method are known	Determines whether a trial will be performed on an ITT basis or following laboratory confirmation.
Mean time for laboratory diagnosis is known	Determines whether a trial will be performed on an ITT basis or following laboratory confirmation.

Table 4.3: Suggested elements for a core minimal dataset of observational based data for high priority pathogens

example, during the influenza A(H1N1)pdm09 pandemic case fatality rate estimates varied widely from 0 to 13,500 per 100,000 laboratory confirmed infections, with a heterogeneity of 99.97% (using I^2 estimate)[293]. A therapeutic trial designed with patient survival as a primary outcome measure could substantially misjudge the required sample size depending on the estimate selected amongst these. Therapeutic trials for the prevention of congenital Zika syndrome will be hindered by the absence of consistently used criteria to define congenital malformations and the subsequent paucity of population level estimates (appendix 2). Not only will this data insufficiency make defining study success challenging, but also creates uncertainty regarding whether any malformations observed in a trial participants are expected given underlying prevalence in the population, or rather represent teratogenic adverse events from drug administration. For MERS-CoV, a lack of biological sampling, including virologic and immunologic assays means that factors associated with more severe disease and virus clearance, a common outcome measure, are not well understood [229].

While these examples are illustrative, there has been no systematic examination of the completeness of the core data needed to conduct trials for the high priority pathogens. For each priority pathogen, the existing data for all items in table 4.3 should be evaluated. In the first instance, it may be sufficient to fill in the information for each pathogen using a traffic light system (where green indicates high quality data available, orange indicates some data, and red indicates little to no data available) based on Delphi survey of field experts. More detailed examination of the literature can then first be undertaken for red criteria. Where missing information is identified, these criteria can be prioritised for inclusion in standardized clinical characterization protocols for emerging diseases designed by research consortia (such as those produced by ISARIC), and should be incorporated into routinely used case reporting forms wherever feasible.

Compared to expensive and lengthy therapeutic development pipelines, advance-

ment of observational data collection protocols represents an efficient and cost effective method of progress that can be achieved through existing WHO mechanisms (such as guideline development group pathways). The end result is streamlined observational data collection early during an outbreak that is relevant for, and focused toward enabling high quality data generation.

5

Improving clinical characterisation for outbreak surveillance, investigation and response systems

Contents

5.1 Preface to the chapter

Chapter 4 discussed the importance of accurate clinical characterisation during an outbreak and demonstrated weaknesses in the characterisation of EVD. The aim of the work described in this chapter is to evaluate and made recommendations to

improve clinical assessment in a setting at high risk for communicable diseases - refugee camps. The work meets objective three of the thesis: to develop methods and tools that facilitate implementation of patient centred research in future outbreaks.

5.1.1 Context

Provoked by political and economic instability and war in regions of Africa and the Middle East, a global forced migration of unprecedented magnitude is occuring. The United Nations High Commissioner for Refugees (UNHCR) reports that by 2015 there were over 65 million people in need of protection due to forced displacement resulting from conflict or persecution[294] – a number that for the first time eclipsed the movement of refugees following World War II. At least 12 million of these people are living in refugee camps in foreign countries[294]. Most of these refugees originate from Syria, Afghanistan and Somalia[295]. While the majority of refugees are hosted in neighbouring countries, since 2014 there has been a sudden increase in migration to Europe[296].

Greece is the location of the work in this chapter, and amongst countries in the European Union (EU), Greece has received the highest number of refugees to date, including 850,000 of the 1,000,000 arrivals into Europe by sea in 2015[296, 297].

Introduction of a new agreement between Turkey and the EU in March 2016 changed the migration pattern, reducing the number of arrivals to Greece[297]. However, this policy has also meant that many refugees who were intending to transit rapidly through the country to preferred countries of resettlement in west Europe are no longer able to cross the border and remain *in-situ*[297]. The consequence is that conditions are deteriorating in some refugee camps[298]. The most recent data available from UNHCR (correct to August 1, 2017) indicates there were 11,535 live arrivals, and nine deaths incoming to Greece during January to July 2017[299];

most were women and children. The most common country of origin of sea arrivals were Syria, Iraq, Democratic Republic of Congo, and Afghanistan.

In Greece, there are 40 official refugee camps operating in various locations around the country (correct to August 1, 2017). Primary healthcare on these sites is managed by a variety of national and international NGOs, the military and the Hellenic Centre for Disease Prevention and Control (KEELPNO). As of July 2017, there were 15 different organisations providing care. The clinical expertise offered, equipment and medications available, and operating hours of each of these clinics varies. A refugee who requires emergency care or hospitalisation is able to directly access the national health service, for free, regardless of their legal status. There is no formal system of information exchange between clinics in refugee camps and hospitals for patient follow up. The number of refugee camps, and the organisations providing care can change unpredictably. Outbreaks in refugee camps in Greece have included Hepatitis A, Shigellosis, and Varicella-Zoster virus[300](personal communication, Angeliki Lambrou, KEELPNO).

Outbreak surveillance, investigation and response in refugee camps in Europe

Given the escalating and rapidly changing landscape of the refugee crisis in Europe, there is debate regarding the most appropriate outbreak surveillance, investigation and response (OSIR) processes to protect refugees from communicable diseases while in camps[301]. The surveillance methods endorsed by the European Centre for Disease Prevention and Control (ECDC) are screening on arrival complemented by syndromic surveillance[302]. These two systems have been adopted in Greece and work alongside a mandatory notification system for reporting under International Health Regulations.

5.1.2 Objectives

The objective of the work in this chapter is to provide an evidence base for improvements to OSIR systems. It does so by evaluating the adequacy of clinical characterisation of potentially communicable diseases in a refugee setting.

5.1.3 Author's contribution

I was the principal investigator for the work contained in this chapter. I wrote the funding application, the protocol, all standard operating procedures, the statistical analysis plan and academic agreements; I was responsible for hiring, training, and managing the team who collected data; I undertook all data analysis and prepared the first draft of the manuscript. I was assisted by the Hellenic Centre for Disease Prevention and Control (KEELPNO) and the National School of Public Health in Greece in management of access to the refugee camps. These organisations, and our collaborators at the London School of Hygiene and Tropical Medicine provided expert advice on the OSIR system in Greece and the refugee crisis.

Publication

The work in this chapter is drafted for journal submission upon thesis hand-in. The provisional publication is as follows:

Rojek, A. M., Gkolfinopoulou, K., Veizis, A., Lambrou, A., Castle, L., Georgakopoulou, T., Blanchet, K., Panagiotopoulous, T., & Horby, P. W. Clinical assessment is a neglected component of outbreak preparedness: Evidence from refugee camps in Greece. in prep.

5.2 Clinical assessment is a neglected component of outbreak response: Evidence from refugee camps in Greece

5.2.1 Introduction

Refugees arriving in may be especially vulnerable to infectious diseases for reasons including poor rates of vaccination, poor immunity to endemic diseases in regions of movement, malnutrition, lack of access to safe drinking water, or living in over-crowded or unhygienic conditions[303–305]. These risk factors can occur in the country of origin, during transit, or during early settlement[304, 305]. The consequence is that infectious diseases are thought to be more common in this population, although definitive population wide estimates are not available for patients arriving within the EU[306–311]. A study conducted in Greece reported that respiratory tract infections were the most common medical problem diagnosed (prevalence of 23%) in refugees arriving at the border with Turkey[312]. MSF clinics at refugee points of entry into Greece and Serbia have diagnosed respiratory tract infections in 41% of patients (n = 33,331) accessing treatment[313]. During a programme of infectious diseases screening in unaccompanied minor refugees in Berlin in 2014-5, potentially communicable diseases were reported in 15.3% of patients[314].

Given the scale of migration, these infections can constitute a significant treatment burden. In Turkey in 2015 there were an excess of 330,000 cases of respiratory tract infections, and 50,000 diarrhoea cases amongst refugees from Syria[303].

There is also a risk of (re)-emergence of infectious diseases in refugees in countries that may be poorly prepared to recognise and treat them[296, 307]. One example is re-emergence of louseborne relapsing fever in refugees in regions of Europe where it

has been eradicated[315]. Refugees are also at higher risk of multi drug resistant infections[308, 316, 317]. It is important to note that in general, this risk to refugees does not imply a risk of ongoing transmission into the community. It is clear that refugees do not constitute a public health threat to the countries of their transit or arrival[302].

The consequences of outbreaks in refugee camps are severe. As an example, WHO estimates that in the event of a novel influenza outbreak in a refugee camp, the attack rate would be 50-60% and complications of severe influenza would be double that seen in the general population[318]. The demand on healthcare systems would be overwhelming and disproportionate, with 30-50% of the population seeking treatment and a hospitalisation rate of up to 10%[318].

Despite this evident vulnerability, little work has assessed the capability to rapidly and correctly identify disease outbreaks in this setting. Specifically, there are concerns that existing surveillance systems fail to adequately monitor infectious diseases in refugees arriving in Europe[306, 319–321]. However, improvement is difficult without an underlying evidence base - when WHO developed their most recent OSIR guidelines for humanitarian settings, they relied on advice from technical experts in light of the paucity of quantitative data[322].

One important step in an OSIR system is outbreak investigation (see figure 5.1). When a surveillance system signals an increase in the number of cases, outbreak investigation occurs[322]. This is when an alert is verified and characterised to plan an appropriate public health response. This step is heavily reliant on accessing clinical information, including the spectrum and severity of patient symptoms, the outcomes of patients (such as case fatality rate or length of hospitalisation), the populations who are most frequently being infected or experiencing the most severe disease, and exposures to potential sources of infection. This step has been described as moving from statistical signal detection to situational awareness[323].

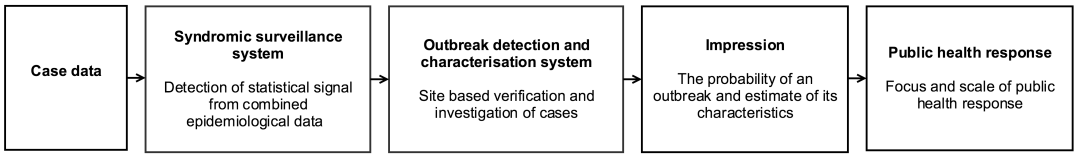

Figure 5.1: Steps in an outbreak response system incorporating syndromic surveillance. Adapted from [323].

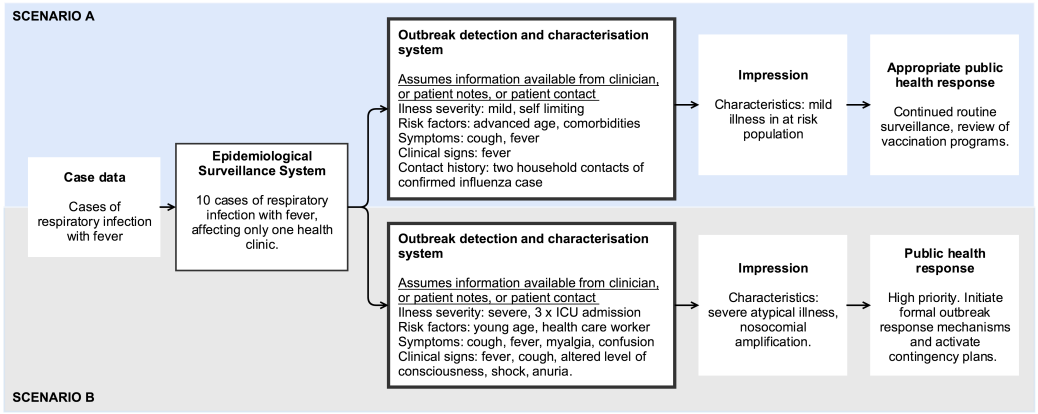

Figure 5.2: Influence of clinical characterisation (box 3) on the outcomes of an OSIR system

As an example of the value of this clinical information (figure 5.2), an alert for respiratory infection with fever may reflect a spectrum of scenarios with very different implications for the public health response. When the clinical impression is biased or incomplete, there risks being a mismatch between the characteristics of the outbreak, and the public health response.

Despite the implications of accurate clinical assessment in informing in public health measures, this process is often performed poorly during outbreaks. Information very often suffers from selection bias, privileging hospitalised severe cases and lacking clear denominators[324]. There is additional evidence to suggest that accurate clinical data may be especially difficult to acccess in a refugee camp. For example, an investigation of a shigellosis outbreak in a refugee camp in Greece suggested the size of the outbreak may have been underestimated due to difficulties in communication without language interpretation, under-diagnosis of cases with

mild symptoms, or denial of symptoms from patients unwilling to risk a delay to departure from the refugee camp[316].

Therefore the aim of this work is to provide quantitative measures of what information may be available to outbreak response teams investigating a cluster of cases in a refugee camp in Europe. It achieves this through observation of routine clinical consultations to identify the extent to which syndromes that may constitute a communicable disease are characterised, and these results recorded. The findings of this work provide an evidence based framework to direct improvements in the outbreak investigation component of an OSIR system. Importantly, this work directs improvement for refugee settings in advance of an outbreak.

5.2.2 Methods

Prospective observational data collection was undertaken in 13 refugee camps in Greece during the period of July 3 to July 28, 2017. The selection of camps was a convenience sample, with preference towards camps operating at the points of arrival of refugees into the country.

Our research team passively observed clinical consultations that were conducted as part of routine care in the refugee camp, and collected data on the clinical information captured in that setting. Because the purpose of the project was to evaluate normal practice, the research team did not seek additional information from patients, or request additional history taking or examination from clinicians, or provide any feedback on the care provided.

Data was recorded for consultations where a refugee presented for the first time with a recent onset (defined by one month) medical illness which was not due to trauma or a known toxin.

The framework for assessment was based on categories of clinical information required for outbreak investigation. These were: assessement of infectious disease exposures (where we recorded three exposures - recent overseas arrival within one month, close (household or nursing) contact with unwell contacts, and known or possible zoonotic exposure), indicators of infectious disease vulnerability (where we recorded two common vulnerable groups - pregnant women and those with comorbid illness), the spectrum of clinical signs and symptoms observed (assessed according to those features included in the case definition for syndromes under syndromic surveillance), and the severity of presentations (based on vital signs using methodology described below). A description of the case definitions used in the syndromic surveillance system used in refugee camps in Greece is provided elsewhere[325]). There were three components of data collection: a) whether the variable was assessed or otherwise ascertained during the clinical consultation (including negative or normal findings), b) whether the result (including negative or normal findings) were recorded in a written or electronic clinical record, and c) the diagnosis or result, if known.

Factors that may have contributed to the quality of information obtained in the consultation were recorded. These were the workload of the clinic, the type of healthcare worker providing care, and the presence of language difficulties[326].

Observers were all registered nurses. All observers received one day of protocol specific training from the principal investigator (AR). The accuracy of observer data collection was tested using standardised, video taped consultations. The mean accuracy of observer reporting was 95.8% (SD 3.5%). To further maintain accuracy, observers were encouraged to select an unsure option if there was uncertainty in an assessment.

For this study, refugees, asylum seekers, migrants in an irregular situation, and persons with a status unknown are referred to using the collective term "refugee(s)".

Statistical analysis

Descriptive statistics are presented as frequencies for categorical variables, means and standard deviations for normally distributed data, and median with range for other continuous variables. The severity of patient illness was assessed using two widely used standardised early warning scores - the United Kingdom's National Early Warning Score (NEWS) [327], and the paediatric equivalent, the Childrens Observation and Severity Tool (COAST) [328, 329]. These scoring systems allocate points to physiological abnormal vital signs to produce an aggregate score that is used to triage patients according to severity of presentation (irrespective of the underlying pathology). This score was modified to exclude scoring based on supplemental oxygen provision, as this is not routinely available in refugee camps.

STATA statistical software (version MP 15) and Microsoft Excel for Mac (version 15.21.1) were used for statistical analysis.

Ethics

The study protocol was granted an exemption by the University of Oxford ethics committee as it constitutes a clinical audit. This authorisation was provided to relevant authorities in Greece who deemed it sufficient to proceed without additional review.

5.2.3 Results

A total of 528 patient consultations were included in the study. 306 patients were male. The median age of patients was 19 years (range 1 month to 70 years) (figure 5.3). The most frequent reason for presentation was an acute respiratory tract illness, followed by a skin condition (figure 5.4).

Assessment of infectious diseases exposures

Figure 5.5 describes the extent to which important infectious disease exposures (of recent international travel, close contact with unwell contacts, and zoonotic exposure) were ascertained and then recorded. There was no assessment of these risk factors in most consultations (97% for known or suspected zoonotic exposure, 82% for close contact with unwell contacts, and 80% for international travel within one month). However, when these risk factors were assessed, patients had zoonotic exposure in 38% of cases, had recently ($<$ 1 month) arrived to Greece in 32% of cases and had close contact with unwell persons 58% of the time.

Assessment of infectious disease vulnerability

Figure 5.5 also displays the extent to which common risk factors for infectious disease susceptibility or increased severity were assessed. Two broadly applicable risk factors were selected - pregnancy and comorbid disease. There was no assessment of pregnancy status in at least 66% of consultations with women of child bearing age, and comorbid conditions were not inquired about in at least 58% of all consultations. 21% of the time that pregnancy was assessed during the consultation, the patient was pregnant. 28% of the time that comorbidities were assessed for, the patient had one or more comorbidities.

Clinical characterisation

Figure 5.6 shows the extent to which features of the most common presenting syndromes were ascertained and recorded (irrespective of the findings). Overall, clinicians did assess many of the key characteristics of syndromes, particularly for the common presentations of gastrointestinal conditions and respiratory conditions. However, in all but one variable (assessment for bulging fontanelles in infants

presenting with a neurological syndrome) clinicians did not document all their clinical findings when assessment occurred.

Table 5.1 compares whether patients were observed to have met (or not met) the case definition criteria for notification in the syndromic surveillance system based on what was observed during consultations, with their actual inclusion or non inclusion in the notification system. In 11% of cases there was agreement between observer assessment and actual reporting. In 23% of cases there was disagreement, meaning that the patient met all criteria in the case definition (based on information ascertained or recorded during the observed consultation) but was not reported, or was reported under a different syndrome. In the remaining 66% of cases there was inadequate information to make a comparison. This classification included cases where the patient did not meet case definition on the basis of observation, but the patient was still reported on the syndromic surveillance form. This was done, rather than considering these cases as a known disagreement, because the notification form always includes provision for a clinician to report a case due to clinical suspicion, even if the syndrome definition is not met. In 32% of cases, observers were unable to link the consultation with reporting - either it was unknown whether the patient was reported, or it was unknown what syndrome they were reported with.

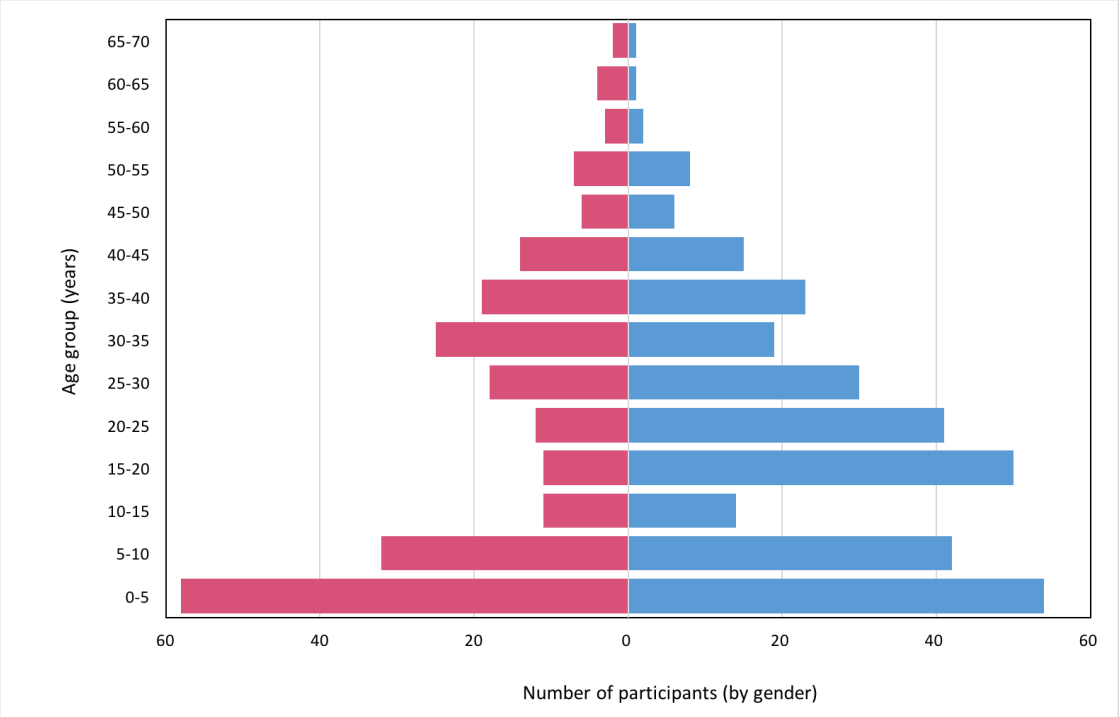

Figure 5.3: Distribution of age and gender of included patients, blue describes male patients, and red describes female patients.

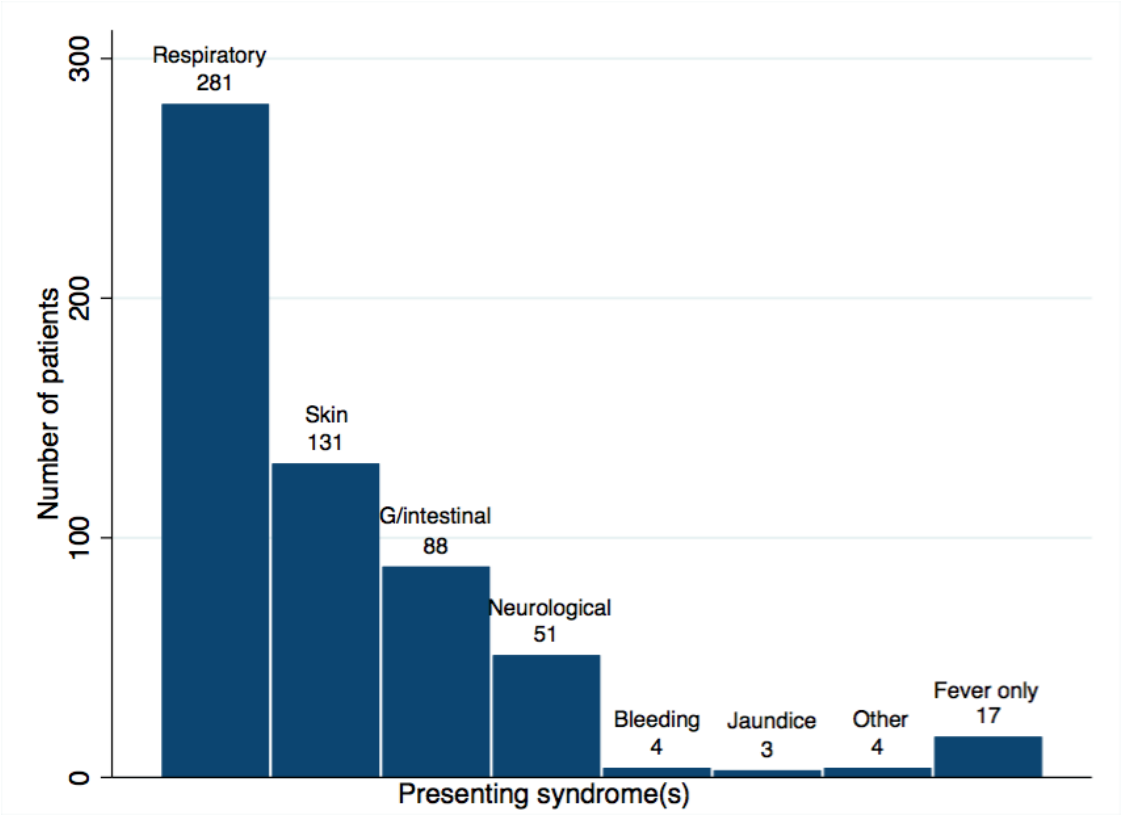

Figure 5.4: Number of refugee patients presenting with different syndromes. G/intestinal is gastrointestinal. Other refers to other syndromes not included in syndromic surveillance (such as urinary tract symptoms).

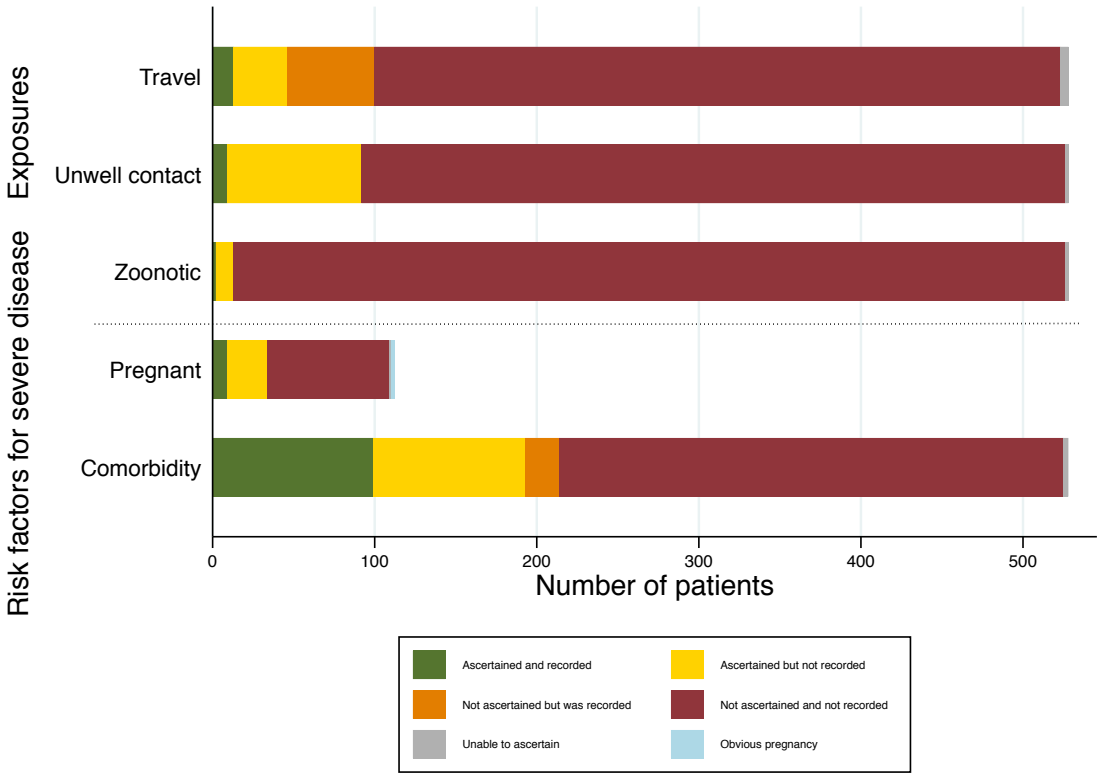

Figure 5.5: Assessment of possible exposure and vulnerability to infectious diseases. Exposure history includes recent travel (defined as international arrival within one month) unwell contacts (household contact or provided nursing care) and zoonotic exposure. Vulnerability includes pregnancy (women 12 - 50 only) and presence of any other medical condition

.

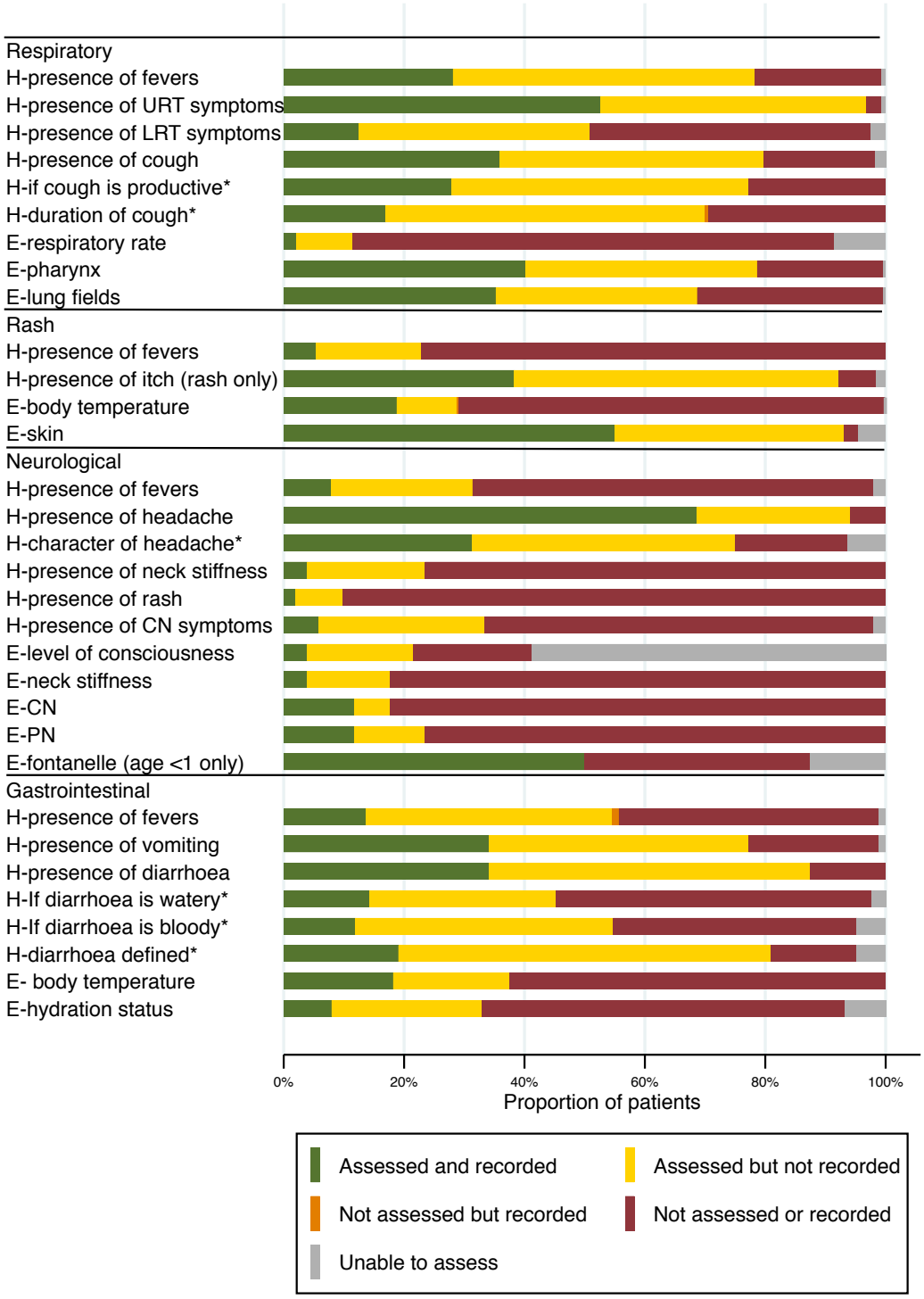

Figure 5.6: Assessment of clinical characterisation of presenting syndromes (limited to syndromes with $>$ 10 patients presenting). The clinical features assessed for each syndrome are based on case criteria for conditions under syndromic surveillance (either the platform used in refugee camps in Greece, or suggested by WHO for use in humanitarian settings). H refers to symptoms assessed by history taking. E refers to assessment by physical examination. CN refers to cranial nerves. PN refers to the peripheral nervous system. * means that this assessment only occurred when the primary symptom was present

Actual inclusion in syndromic surveillance report	Syndromic surveillance category according to observation of consultation and notes									
	Respiratory infection with fever	G/enteritis without blood	Bloody diarrhoea	Rash with fever	Suspected scabies	Suspected pulmonary TB	Jaundice of acute onset	Meningitis/ encephalitis	Insufficient information to assess	Case definition not met*
Respiratory infection with fever	17	0	0	0	0	5	0	0	23	7
G/enteritis without blood	0	2	0	0	0	0	0	0	8	4
Bloody diarrhoea	0	0	0	0	0	0	0	0	1	0
Rash with fever	0	0	0	0	2	0	0	0	0	0
Suspected scabies	0	0	0	0	9	0	0	0	0	3
Reported but syndrome unknown	4	1	0	0	6	1	0	0	17	3
Unsure if reported	27	3	0	1	15	2	1	0	70	19
Not reported	36	4	1	0	62	4	1	4	134	31

Table 5.1: Assessment of clinical characterisation of syndromes. Comparison is made between the reporting of the patient in the syndromic surveillance system (rows), with whether that patient is believed to have met the case definition based on the information collected during the consultation (columns). White boxes indicate agreement between reporting status and case definition based assessment, light grey boxes indicate a potential for discrepancy between reported status and case definition assessment and dark grey boxes indicate that a patient was not reported despite meeting case definition. Where I state 'insufficient information to assess', this indicates that there was insufficient assessment to included, or exclude a patient based on the case definition. *listed as potential discrepancy as inclusion may have been on the basis of other clinical suspicion. G/enteritis is gastroenteritis.

Severity assessment

In very few patients (fewer than 4% of children, and 1% of adults), were a full set of vital signs available to the observer (table 5.2). Therefore, the extent to which the severity scores presented (for patients with one or more vital signs taken) reflect severity across the entire population is not known.

Factors that may have influenced consultation quality

The median time per consultation was 10 minutes (1-45 minutes). Our observers could not reliably document how busy or crowded the clinic was. 521 (98.6%) of consultations were undertaken by doctors, and 7 (1.3%) by registered nurses. A professional interpreter or cultural liaison was used for 478 (90.5%) of consultations, and a family member interpreted for 9 (1.7%) consultations. There was no interpreter available, but language difficulty experienced in 12 (2.3% of consultations) and the clinician spoke the same language(s) as the patient in 29 (5.5%) of consultations. Observers were asked to list other factors they believed may have affected the consultation and answers included multiple patient consultations occuring at the same time (n = 5), other communication problems (n = 5), a distressed patient or acute concerns about patient mental health (n = 3), too many people (six or more) being present in the room (n = 2), the status of the patient as an unaccompanied minor (n = 1), constant interruptions (n = 1) and, a patient being unwilling to have a physical examination (n = 1).

Paediatric	No vital sign data	Aggregate COAST score of patients (and clinical relevance)						Proportion where all variables in assessment present
		0	1	2	3	4	5 or 6	
		Routine monitoring		Prioritise in queue	Senior doctor review	Urgent review & move to resuscitation area	Emergency	
< 1 years	18	6	4	0	0	0	0	4.00%
1 to 4 years	47	30	6	1	0	0	0	0
5 to 12 years	42	44	7	1	1	0	0	2%

Adult	No vital sign data	Aggregate NEWS score of patients (and clinical relevance)						Proportion where all variables in assessment present
		0	1	2	3	4	5 + (or one parameter 3+)	
		Low clinical risk					Medium clinical risk	
> 12 years	268	16	25	7	3	2	0	1%

Table 5.2: Assessment of severity of patient presentations. Two vital signs based scoring systems are used, the National Early Warning Scoring system, which is used in adult patients, and the Children's Observations And Severity Tool for children, as described in the methods. Data is only shown for patients where one or more vital sign was recorded.

5.2.4 Discussion

Herein we report data on clinical information likely to be available for outbreak investigation in a humanitarian setting.

We found that when acute medical syndromes were encountered in refugee camps, clinical characterisation of the predominant syndrome was, in general, performed well, especially for common syndromes (respiratory and gastrointestinal syndromes). For example, 81% of the time patients had diarrhoea, it was defined in the consultation (as three or more loose stools per day), and the type of cough (productive or non productive) was assessed in 77% of patients presenting with this symptom. Despite this, there were occasions when clinical assessment was not comprehensive. The consequence was that for many of these cases (n=134), it was difficult to ascertain if the patient was presenting with a syndrome under surveillance.

With very few exceptions, case records did not contain all the information assessed during the consultation. This is an important opportunity missed, because clinicians are spontaneously ascertaining information that would be valuable for an outbreak team. Our concern is that in refugee camps, alternative means of investigating a case may not be readily available. Interviewing of a patient or their family is often used in other situations, but this may not be accurate in a refugee camp as refugees may not wish to disclose symptoms[316]. Furthermore, the extent to which refugees who are migrating through Europe can be traced for information verification is unknown. In addition, the high turnover of clinicians in some camps means these individuals may not be accessible to outbreak investigators either. Tools to facilitate rapid recording of information should be urgently developed.

We report low ascertainment of infectious diseases exposures. This is despite the differential diagnosis for illnesses in refugees in Europe varying by their country of origin, transit route and living conditions[305]. For well characterized diseases,

identification of the source of the outbreak directs control. For an emerging disease, it is integral for hypothesis generation. Likewise we found incomplete assessment of whether refugees belonged to a vulnerable population for infectious diseases. These findings agree with reports that statutory surveillance systems for refugee populations in Europe cannot stratify patient populations during outbreaks[319]. This impacts prioritisation of resources and care during an outbreak.

Our assessment of severity of patient illness found that fewer than 5% of patients in any age group had a full set of vital signs taken, which limits external, objective assessment of patient severity. This finding is not unexpected - the lack of consistent and understandable measures of patient severity during the H1N1pdm09 pandemic was one of the most notable failures of the outbreak, that resulted in an over-estimation of disease severity[17]. While there are other means of assessing patient severity we used a widely used and validated early warning system.

We do not suggest that patients assessed to meet the case criteria for a notifiable disease, but who were not reported, were misdiagnosed. There are various valid reasons why this may occur (such as there being a clear alternative explanation for symptoms or signs). However, universal adoption and strict adherence to case definitions is encouraged by public health authorities who expect false positive notifications as this is a preferable outcome to under-reporting [322]. Therefore, far from identifying errors in clinician practice, our findings of discrepancies between assessor reporting and clinician reporting are instead broadly illustrative of common difficulties in interpretation and use of syndromic surveillance between different stakeholders.

Our understanding of factors that impede comprehensive patient evaluation is limited. Unfortunately we could not reliably measure how busy clinics were. For the majority of consultations, language did not appear to be an issue, but there were reports of other communication difficulties during consultations. Frequent

interruptions and multiple simultaneous consultations occurring are likely to impair consultation quality, but reflect the reality of the working environment.

Limitations

The scope of this work was limited to patient presentations in some official refugee camps in Greece. The representativeness of these findings for refugees treated in other healthcare settings, or other countries is unknown. Furthermore, we treat refugees as a homogeneous presenting group, although the risk of infectious diseases could differ within this population. We used a broad classification to identify patients with presenting syndromes that may include an infectious disease. It is possible, for some of these patients, that the presenting syndrome clearly posed no possibility of being an infectious disease. However, sub-group analysis proved difficult - while we intended to perform this on the basis of febrile status, there was a low proportion of cases where fever was ascertained, and some syndromes do not require fever in their case definition. We could not assess for the following syndromes reportable in Greek refugee camps: malaria, diphtheria, or sepsis, as ascertainment of some elements of the case criterion could not be achieved by passive observation (e.g. the presence of a pharyngeal pseudo-membrane for diphtheria) or they relied on laboratory diagnosis. In retrospect, presenting neurological syndromes should have been divided into headache and other nervous system presentations (such as acute paralysis or weakness) as the types of clinical assessment required for diagnosis differ significantly between these presentations. We were interested in clinician assessment of patient vaccination status, but could not do so due to the wide variability in how this was assessed.

Future directions

Opportunities exist to improve the clinical information available for OSIR in refugee camps. A focused examination of the barriers that clinicians experience to better evidence generation is warranted. In the near future we will convene an expert working group (consisting of academics, front-line doctors and nurses, representatives from KEELPNO, and representatives from collaborating NGOs) with the objective of adopting or producing tools and methods that facilitate the acquisition and recording of clinical information that meet the needs of both clinicians and public health responders.

5.2.5 Conclusions

Our findings identify several limitations of the data collected during clinical assessment in the refugee setting in Greece, which will limit outbreak response in this context.

5.3 Contribution of the chapter

Existing OSIR guidelines for humanitarian settings, including WHO guidelines[322], and the Sphere humanitarian charters and minimum standards for humanitarian response (www.sphereproject.org) focus entirely on the constitution and agreed activities of an outbreak response team. Furthermore, there has been an absence of quantitative data on which to design OSIRs in humanitarian settings. The work in this chapter will be most valuable, if, in the expert working group, we are able to produce tools and methods that are pragmatic and enable and facilitate enhanced data collection in a manner that does not detract from the immediate priorities of treating patients. Further work is also required to understand clinician knowledge,

attitudes and practices regarding their contribution to OSIR.

Beyond the primary findings of the work in this chapter, it raises additional points of discussion relevant to this thesis, and specifically the issue of how to facilitate clinical research in this high risk setting. Presently, the OSIR in Greece functions relatively independently of the clinical interface where patients with a communicable disease presents. We are not the first to note that outbreak response is characterised by an artificial separation of the public health, clinical and scientific response in this setting. In particular, a previous review of infectious disease preparedness for refugees in Europe has decried the extent to which parallel but incompatible information sources exist for syndromic surveillance, mandatory notification systems, case based or clinical systems and laboratory systems[306, 319].

This is an understandable consequence of engrained disciplinary divisions and regulatory frameworks but is inefficient given that the ultimate aims of all groups are to improve patient outcomes and control the outbreak. Under even a cursory examination, it is clear that the boundaries between the public health, clinical and scientific response are blurred, with the necessary evidence overlapping and being collected from the same patient. What distinguishes research from clinical or public health practice is often difficult to define, and rather than trying to draw arbitrary boundaries, we should aim to integrate the data needs of all disciplines.

The quality of evidence can be improved by designing unified data collection protocols that are driven by an explicit link to the public health and clinical decisions that need to be taken. Whilst it would be naïve to think there are template data capture tools or protocols that will perfectly fit any outbreak, it would be a major step forward if data capture instruments were to be developed that are explicit about the content, quality and scale of data needed to take decisions. The successful implementation of protocols for integrated clinical data collection will require action to lower the barriers to the collection and sharing of standardised

data. Such action should include the adoption of generic disease severity scores such as those used in this chapter; and the development of user-friendly, scalable and open-access software for data capture and sharing. Calls already exist for the EU to convene expert working groups to agree on core variables and improve the comparability of data collection on infectious diseases in refugees[319].

If I were to conduct this work again, I would improve the breadth of the work by assessing the extent to which patient dispensation is recorded, and the extent to which patients can be followed up to determine outcomes for an outbreak investigation. This information is not presently known and may be especially difficult to obtain in refugee camps due to the transient nature of the population, and a myriad of reasons that refugees may not wish to provide contact information to authorities[306]. While audit of whether clinicians document relevant contact details should be feasible, assessment of the extent to which patients can be followed up is more complicated - patients who consent to follow up from a research group may not be reflective of the underlying population. In addition, it would have been interesting to assess the surveillance and reporting of infectious diseases for the growing population of patients who do not reside in official refugee camps and for whom little information on risk and response exists.

6

Concluding Remarks

In the turmoil of outbreaks and the pressure to protect public health and economic interests, it is sometimes forgotten that patients lie at the heart of every outbreak. These patients, their families and the clinical teams caring for them are often struggling with frightening uncertainty and inadequate support and resources. The aim of the work in this thesis was to enable expedient, rigorous science that improves survival for these patients, and certainty for their clinicians.

The Lancet's health in humanitarian crises series recently stated 'this comment is a call to humanitarians . . . to recognise that the objective of this work (scientific data collection) is not to produce evidence, but rather to provide the most effective interventions for the most marginalised and most vulnerable in our societies' [330]. I echo this sentiment for the EEID literature, where previously, clinical practice has been reliant on poor quality data. This data may distort understanding of disease. It also constituted a missed opportunity to inform high quality evidence generation.

In this thesis I have presented work that aims to direct improvements in the patient centred research response to EID outbreaks.

Chapter 2 demonstrated that the research responses to SARS and the influenza A(H1N1)pdm09 pandemic constituted missed opportunities to conduct clinical treatment trials, and to harness observational data to provide meaningful evidence. I recommend that observational data registries are designed and implemented during outbreaks in order to better monitor and inform patient treatment.

Chapter 3 showed that it is feasible to conduct clinical trials during an outbreak, but that further innovation is required to expedite and enhance operational feasibility. I recommend that these innovations include testing of trial design options, accrual of evidence in analogous diseases during inter-epidemic periods, and prioritisation of supportive care trials.

Chapter 4 demonstrates how the limited observational evidence available for EIDs, and the variability in the way this information is presented risks biasing clinical characterisation of these infections. I support calls for a harmonised IPD database for EID clinical information and I recommend that audit of observational data is undertaken for WHO priority pathogens to identify clinical research priorities to support countermeasures R & D.

Chapter 5 demonstrates that data informative for outbreak response is collected at the clinical interface, but frequently not recorded. I recommend that pragmatic clinical data collection tools are adopted or designed for this setting in order to improve OSIR performance.

This work contributes to an increasing emphasis on clinical research during outbreaks in the years following the west Africa EVD epidemic. Clearly, there remain a great number of opportunities to further strengthen capabilities. In light of my recommendations, we have begun work on a number of activities with this

intent. This includes collaboration with statistical modelling groups to advise on the clinical trial designs most likely to reach definitive conclusions under various epidemiological scenarios. We continue to conduct laboratory analysis (including pharmacokinetic and cytokine studies) on samples from patients treated with TKM-130803. It is hoped PK studies will help to elucidate the disparity in the efficacy of this product in humans compared with non human primates, and cytokine analysis will contribute to knowledge of the immune response to EVD. An expert working group will convene shortly to discuss the findings of the work presented in chapter 5 to determine how the clinical information captured in refugee camps can be capitalised on to better inform OSIR.

To conclude, the work in this thesis has identified ways to improve our ability to collect high quality evidence during an infectious disease outbreak, with the intent of improving patient outcomes. While the intent is specific, the implications may be broad given the economic and societal implications of outbreaks and hence this is a meaningful contribution to the global health agenda.

Appendices

A

Supplementary manuscript 1

Insights from clinical research completed during the west Africa Ebola virus disease epidemic

Amanda Rojek, Peter Horby, Jake Dunning

The west Africa Ebola virus disease (EVD) epidemic was extraordinary in scale. Now that the epidemic has ended, it is a relevant time to examine published studies with direct relevance to clinical care and, more broadly, to examine the implications of the clinical research response mounted. Clinically relevant research includes literature detailing risk factors for and clinical manifestations of EVD, laboratory and other investigation findings in patients, experimental vaccine and therapeutic clinical trials, and analyses of survivor syndrome. In this Review, we discuss new insights from patient-oriented research completed during the west Africa epidemic, identify ongoing knowledge gaps, and suggest priorities for future research.

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Introduction

The world's largest ever epidemic of Ebola virus disease (EVD) probably commenced in December, 2013, following the infection of the presumed index case, a 2-year-old child living in rural Guinea.[1] The subsequent outbreak soon crossed into Sierra Leone and Liberia and case numbers escalated rapidly. When WHO acknowledged in August, 2014, that the outbreak was a public health emergency of international concern, there were already 1711 reported cases and there had been 932 deaths.[2] By the end of the epidemic, 28 646 cases and 11 323 deaths had been reported,[3] but the true numbers are likely to be much higher. The epidemic had far-reaching effects in west Africa, including enormous economic costs and significant strains on already stretched health-care systems.[4,5] A staggering 881 health-care workers were infected and 513 died.[6]

The focus of global response efforts was, quite rightly, to provide humanitarian assistance and medical care, and to interrupt chains of transmission.[7] But there were also calls from WHO, funding bodies, and governments to urgently increase the scale of scientific research to respond to the rapidly growing EVD epidemic.[8] Before 2014, outbreaks were short-lived, occurred in remote locations, and involved relatively small case numbers. Such factors, coupled with little research interest and funding, meant that the general understanding of EVD was limited. The west Africa epidemic provided an important opportunity to improve patient outcomes through clinical studies that would enhance knowledge and allow investigation of potential interventions. There were major hurdles to overcome, however, including logistical challenges,[9,10] and ethical and societal considerations[11,12] that could affect the ability to reach conclusions within the lifetime of the epidemic.

This Review summarises published findings from clinical research completed during the epidemic, and then discusses the implications for countries at risk of EVD outbreaks, ongoing clinical research gaps, and priorities moving forward. There was a broad range of research done during this period, so we have placed emphasis on patient-centred developments and progress made investigating Ebola virus vaccines (appendix).

Clinical features of EVD

In the west Africa epidemic, the greatest burden of EVD was in young adults (median age 32 years, IQR 21–42).[13] It is unclear whether this burden represents an increased risk in young adults (perhaps because of increased exposure) or a case ascertainment bias (if children or the elderly were less likely to be in the official count). There was no marked gender difference in disease prevalence (48·8% of probable and confirmed infections were in men).[14]

We now know that young age is a predictor of death (odds ratio [OR] per year of life 0·91, 95% CI 0·85–0·97) and that children tend to deteriorate rapidly, with a median of 3 days from admission to an Ebola treatment centre (ETC) to death in a cohort of 300 children.[15,16] Likewise, some data show that mortality is higher in patients older than 45 years and in men.[13,14,17–20] The previously published case fatality rates (CFRs) for maternal (90%) and neonatal EVD (100%) might be an overestimation,[21] since there have been subsequent case reports of maternal[22,23] and, very rarely, neonatal survival.[24] Without systematic data collection, however, the prognosis for pregnant women is uncertain.

Although first described as Ebola haemorrhagic fever, because of the frequency of bleeding observed during the initial outbreaks of 1976,[25,26] a spectrum of illness was evident in the west Africa epidemic and haemorrhage, when present, was a late finding associated with fatal disease.[27,28] The hallmark of advanced disease in this epidemic was severe gastrointestinal illness.[13,18,29–33]

The most frequent symptoms at presentation (table 1) were fever, fatigue, anorexia, vomiting, diarrhoea, headache, and abdominal pain.[13,18,31–33] Anecdotal reports of large volume, cholera-like diarrhoea emerged from ETCs in west Africa, and volumes of up to 10 L of diarrhoea per day were observed in medically evacuated patients.[29,30] Notably, fever was absent in at least 10% of patients,[13,18,31–33] which has important implications for clinical triage and case definitions that include fever as a prerequisite symptom. Less common clinical manifestations, including confusion, conjunctivitis, and hiccups,[20,33] had good discriminatory importance in identifying EVD cases in all patients presenting to ETCs

See Online for appendix

	Signs and symptoms[43] reported in more than 10% of patients with acute Ebola virus disease	Investigational findings that have been reported during acute Ebola virus disease	Signs and symptoms during survivor syndrome from Ebola virus disease
General	Fever, fatigue, hiccups	Raised pro-inflammatory markers, including CRP; elevated lactate[34]	Fatigue,[35-37] depression,[36-39] anxiety,[36,37,39] insomnia[35,36,38]
Neurological and visual	Headache, confusion	Detectable Ebola virus RNA in the cerebrospinal fluid;[40,41] diffuse swelling, microvascular occlusions as observed by MRI[42]	Difficulty concentrating, mood changes, and memory loss;[38,39] headaches;[35,36,38,43] dizziness;[38] difficulties hearing;[35,37–39,44] visual disturbances;[35–37,39,43,44] peripheral paraesthesia or dysaesthesia[37]
Cardiovascular	Chest pain	Bradycardia,[45] arrhythmias as shown by electrocardiogram;[46] myocarditis shown during MRI[47]	Chest pain,[36,38,43] palpitations[35,37,38]
Pulmonary	Cough, dyspnoea, sore throat	Pulmonary oedema and pulmonary effusion as observed on x-ray and USS[46]	Dyspnoea[37]
Gastrointestinal	Anorexia, vomiting, diarrhoea, abdominal pain, odynophagia	Paralytic ileus and bowel wall oedema shown by USS[29,48]	Anorexia,[35,38] abdominal pain,[35,36,38,43] constipation[38]
Hepatobiliary	Jaundice	Transaminitis with high AST:ALT ratio[34,49]	..
Renal, urological, and electrolytes	..	Acute kidney injury,[34,50,51] raised creatine kinase,[34] hypokalaemia[34,49,51] or hyperkalaemia,[34] hyponatraemia,[34] hypocalcaemia,[49] hypoglycaemia[16]	Decreased libido; sexual dysfunction and testicular pain[36,38]
Haematological	Clinically significant haemorrhage uncommon, likely to be more frequent in pregnant women.	Leucopenia, thrombocytopenia, raised INR, haematoconcentration[34]	Anaemia[35]
Skin and musculoskeletal	Myalgia, arthralgia, conjunctivitis	..	Arthralgia;[35,36,39,43,44] myalgia;[36] alopecia, skin peeling, and pruritus[35,36,38,39,43]

USS=ultrasound scan. ALT=alanine transaminase. AST=aspartate transaminase. CRP=C-reactive protein. INR=international normalised ratio.

Table 1: **Clinical manifestations of investigational findings in Ebola virus disease, reported by studies done during the west Africa epidemic**

and therefore remain helpful for presumptive clinical diagnosis in the context of a known outbreak.

A cross-sectional seroepidemiological study done in Sierra Leone found that 14 (7·5%) of 187 individuals who had not been diagnosed with EVD had detectable anti-Ebola glycoprotein antibodies.[52] 12 of the 14 denied any symptoms compatible with EVD. These results, when considered alongside related data from previous outbreaks,[53,54] suggest that a proportion of Ebola virus infections are subclinical, although the contribution of such cases to transmission or herd immunity is unknown and the specificities of serological assays need to be considered.

WHO's estimated CFR for the epidemic was 70% (95% CI 69–72).[32,55] Overall, mortality was lower in patients admitted to hospital (CFR 61%, 95% CI 59–62) compared with patients not admitted to hospital (88%, 86–90).[32] Small hospital series have reported substantially improved survival (eg, CFR 32% in a hospital in Sierra Leone[56]), but these data should be interpreted with caution, since there are many potential explanations for the variability in CFR. For example, although medical intervention might have conferred a survival benefit, the influence of case selection bias (arising from self-presenting patients who are not representative of patients with EVD in the community) or a survival bias (when the most unwell patients succumbed to disease before admission) has not been fully assessed. The 19% CFR seen in patients treated in Europe and the USA was much lower than that reported in west Africa;[57] although not confirmed, possible explanations include fewer untreated comorbidities and lower levels of viraemia at admission, and access to advanced physiological support and experimental therapies that were not available routinely in the three most affected countries in west Africa.

Complications of acute illness

EVD can be a severe and complex multisystem disease, with inflammation, vascular leakage, hypovolaemic shock, electrolyte disturbance, and direct organ damage all contributing to illness. Most existing knowledge about the pathogenesis of EVD has come from in-vitro studies and animal models (reviewed elsewhere[58–60]), and limited histopathological data from previous human cases of EVD.[60] Improved characterisation of the broad spectrum of organ involvement (table 1) is an important contribution to knowledge about EVD from this outbreak.

Gastrointestinal complications

The mechanism of severe diarrhoea in EVD is unclear. Although clinical descriptions of large volume, so-called rice water diarrhoea draws analogy with cholera and implies a secretory process, previous autopsy findings indicate that intestinal wall inflammation also occurs.[60]

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There have been small gains in explaining why patients experience abdominal pain (including peritonism in a subset of cases),[29,46,48] with case reports from resource-rich countries identifying paralytic ileus by ultrasonography.[29,48] In one case, marked bowel wall oedema was observed; the treating clinicians speculated that both viral-mediated damage and iatrogenic hypoproteinaemia might have contributed to this finding.[29] They also suggested that an inflamed gastrointestinal tract was likely the source of the bacteraemia observed in this patient, but there are few similar data to suggest whether this was a common phenomenon in west Africa.

Renal complications

Renal dysfunction is more common than previously thought. In one series of 150 patients, acute kidney injury (defined according to the Risk, Injury, and Failure; Loss; and End-stage kidney disease [RIFLE] criteria) occurred in 50% of patients and was an independent predictor of mortality (OR 5·84, 95% CI 1·15–29·58);[34] a similar pattern has been seen in other cohorts.[19,50,51] Importantly, these studies suggest that renal dysfunction occurs earlier in the disease trajectory than previously recognised and, at times, before the onset of severe vomiting and diarrhoea. For these patients, this early onset of disease manifestation indicates a mechanism partly independent of prerenal hypovolaemia because of gastrointestinal losses.[34,61] There are probably various contributors, including renal hypoperfusion from septic shock or, in patients with disseminated intravascular coagulopathy, thrombus formation in the renal microvascular system, or rhabdomyolysis.[34] In particular, the risk of acute kidney injury from rhabdomyolysis has yet to be fully elucidated. Although approximately half of patients with EVD experience myalgia[32] and suggestive laboratory findings of raised creatine kinase[34,57,62] and hyperkalaemia have been reported, identification of true rhabdomyolysis has been limited by insufficient urine myoglobin measurement. Furthermore, there have been few mechanistic studies of Ebola-virus-induced muscle damage.

Hyperkalaemia has been reported in 13% of patients with EVD in one series based in west Africa.[34] This finding is plausible given the prevalence of acute kidney injury and the hypothesis of rhabdomyolysis, but hyperkalaemia has been reported infrequently in other series, both in west Africa[51] and in medically evacuated patients (albeit confounded by frequent use of renal replacement therapy in this setting). Therefore, there is no certainty and caution is required when interpreting potassium findings obtained under field conditions, since erroneous readings of hyperkalaemia due to specimen haemolysis is possible. Hypokalaemia is common,[34,49,51] and although this is not an unexpected finding, given the severity of gastrointestinal losses in EVD, the variability in reported blood potassium disturbances highlights the necessity of biochemical testing to inform clinical decision making. Although data are limited, other commonly observed metabolic abnormalities include hyponatraemia, hypocalcaemia, and hypomagnesemia.[34,49,57] Additionally, severe and frequent hypoglycaemia has been described in children with EVD.[16]

Hepatic complications

There is little new knowledge regarding liver injury in EVD. Normal bilirubin concentrations were considered normal in patients with EVD in west Africa, but transaminitis was common, typically with a high aspartate transaminase to alanine transaminase ratio.[19,34,49,57] It is not clear whether the increased ratio represents liver damage, muscle damage, or both.[34,57,62] A high AST concentration during the first week of illness was shown to be associated with fatal outcome.[63] In the same study, AST correlated with the Ebola virus cycle threshold value during PCR analysis, suggesting it could be used as a surrogate marker of viral load.[63]

Respiratory complications

Dyspnoea and tachypnoea were observed frequently in west African patients with EVD. Difficulty in breathing was reported in between 41%[20] and 50% of patients.[64] Tachypnoea was observed in all 35 patients in one cohort.[19] Other groups have reported much lower rates of dyspnoea,[10,33,65] but there is likely to be variability in reporting since the intensity of monitoring is varied and dyspnoea is a subjective symptom. Acute lung injury has been observed in patients with EVD who were medically evacuated and had access to more intensive monitoring. In this setting, hypoxaemia was observed in 14 (52%) of 27 patients and non-invasive or invasive mechanical ventilation was required in nine patients (33%).[57] Tachypnoea could occur secondary to acidosis, which is common in EVD,[19,34,51] but pulmonary oedema associated with vascular leakage or fluid overload might also contribute to this condition.[30,48] Direct viral pneumonitis was suggested as the cause of acute respiratory failure in one case, as shown by interstitial pulmonary infiltrates and the detection of Ebola virus in bronchial aspirate fluid.[66]

Cardiovascular complications

Further reports of inappropriate bradycardia in patients with EVD surfaced during this epidemic.[45] Because some patients in this report were also encephalopathic, the authors suggested a possible central neurological cause, as opposed to the previous hypothesis of toxin-mediated damage.[45] Arrhythmias have been reported in medically evacuated patients[46] and have been the presumed proximal cause of sudden death in some patients with EVD during acute illness or during early recovery[67] in west Africa. Electrolyte disturbances could be possible precipitants, but there is also evidence that viral myocarditis can occur during acute illness and recovery.[35,47] Additionally, a hypercoagulable state has been shown during early

recovery;[68] although this raises the possibility of venous thrombosis and pulmonary thromboembolism,[35] evidence of these complications is incomplete. Additionally, higher haemoglobin concentration and haematocrit were associated with mortality in a west Africa cohort;[34] this finding might have resulted from haemoconcentration, but whether the increase in blood viscosity has clinically important consequences is unknown.

Neurological complications

Neurological complications were common in patients in west Africa and included headache (61%), confusion (13%), and coma or unconsciousness (6%).[32] A third of patients treated in Europe and the USA were encephalopathic at some point during their illness.[57] Encephalitis during acute illness and early recovery has been described, with detection of Ebola virus RNA in cerebrospinal fluid.[40,41] This association alone is insufficient to assume an infective mechanism, but is supported by isolation of virus from cerebrospinal fluid in a survivor with meningoencephalitis.[69] Detailed radiological investigation is challenging even in resource-rich settings, but MRI brain imaging done at day 33 of illness has shown microvascular disease and ischaemia in a patient with meningoencephalitis.[42]

Inflammatory response

The association between high viral load in blood and increased mortality is now well established,[18–20,34,64,70–72] and has been shown to follow a sigmoid (logistic) function.[73] Severe EVD is associated with an intense inflammatory response, characterised by high concentrations of pro-inflammatory mediators.[60,74,75] The kinetics of soluble immune mediators and biomarkers in serial blood samples obtained from seven patients with EVD treated in the USA showed an association between more severe disease and biomarkers suggestive of endothelial or coagulatory dysfunction. These patients also showed a comparative absence of biomarkers indicative of an immune response (compared with those found in patients with less severe EVD).[76] Two case series reported high concentrations of C-reactive protein and lactate, especially in fatal cases.[34,57]

Co-infections and sepsis

There are new and unexpected findings describing how concurrent infections affect EVD prognosis. Analysis of blood samples from 1182 patients infected with Ebola virus found that patients with *Plasmodium* spp parasitaemia were 20% more likely to survive, even after accounting for the mortality risk factors of marked Ebola virus viraemia and increasing patient age.[77] In the same study, the survival advantage was independent of treatment with antimalarial drugs, and administration of different antimalarial drugs failed to improve survival in mice infected with EVD. The authors hypothesised that concurrent *Plasmodium* sp infection might moderate the host immune response, perhaps by reducing the exuberant cytokine response observed in EVD.[77] By contrast, a separate study did multivariate analysis on data from 1047 cases and found that malaria parasite co-infection was an independent determinant of fatal outcome, but only for children who were aged 5–14 years; all patients in this study received antimalarial therapy.[78] The reasons for these discrepant findings are unclear.

A study of 49 patients with EVD found that co-infection with GB virus C was associated with improved survival.[79] Similar to the hypothesis for the effect seen with concurrent *Plasmodium* sp infection, GB virus C might also have beneficial immunomodulatory functions in EVD infection.[77] Studies showing the effect of HIV co-infection on survival in EVD have either not been done or have yet to report their findings.

Physiological and biochemical findings that would fulfil the commonly accepted criteria for septic shock have been described for patients with EVD treated in Europe and the USA. It seems likely that sepsis and septic shock also occurred in many patients with EVD treated in west Africa, but there are insufficient data to confirm this. Sepsis could be caused by Ebola virus infection alone, or by bacterial co-infections (these have not been investigated systematically).[29]

Supportive care

Supportive care remains the principal management strategy for patients with EVD. Several authorities advocate focusing efforts on correcting gastrointestinal fluid losses and electrolyte imbalances, and preventing hypovolaemic shock.[80] Recommended components of care often included oral or intravenous fluids, analgesia, antiemetics and antidiarrhoeal medications alongside empirical antimicrobials and antimalarials.[81,82]

The lower case fatality rate in patients treated in the USA and Europe (19%) suggests that intensive supportive care strategies can contribute substantially to improved survival.[57] Trials of supportive care were not completed during the west Africa EVD epidemic, however, and the evidence base for defining optimal supportive care for EVD remains insufficient.[83] Intensive intravenous fluid resuscitation was shown previously to be harmful in severe paediatric infections in resource-limited settings, albeit in a different context to EVD; therefore, a universal fluid resuscitation protocol for ETCs could potentially cause harm in some patients.[84] The complexities of detecting and correcting abnormalities in fluid distribution and organ perfusion have been shown by studies of patients treated in the USA and Europe.[57,85] Some have questioned whether EVD-associated sepsis differs significantly from bacterial or fungal sepsis and, accordingly, whether applying general principles of sepsis management (or administering experimental sepsis treatments) to patients with EVD could improve survival.[80,86]

Several interventions have been used routinely in some ETCs but not in others, with little evidence of

their benefit or risks. For example, the role of empirical vitamin K remains unclear,[87] given the limited understanding of the frequency and mechanisms of coagulopathy in EVD. Non-steroidal anti-inflammatory drugs were prescribed in some centres,[56] despite their potential to worsen gastrointestinal and renal complications. Loperamide is known not to confer benefit in patients with cholera, but it is uncertain whether a similar, secretory process causes the large volume diarrhoea described in EVD. Additionally, paralytic ileus is a known complication of EVD and a contraindication to loperamide use, but might go unrecognised in a typical ETC setting. The apparent variability of electrolyte disturbances also raises concerns about routine empirical electrolyte supplementation in the absence of blood electrolyte monitoring.

Any future trials of supportive care strategies in EVD will be challenging if new outbreaks are more typical (ie, smaller and of shorter duration), but high-quality supportive care is clearly a major factor influencing survival and it is important that recommended supportive care strategies are evidence-based. An expert consensus statement on the optimal package of supportive care for EVD in various settings would be a helpful interim measure, even more so if this identified the most important evidence gaps to guide the design of prospective clinical studies should a situation arise where such trials were possible.

Survivors

The enormity of the west Africa outbreak has led to an unprecedented number of EVD survivors. The most frequently reported post-EVD complications in this epidemic (table 1) are consistent with previous outbreaks.[88,89] These include arthralgia, visual disturbances (including uveitis and loss of visual acuity), hearing impairments, myalgia, fatigue, abdominal pain, and sleep disturbances.[35,36,38,43,44] Neurological deficits were reported infrequently before this outbreak, but now appear to be an important contributor to morbidity.[39] Psychological distress in response to a life-threatening illness could also contribute to neurocognitive manifestations.[90] Survivors report very poor social acceptance by their communities and are often stigmatised.[36,38] Although this is known to affect survivor confidence and social engagement,[38] long-term psychological needs are unknown.

The pathogenic mechanisms that underlie EVD sequelae remain poorly understood. There is a long-held assumption that autoimmune or post-infectious inflammatory processes play prominent roles, but an association between viral replication in immune privileged sites and late complications in some survivors is newly established.[69,91]

Ebola virus was isolated from the aqueous humour of a survivor with panuveitis, 14 weeks after diagnosis.[91] The total duration of viral sequestration was unknown, but was less than 18 months.[92] Additionally, infectious virus was detected in the cerebrospinal fluid of a survivor with meningoencephalitis, 9 months following acute illness.[69] At this time, there was also a transient viraemia, thought to represent a so-called spillover of Ebola virus from its site of replication in the CNS.[24,69] Both of these patients were medically evacuated to settings with advanced care and received experimental therapies. Therefore, it is unclear if the nature, timing, and severity of these complications are representative of sequelae seen in west Africa. Follow-up of 151 survivors in Sierra Leone showed that late recrudescence, defined as illness or death that could not be attributed to a non-EVD related cause after a period of full recovery from confirmed EVD, was rare (maximum estimate of 0·7%).[93]

Persistence of Ebola virus in body fluids had been shown before this outbreak[94] but the long duration of persistence has been an unexpected finding.[95] For example, viral RNA is detectable in semen up to 18 months following discharge from an ETC.[96] There are few data available to estimate the proportion of male survivors affected. In one small convenience sample of survivors who were at varying durations into recovery, the overall prevalence of viral RNA positive semen was 49%.[97] Determinants of viral persistence in semen require further study.

There is also new evidence that women who recover from EVD during pregnancy can harbour persistent virus in the amniotic fluid and placenta and deliver an infected, stillborn fetus.[23,98,99] Additionally, there are reports of viral persistence in other body fluids that would not be considered to be immune privileged, albeit for a briefer timeframe. Case reports suggest shorter-lived persistence of viable virus (and viral RNA) in urine and viral RNA in sweat[85,100] and contribute to existing knowledge of persistence in vaginal, rectal, and conjunctival swab specimens and in breast milk.[94,101,102] Some caution is required when interpreting these small case studies; for example, the method used to collect a positive urine sample from a male patient was not described, raising the possibility of cross-contamination by virus present in semen.[85] Nonetheless, the viral kinetics of persistence in these fluids require closer examination, particularly when there are implications for guidance on preventing sexual transmission or potential transmission by breastfeeding.

The phenomenon of viral persistence means that, in limited circumstances, survivors can act as a reservoir for ongoing disease transmission. Convincing evidence now exists to show that men can transmit Ebola virus to women during sexual intercourse.[103,104] The prolonged duration of viral persistence in semen raises the possibility of sexual transmission occurring long after the resolution of acute illness. There is evidence that a flare of EVD in Guinea, which occurred months after the end of the Guinean outbreak, was caused by male-to-female sexual transmission (at approximately 470 days

after initial illness in the male partner).[103] There are no population-level data that predict the risk for sexual partners of EVD survivors, but the low incidence of new flares of disease provides some indication that transmission leading to disease is uncommon. There is no published, definitive evidence of female-to-male sexual transmission having occurred, or of mother-to-child transmission by breastfeeding.

There are several ongoing research priorities for survivors. Long-term studies are a priority because the longest survivor follow-up reported to date has been just over 2 years,[88] with ongoing symptoms reported at that time. Of note, there are no descriptions of the effect of EVD on childhood development and outcomes, and although the small amount of evidence so far suggests that EVD survivors might be at greater risk of pregnancy-related complications including stillbirth, these data require comparison with age-matched controls.[24,105] The risk of EVD recurrence and subsequent transmission by survivors is a key concern and so biological sampling in survivor cohorts is important to direct guidance on prevention strategies.[106] Although a biological sampling approach (based on sequential negative samples) seems reasonable, we first need to know the natural history of persistence (ie, whether detection of Ebola virus in semen can follow non-detection in earlier samples). Clinical trials of experimental drugs to clear persistent virus have commenced (registration numbers NCT2818582 and NCT02739477). To date, many of the available viral persistence studies have relied on reverse transcriptase PCR to identify viral presence, but future studies should also focus on identifying live virus, which is more indicative of potential transmission risk.

Therapeutics

Experimental treatments

Before the west Africa epidemic, experimental therapeutics had not been studied in patients with EVD, although transfusion of blood from convalescent patients had been tried.[107] The sheer scale of the west Africa epidemic demanded that effective, specific treatments should be identified and made available to patients as soon as possible. Accordingly, an expert panel was convened by WHO in September, 2014, to prioritise promising candidates for clinical trials.[12]

Disappointingly, no clinical trial of potential therapeutic agents has produced conclusive evidence of a beneficial effect (table 2). None of the trials have shown safety concerns for the respective agents, but safety and tolerability will need to be confirmed in subsequent studies. A phase 2 clinical trial of the antiviral favipiravir showed no survival benefit for patients with EVD and with a high viral load (cycle threshold <20), but suggested that further efficacy studies in patients with less advanced disease (cycle threshold ≥20) may be warranted.[50] A trial of the antiviral brincidofovir in Liberia was stopped before a conclusion could be reached after the drug company withdrew involvement in Ebola trials, in the setting of falling case numbers.[110] A phase 2, single-arm trial of the small interfering RNA lipid nanoparticle compound TKM-130803, done in Sierra Leone, showed no survival advantage in patients with severe EVD, compared with survival in historical (untreated) controls.[109] The Ebola-Tx trial showed no survival benefit in patients who received convalescent plasma compared with historical controls.[71] A separate report of the antibody titres in the transfused plasma found that concentrations of neutralising antibody were generally low and no significant association was found between antibody concentrations in the transfused units and patient survival.[111] A multicentre, randomised trial of the ZMapp triple monoclonal antibody cocktail found that the CFR in patients receiving ZMapp in addition to standard care (22%) was lower than in patients receiving standard of care alone (37%). Although this finding did not meet the prespecified statistical threshold for efficacy, the posterior probability that the addition of ZMapp improved survival was 91%.[108]

Other patients with EVD received experimental therapies on a compassionate basis, outside of clinical trials.[30,57,112,113] Many of these patients were treated in resource-rich countries and received a combination of experimental agents alongside intensive care support and nursing care, so it is difficult to assess safety or efficacy. A small number of patients in west Africa received repurposed agents (including lamivudine, amiodarone, atorvastatin, irbesartan, clomifene, and favipiravir) without enrolment in a registered trial.[114,115] Anecdotal reports of survival benefit have been reported for some of these agents,[114,116] but it is impossible to draw any meaningful conclusions.

A retrospective study of patient outcome data from an ETC in Liberia found a temporal association between the use of antimalarial combination artesunate–amodiaquine and a period of reduced EVD mortality.[117] Patients received this combination when there was a supply failure of the first line agent (artemether–lumefantrine), rather than for hypothesis-driven reasons. This supply failure, along with other limitations described by the authors, makes it difficult to interpret the findings from this study, but additional studies are warranted since in-vitro activity of amodiaquine against Ebola virus provides biological plausibility.[118]

Despite the largely negative outcomes from clinical trials, it must be recognised that the ability of researchers to overcome regulatory and operational barriers to complete trials to internationally accepted standards represents real progress, compared with previous outbreaks caused by high-hazard or emerging pathogens. Several ongoing challenges remain, however. For some drugs, the 100% survival rates seen in non-human primate models[119,120] were not replicated in clinical trials. The reasons underlying these discrepancies should be explored, to maximise the use of the animal model in

	Trial design	Research question (PICO model)	Registration number (declared status as of November, 2016)	Result
ZMapp	Open label RCT with adaptive trial design	Intervention: 50 mg/kg ZMapp, intravenous, every 3 days, total of three doses; comparison with optimised care alone (including favipiravir in Guinea); outcome measured as day 28 survival	Registered as PACTR201503001065306, NCT02363322 (completed)	No statistically conclusive benefit[108]
TKM-130803	Open label, single arm, Component of a multi-stage approach	Intervention: 0·3 mg/kg of TKM-130803, intravenous, once daily, total of seven doses; comparison with historical controls; outcome measured as day 14 survival	Registered as PACTR201501000997429 (completed)	No overall survival benefit[109]
Favipiravir	Open label, single arm	Intervention: 6000 mg (day 0) and 2400 mg (days 1–9), oral, daily of favipiravir, total of ten doses; comparison with historical controls; outcome measured as day 14 survival	Registered as NCT02329054 (completed)	No overall survival benefit[50]
Convalescent plasma	Open label, single arm	Intervention: 400–500 mL of convalescent plasma from two donors, administered as two consecutive (200–250 mL) transfusions; one treatment cycle in total; comparison with historical controls; outcome measured as day 14 survival	Registered as NCT02342171 (completed)	No overall survival benefit[71]
Convalescent plasma	Open label, single arm	Intervention: 180–220 mL of convalescent plasma from two donors, administered as two consecutive (90–110 mL) infusions; up to three treatment cycles, at least 48 h apart; no comparison made; outcome measured as Ebola virus load	Registered as NCT02333578 (recruiting)	NA
Convalescent plasma	Open label, single arm	Intervention: INTERCEPT plasma; dose not defined; comparison not defined; outcome measured as 1 year survival	Registered as NCT02295501 (open to enrolment)	NA
Convalescent plasma	Open label, random allocation	Intervention: single transfusion of convalescent plasma; dose not defined; comparison with Ringer's Lactate solution; outcome measured as all-cause mortality as 14 days after treatment	Registered as ISRCTN13990511 (ongoing; no longer recruiting)	NA
Brincidofovir	Open label, single arm trial, component of a multistage approach	Intervention: 200 mg brincidofovir oral, initial dose, then 100 mg, oral, twice weekly; total of five doses; comparison with historical controls; outcome measured as day 14 survival	Registered as PACTR201411000939962 (recruitment suspended)	No statistical conclusion[110]
Azithromycin, Sunitinib, Erlonitib, Atorvastatin, Irbesartan	Multi-arm RCT with adaptive trial design	Intervention: azithromycin (1500 mg, oral, daily for 5 days) *vs* sunitinib (50 mg, oral, daily for 7 days) and erlonitib (150 mg, oral, daily for 7 days) *vs* atorvastatin (40 mg, oral, daily until discharge) and irbesartan (150 mg, oral, daily until discharge); comparison with intravenous fluids and laboratory testing alone; outcome measured as day 14 survival	Registered as NCT02380625 (not yet open to recruitment)	NA
Interferon β	Open label, single arm	Intervention: subcutaneous interferon β once daily for up to 10 days; comparison not defined (safety and effectiveness study); undefined outcome	Registered as ISRCTN17414946 (completed)	NA
Amiodarone	Open label, RCT	Intervention: amiodarone (20 mg/kg, intravenous, on days 1–3 then 200 mg, oral, three times daily, on days 4–10); comparison with supportive care alone; outcome measured as day 10 survival	Registered as NCT02307591 and PACTR201501001014425 (withdrawn)	NA

Where a dose of an intervention has been stated, it refers to the stated adult dose. Refer to trial protocols for weight adjustment. PICO=participant, intervention, comparison, outcome. RCT=randomised controlled trial. NA=not available.

Table 2: **Patient-based clinical trials of experimental therapeutics registered on clinical trial databases during the west Africa Ebola virus disease outbreak**

drug development. Explanations might include inherent biological differences between species, animal models that do not match human illness,[109,121] differences in exposure route and infectious dose, or that some patients present late in the course of illness with complex end-organ manifestations that cannot be simulated completely in an animal model.

Vaccines

The epidemic also prompted accelerated efforts to take leading vaccine candidates to clinical trials, and to advance preclinical pipelines for less-developed candidates.[122] Overall, four candidate vaccines met WHO criteria for fast-tracked clinical assessment: the replication competent recombinant vesicular stomatitis virus (rVSV) vaccine expressing Zaire Ebola virus gylcoprotein (ZEBOV), the replication-deficient chimpanzee adenovirus serotype 3 vector vaccine (ChAd3-ZEBOV), followed later by another adenoviral vectored vaccine (Ad26-ZEBOV) with a heterologous boost (modified vaccinia virus Ankara, MVA), and a nanoparticle vaccine (Novavax).[123] The first clinical trial in a highly affected country commenced in February, 2015; with the exception of the nanoparticle vaccine, for which the phase 1 trial is ongoing, all of the candidates have been investigated in clinical trials in the region (table 3).

The phase 3 *Ebola* ça *suffit* rVSV-ZEBOV trial done in Guinea yielded remarkable interim findings.[124] This study used a novel approach of ring vaccination, a method that was first used during smallpox eradication programmes and involves vaccination of high-risk contacts (defined geographically or socially) of known EVD cases, with the aim of interrupting transmission. Rings of contacts received either immediate or delayed (21 days postexposure) vaccination in a cluster randomised trial. This pragmatic approach aimed to balance the

	Trial design	Research question (PICO model)	Registration number (declared status as of November, 2016)
rVSV ZEBOV			
Ebola ça suffit!	Open label, cluster randomised, ring vaccination	Participants include contacts of confirmed EVD patients; intervention with immediate vaccination with rVSV ZEBOV; comparison with delayed (day 21) vaccination; outcome measured as safety and efficacy	Registered as PACTR201503001057193 (interim results available[124])
Ebola ça suffit!	Open label, single arm	Participants include adult front-line workers; intervention with immediate vaccination with rVSV ZEBOV; comparison with delayed (day 21) vaccination; outcome measured as safety and efficacy	Registered as PACTR201503001057193 (closed to recruitment, follow up complete[125])
STRIVE	Open label, randomised, with two substudies	Participants include adult front-line workers; intervention with immediate vaccination with vVSV ΔG ZEBOV; comparison with delayed (18–24 weeks) vaccination; outcomes measured as safety, efficacy, and immunogenicity	Registered as NCT02378753, PACTR201502001037220 (ongoing but not recruiting)
Multiple			
PREVAC	Double-blind RCT	Participants include children and adults; intervention with immediate vaccination with rVSV-ZEBOV (with or without rVSV boost) or Ad26.ZEBOV + MVA-BN-Filo boost; comparison with placebo; outcomes measured as safety and immunogenicity	Registered as NCT02876328 (not yet open for recruitment)
PREVAIL	Double-blind RCT	Participants include adults with Ebola virus infection; intervention with immediate vaccination with VSVG-ZEBOV or ChAd3-EBO Z; comparison with placebo; outcomes measured as safety and immunogenicity	Registered as NCT02344407 (ongoing, but not recruiting, no results available)
Ad5-EBOV			
Ad5-EBOV	Double-blind RCT	Participants include healthy adults aged 18–50 years in Sierra Leone; intervention with high dose, or low dose immediate vaccination with Ad5-EBOV; comparison with placebo; outcome measured as safety and immunogenicity	Registered as NCT02575456, PACTR201509001259869 (completed, no results available)
Ad26. ZEBOV + MVA-BN-Filo			
EBOVAC	Open label, single arm, followed by double-blind RCT	Participants include healthy adults and children in Sierra Leone; intervention with immediate vaccination with Ad26-ZEBOV and with MVA-BN-Filo boost; comparison with placebo (meningococcal vaccine during immediate vaccination) during the second stage of the RCT; outcome measured as safety, immunogenicity, and efficacy	Registered as NCT02509494, PACTR201506001147964 (recruiting)

EVD=Ebola virus disease. PICO=participant, intervention, comparison, outcome. RCT=randomised controlled trial.

Table 3: **Vaccine trials recruiting in the most affected countries during the Ebola virus disease outbreak in west Africa**

requirement for high-quality efficacy and safety data against ethical concerns about using placebo designs in highly susceptible populations in the midst of an EVD outbreak.[126] Preliminary results suggest excellent efficacy (100%, 95% CI 75–100). There were no new infections after 6 days in participants that were immediately vaccinated (n=2014), compared with 16 infections in the delayed vaccination group (n=1930).[124] In light of these findings, randomisation was stopped and all subsequent participants received immediate vaccination. Concerns have been raised about the reactogenecity of rVSV-ZEBOV following observed, transient fever (up to 30%), arthritis (3–22%), rash, and dermatitis in phase 1 trials in Africa and Europe.[127] Whether these findings apply to other populations is unknown, as is the effect of potential side-effects on the acceptability of the vaccine among individuals at varying levels of risk of EVD. A substantial practical challenge to rolling out this vaccine in an outbreak would be differentiating those with transient vaccine-related fever from those who are developing symptomatic EVD. Additionally, the transient viraemia triggered by vaccination could also result in a false-positive PCR result with some tests.[128]

Adenovirus vector vaccines were the second type of vaccine to reach clinical trials in the affected countries. Phase 1/2a trials of ChAd3-ZEBOV showed safety.[129–131] However, a trial with study groups in the USA and Mali showed that a single dose of vaccine elicited sufficient immunogenicity likely to be effective in postexposure prophylaxis scenarios, but that a heterologous prime and boost (with modified vaccinia Ankara expressing Zaire Ebola virus glycoprotein) would be more appropriate when an extended period of protection was required.[129] The superior protective efficacy of a heterologous prime-boost regimen has been shown in other phase 1 trials of ChAd3[132] and Ad26-ZEBOV[133] and, in practical terms, might make it important for groups who have prolonged exposure periods—eg, health-care workers

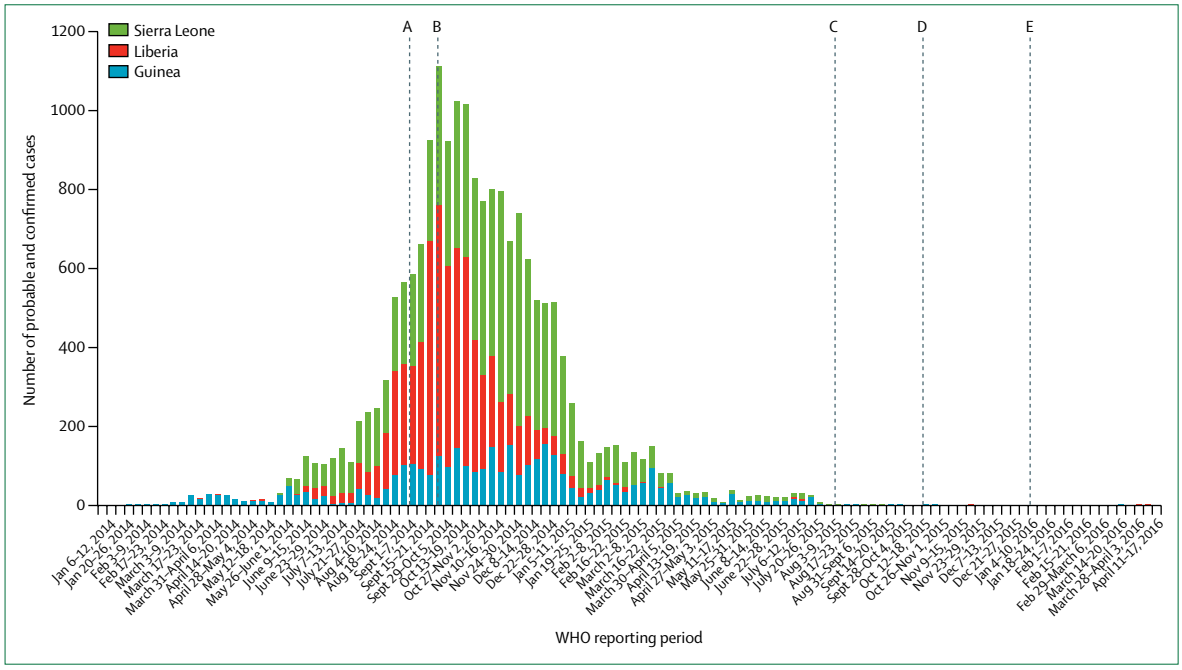

Figure: **Significant research advances during west Africa Ebola virus disease epidemic**
Adapted from Bausch and Rojek (2016).[134] (A) WHO holds consultation on potential Ebola therapeutics and vaccines.[135] (B) WHO Response Team publishes first large observational patient data set.[13] (C) Interim results of VSV-ZEBOV vaccine trial published.[124] (D) Molecular evidence for sexual transmission published.[104] (E) First clinical trial of experimental treatment (convalescent plasma) published.[71]

and burial teams.[129] As we learn more about viral sequestration and sexual transmission, more durable vaccine-induced immunity might be required to provide longer-term protection of sexual partners or survivors of EVD. However, the inclusion of a boosting component will add to the logistical complexity of mass vaccination. The results of field trials of adenovirus vector-based vaccines are awaited (table 3).

Other ongoing vaccination trials in the region commenced too late to identify effectiveness. However, they should be able to provide important safety and immunogenicity data, including comparative data for different candidate vaccines. This presents a dilemma with respect to licensure of these vaccines. Although it is possible that promising Ebola vaccines could receive regulatory approval if human safety and immunogenicity data are supported by evidence of efficacy in non-human primate studies, the limitations of the present animal model and an imprecise understanding of immune correlates of protection mean there is little certainty in this process. The ongoing development and assessment of different vaccines are important, because it is unlikely that a single vaccine will meet all of the criteria in the WHO target therapeutic profile.[123]

Conclusions

There have been several notable successes in the scientific response to this epidemic, including improved characterisation of EVD complications and the completion of clinical trials of experimental therapeutics (figure). Progress was slow in other areas. Despite the large number of patients, the reporting of clinical manifestations was fragmented and many published studies have described small cohorts or single cases. Data collection was frequently ad hoc or retrospective, highlighting the need to embed clinically relevant research in outbreak preparedness and response. Knowledge of how EVD affects susceptible populations, such as pregnant women and children, has not progressed substantially. We do not know the true benefits (or potential harms) of administering specific components of supportive care. Reporting on the outcomes of patients treated in resource-rich countries has been descriptive and repetitive, and only one medically evacuated patient was recruited to a clinical trial.

An important question is how to apply findings from studies that have generated new information, particularly when the results are inconclusive. For example, despite the absence of incontrovertible evidence of efficacy, it is possible that ZMapp will be included as standard of care in future EVD outbreaks; if this happens, it is likely that trials of any new agents will need to show superiority of the new agent given alongside ZMapp, compared with ZMapp alone. Such trials will also need to stratify by viral load on admission.[73]

Individual components of supportive care interventions have not been assessed in EVD-specific trials. The rationale of providing intravenous fluid replacement to patients with substantial gastrointestinal fluid losses is clear, but there is scope to compare different empirical

Search strategy and selection criteria

We searched PubMed for articles published from the beginning of the west Africa outbreak on Jan 1, 2014, to Nov 30, 2016, using search terms "Ebola" or "Ebola virus" or "Ebola Virus Disease" or "Ebola Haemorrhagic Fever" using British and American spelling variations. We reviewed the articles from the search that had an abstract available in English in addition to relevant references cited in those articles and conference and international meeting reports. We reviewed all publications that contained original research or patient data for quality and relevance. To identify ongoing unpublished clinical research, we searched clinical trial databases ClinicalTrials.gov, the Pan African Clinical Trials Registry, and the ISRCTN registry.

Two authors (AR, JD) categorised all papers according to predefined subject area, using publication review software (appendix). There were no discrepancies in individual categorisation that required mediation from the third author (PH). Papers were selected for inclusion on the basis of clinical relevance by joint review of two authors (AR, JD). A few papers that were published before the outbreak were included where comparison with existing knowledge was considered necessary; these were identified from the libraries of the authors.

For the **software** see http://rayyan.qcri.org/

See **Online** for appendix

fluid replacement regimens and investigate the optimal timing of fluid replacement. Although the observational studies of patients treated in Europe and the USA suggest that physiological support does contribute to survival, many of the advanced interventions used will be difficult to translate to the typical ETC environment and so a key component of assessment will be feasibility and practicality.

For pharmaceutical interventions that could alter the course of future outbreaks, the greatest hope comes from the *Ebola* ça *suffit!* ring vaccination trial. The final results from this trial[136] were published after the literature search for this Review, and the results confirm the highly promising interim findings.[124] It is likely that ring vaccination strategies will be adopted in future outbreaks caused by Zaire Ebola virus strains.

Additional findings from the west Africa epidemic are expected and it is hoped that new data will contribute to the knowledge base. The degree to which findings from this epidemic can be applied to future outbreaks, including those caused by different species of Ebola virus, is unknown; a comparison of key clinical findings from different outbreaks would be useful, but would rely on high-quality, comparable datasets being available. Future, smaller EVD outbreaks can be expected in at-risk countries and clinical studies will need to be rapid and efficient; greater yields may be obtained if research priorities are agreed in advance, with centralised coordination of studies.

In all of the fields reviewed, we have discussed areas of priority for future investigation. To achieve the most rigorous outcomes from future studies, there must be an improved commitment to producing protocol-directed, hypothesis-driven research whenever possible. When this is infeasible, recommendations should be based on careful, systematic data collection and use of shared platforms that facilitate data collation across different sites. This data collation will require not only a commitment from scientists but also funding and publishing mechanisms that facilitate and reward collaborative science.

Contributors

AR performed the literature search, according to the stated search strategy. AR, PH, and JD reviewed and selected articles to include in the Review, based on the stated selection criteria. AR produced the figures and tables. All authors contributed to writing the Review.

Declaration of interests

We declare no competing interests. The authors were investigators for two clinical trials described in this Review (TKM-130803 and brincidofovir trials).

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B

Supplementary manuscript 2

Annals of Internal Medicine RESEARCH AND REPORTING METHODS

Clinical Trials of Therapeutics for the Prevention of Congenital Zika Virus Disease: Challenges and Potential Solutions

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Zika virus (ZIKV) infection in pregnancy is associated with adverse fetal outcomes, such as microcephaly and other congenital malformations. No therapeutic options are available to pregnant women with ZIKV infection to prevent these effects. Drug trials in pregnancy raise several scientific, ethical, and logistic challenges, which are compounded further in ZIKV because of limited knowledge of the disease pathophysiology and a product development pipeline in its infancy. We evaluate the major challenges in choosing therapeutics to prevent congenital ZIKV disease and conducting clinical trials of these treatments, with a focus on preventing congenital central nervous system malformations. These challenges must be characterized and planned for now so that clinical trials can progress expediently and effectively in the future.

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Zika virus (ZIKV) is a flavivirus transmitted by the *Aedes* mosquito (1). As of 29 December 2016, 75 countries, including the United States, reported mosquito-borne transmission (2). The evidence for a causal association between maternal ZIKV infection and congenital malformations is now accepted (3). A more extensive spectrum of birth defects, including central nervous system (CNS), genitourinary, gastrointestinal, and cardiac malformations, likely will become apparent over time (4). The World Health Organization (WHO) recently announced an emergency, fast-tracked research and development agenda, including the need for ZIKV therapeutics to be evaluated in clinical trials (5). Trials of therapeutics in nonpregnant adults would offer limited estimates of safety and effectiveness in preventing adverse fetal outcomes; therefore, trials in pregnant women are specifically needed. However, drug trials in pregnancy raise ethical, scientific, and logistical challenges (6), which are compounded in ZIKV because of limited knowledge of the epidemiology and pathogenesis of adverse fetal outcomes. We discuss the issues surrounding the choice and investigation of therapeutics to prevent congenital ZIKV disease (CZD), with a focus on CNS malformations.

Candidate Drug Selection

The theoretical mechanisms by which maternal ZIKV infection disrupts fetal CNS development are numerous and complex (Figure). A major obstacle to choosing therapies for research is our limited understanding of the predominant mechanisms underlying CZD, intrauterine growth restriction (IUGR), and fetal loss (7). In this section, we discuss some general aspects pertinent to drug selection.

Targeting Viral Replication

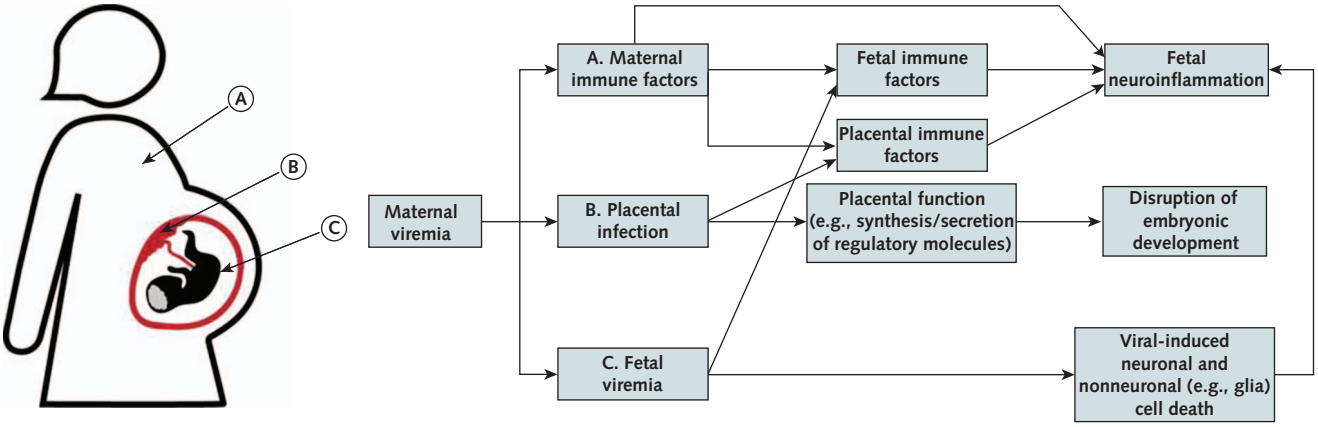
Targeting viral replication is an upstream approach that avoids narrowly targeting only one of several potential pathogenic downstream consequences of infection (Figure). It also prevents some of the risks associated with targeting host factors (discussed later). The full spectrum of congenital disease likely is broader than currently appreciated and may not become apparent until childhood. Several factors, including host responses, nutritional factors, and inoculation dose, may influence this spectrum. Directly inhibiting viral replication would minimize the therapeutic variability among individuals as a result of such factors.

Direct-acting antiviral small molecules are prime candidates for investigation. High-throughput screens and computational drug discovery have led to identification of candidate drugs for dengue, a related flavivirus (8). Drug repurposing therefore may be an efficient approach and has been used in other epidemics, such as the Ebola outbreak (9). Priority should be given to antiviral and nonantiviral drugs that have shown in vitro activity against other flaviviruses and Flaviviridae and have already been approved by regulatory agencies in other contexts (8). Several drugs approved by the U.S. Food and Drug Administration (FDA), some of which have activity against dengue, hepatitis C, chikungunya, and Japanese encephalitis, were recently screened for anti-ZIKV activity (10–13). Sofosbuvir (11), niclosamide (12), ivermectin (13), mefloquine (13), daptomycin (13), bortezomib (13), and mycophenolic acid (13), among others, have anti-ZIKV activity in vitro. The viral polymerase inhibitor 7-deaza-2′-C-methyladenosine also has in vivo activity against ZIKV in mice (10). The use of ZIKV-specific, target-based chemogenomic approaches would help further narrow the list of promising candidates for testing in vitro and in vivo (8).

Targeting Host Neural Receptors for the Virus

Zika virus has been isolated from brain tissues of human fetuses with microcephaly (14), confirming that fetal infection may occur. The virus may be transferred via placental infection or by passage across the placenta (15), followed by entry into the CNS and replication within neurons and glia (16). Although the cell surface receptors by which ZIKV enters neurons have not yet been fully functionally characterized, AXL is a key receptor for viral entry into other cells (such as fibroblasts and dendritic cells) (17), and AXL and other putative flavivirus entry receptors are strongly expressed in neural stem cells (NSCs) of the developing brain (18). In vitro human cell and in vivo mouse studies have demonstrated that ZIKV preferentially infects forebrain-

Figure. Theoretical mechanisms underlying Zika virus disruption of central nervous system development.



specific NSCs, resulting in cell death, cell-cycle dysregulation, and microcephaly (19, 20). Although prolonged ZIKV infection of the fetoplacental unit may occur (14), persistence of virus within the fetal CNS may not be necessary for microcephaly to result. The stem cell pool has a finite ability to regenerate (21); therefore, partial destruction of the NSC pool may have cascading consequences on brain development throughout gestation. Primary microcephaly itself is hypothesized to result from depletion of the founder NSC populations (22). These findings highlight AXL, as well as other entry receptors, as an investigative target for blockade. However, normal AXL signaling supports various aspects of CNS development and function (23), making it a problematic target. More generally, because many flavivirus entry receptor genes likely play a role in normal CNS development (24), targeting them is an unpredictable strategy.

Targeting the Host Immune Response

Both fetal and maternal immune responses may have profound effects on fetal neurodevelopment (25, 26). The development of the fetal immune system begins in earnest in the second trimester (27), suggesting that the fetal immune response to ZIKV likely does not play a substantial role in disease during early maternal ZIKV infection. However, the spectrum of congenital malformations is potentially wide, and congenital disease may occur as a result of mid to late maternal infection (4), raising the possibility of several pathogenic mechanisms. Maternal immune factors also might play a role at any stage of pregnancy.

For example, ZIKV-induced activation of Toll-like receptor 3 (TLR3) results in stunted growth of human embryonic stem cell–derived cerebral organoids (an in vitro model for human embryonic CNS development) (28), which is prevented by TLR3 inhibition (28). In the CNS, TLR3 is a key innate immune antiviral pathogen recognition receptor (29), whose activation results in the transcription of genes involved in both immunity (29) and neuronal development (Salam AP. Unpublished data). Besides TLR3 signaling, other immune pathways likely are activated by ZIKV, and some of them also will disrupt CNS development (30). Thus, targeting host immune pathways may represent a therapeutic strategy for preventing disruption of CNS development. However, unperturbed TLR3 and CNS immune function play a critical role in CNS development (30); therefore, any attempt to modulate TLR3 function in utero, as with AXL, may lead to unpredictable consequences.

Targeting Placental Dysfunction

Altered placental function due to placental infection or maternal immune factors may lead to the release of placental immune factors that are neurotoxic to the fetus or disrupt the synthesis of placental molecules necessary for healthy embryonic development (15). In pregnant mice, systemic ZIKV infection results in placental damage and insufficiency, leading to IUGR, fetal loss, and microcephaly (20, 31). In addition, ZIKV impairs placental autophagy (17), which normally limits viral replication (32). Placental vulnerability to infections seems pronounced early in pregnancy (33), consistent with the greatest risk for microcephaly occurring during ZIKV infection in the first and early second trimesters (34). Like targeting the host immune response or cell entry receptors, however, placental function exists in a delicate homeostatic balance (35); therefore, modulation of placental function is potentially unpredictable. Several animal studies, however, successfully tested the feasibility of targeting placental dysfunction in the context of preeclampsia and IUGR. Human clinical trials investigating the effects of such agents as arginine, sildenafil, pravastatin, and oxygen supplementation therapy on IUGR are ongoing (36).

The Use of Pathogen-Specific Antibodies

The administration of pathogen-specific antibodies (PSAs), such as human convalescent plasma or human immune globulin, is established for several infections in general (37) and, to a limited extent, for some during pregnancy (38, 39). Pathogen-specific antibodies bind to ligands on viral surfaces, preventing the virus from

entering cells ("neutralization") or activating the immune system, resulting in viral destruction and lysis of infected cells. In immunologically naive persons, PSAs are used to prevent maternal measles and varicella-zoster virus after exposure (38, 39); however, evidence that PSAs can prevent or limit the adverse consequences of established maternal infection on fetal outcomes is inconclusive. For example, administration of cytomegalovirus (CMV)-specific hyperimmune globulin in maternal primary CMV infection was associated with reduced maternal-to-fetal transmission and congenital infection severity in several prospective, observational studies (40–42). Nevertheless, a recent small-scale randomized trial failed to demonstrate a statistically significant benefit (43); however, the researchers did not assess hearing loss in infants (a common consequence of maternal CMV infection), the study may have been underpowered, the timing of primary CMV infection relied partly on subject recall, and a delay of up to 6 weeks occurred between the estimated primary infection and therapy. Two larger phase 3 trials are ongoing (44, 45).

One benefit of the use of nonengineered PSAs is their relative safety. The most serious side effects are transfusion reactions, but these are rare. In addition, PSAs generally are considered safe for the fetus (46), which highlights them as a potential "ready-to-go" therapeutic candidate. Given their approved use in other maternal infections, PSAs may not have to undergo additional preclinical safety testing. The main limitations of nonengineered PSAs are primarily logistic and include issues surrounding collection, storage, and transport. Further, in vitro evidence suggests that antidengue antibodies may result in antibody-dependent enhancement of ZIKV replication (47); therefore, the donor pool may be required to have high anti-ZIKV antibody but low or absent anti-dengue antibody titers. This donor pool may be difficult to populate given that dengue is endemic in the most affected country, Brazil (48). Although engineered PSAs against ZIKV would circumvent this issue, they would require extensive preclinical and clinical safety testing.

Pharmacokinetics and Pharmacodynamics in Pregnancy

Pregnancy results in dramatic physiologic changes that affect drug absorption, distribution, metabolism, and excretion (49). Elevated progesterone levels prolong small-bowel transit time by 30% to 50% (49). Hemodilutional hypoalbuminemia (49) affects drug bioavailability. Hormonal alterations in drug-metabolizing enzymes (49) and changes in renal blood flow (49) affect drug metabolism and clearance. Further, the chemical characteristics of drugs affect the way they cross the placenta, including simple diffusion, facilitated diffusion, pinocytosis, and active transport (50). Thus, for de novo ZIKV therapeutic agents and some repurposed drugs, substantial preclinical research using models of human placental function will be required. Placental function shows dramatic interspecies variation (51), limiting our ability to draw definitive conclusions from animal models alone. In vitro models, such as explants of human placenta, may offer additional insights (52). Placental infection and inflammation may alter drug and immunoglobulin passage across the placenta (53), and preclinical testing should include models of placental function in the context of ZIKV infection. Whether a ZIKV therapeutic agent crossing the placenta, as well as the fetal blood–brain barrier, is desirable will depend on the therapeutic strategy. Our understanding of human fetal blood–brain barrier development is very limited, however, and complicated by the fact that inflammation may alter blood–brain barrier permeability (54).

Therefore, maternal pharmacokinetic monitoring of any therapeutic agent is important. Pharmacokinetic monitoring of PSAs may be complicated by cross-reactivity with other flavivirus antibodies. Fetal pharmacokinetic monitoring would be advantageous depending on the therapeutic strategy and target, although it requires amniotic fluid sampling, which is invasive, requires trained personnel, and has risks. If therapy were to continue close to delivery, umbilical cord blood might be sampled for pharmacokinetic monitoring.

Teratogenic Risk

Some of the candidate agents identified by drug repurposing screens cross the placenta and are rated as class B by the FDA, meaning that animal reproduction studies failed to demonstrate fetal risk, and no adequate, well-controlled studies were done in pregnant women. In view of the limitations of rodents as a model for human CNS development, nonhuman primate models may be needed (55); however, preclinical testing in such models is lengthy and limited by small numbers of animals. Predictive drug–target gene models of clinical teratogenicity might help prioritize the spectrum of candidate drugs for repurposing (56). For example, drugs with medium in vitro and in vivo efficacy but low predicted teratogenic risk might take priority over those with high in vitro and in vivo efficacy but medium–high predicted teratogenic risk.

The knowledge threshold for teratogenic risk ideally would be as—if not more—stringent as normally expected; however, a strict threshold might severely limit the choice of therapeutic agents that could be studied. Thus, conducting a clinical trial of a drug for which slightly less information than usual is available regarding teratogenic risks may be appropriate. Either way, the actual risk for teratogenicity associated with any drug has to be balanced against the risk for CZD. Accepting a higher-than-normal risk for teratogenicity may be appropriate, as long as the risk is lower than that for severe CZD. Thus, therapeutics with a low risk for major teratogenicity or medium risk for minor teratogenicity still may benefit fetuses at high risk for severe CZD. Defining the fetal populations with the greatest risk for severe malformations therefore is critical.

Table 1. Suggested Enrollment Criteria

Inclusion (all criteria must be met)
Positive result on β-human chorionic gonadotropin testing
1–22 weeks' gestational age according to last menstrual period or ultrasonography, if available
Meets the clinical case definition for a suspected case
Onset of symptoms within 72 h
Positive results on blood PCR testing for ZIKV
Exclusion (≥1 criteria must be met)
Family history of congenital malformations (unless confirmed to be a result of ZIKV infection)
Suspected or confirmed TORCH infection previously during the same pregnancy
Concomitant co-infection with dengue or chikungunya viruses
Chronic medical conditions (e.g., diabetes and hypothyroidism)
Long-term medications
Alcohol, tobacco, or recreational drug use during pregnancy
Existing ultrasonographic evidence of congenital malformations before enrollment

PCR = polymerase chain reaction; TORCH = toxoplasmosis, other (syphilis, varicella-zoster, and parvovirus B19), rubella, cytomegalovirus, and herpes; ZIKV = Zika virus.

STUDY POPULATION

Table 1 suggests an appropriate study population. Several knowledge gaps exist that limit the precise definition of the group most likely to benefit. When the virus crosses the placenta following maternal infection (15), how long viral replication persists in the mother and fetus (14) and whether latent viral reservoirs exist within the mother and fetus are unknown (57). Whether the development of microcephaly depends on prolonged exposure of the fetus to ZIKV or whether only a short period of exposure is necessary to initiate a cascade of developmental disruption is unclear. Initial therapeutic trials therefore should focus on inhibiting viral replication in symptomatic pregnant women with evidence of acute ZIKV infection. Although fetal malformations occur in asymptomatic maternal infections (58), screening asymptomatic women regularly during pregnancy (for example, at least weekly) as an initial trial strategy would be logistically demanding. Screening or prophylaxis in high-risk asymptomatic women might be a viable strategy in the future.

Serologic assays should not be used to confirm infection because of cross-reactivity with other flaviviruses and the predicted time course of anti-ZIKV antibody responses (59). Also, they offer no information regarding ongoing viral replication. A positive ZIKV polymerase chain reaction (PCR) test in blood or urine therefore should be considered as evidence of infection. Patients with co-infections, such as dengue or chikungunya, should be excluded, and a multiplex PCR test for ZIKV, dengue, and chikungunya would be useful. However, PCR assays also are limited in their ability to assess viral clearance, because undetectable RNA in blood or urine does not exclude ongoing replication in other compartments. Because antibody-dependent enhancement may enhance ZIKV infection, patients with evidence of previous dengue infection (that is, positive IgG but negative IgM and PCR) may be among those who benefit the most from therapy.

A sensible limit on time elapsed between symptom onset and start of therapy will need to be defined. Starting therapy too soon would limit study enrollment and potential translatability to clinical practice; waiting too long might result in therapy being started too late to prevent congenital disease. Different therapeutic targets and agents likely have different therapeutic time windows; for example, PSAs seem to work best when given very early in the disease course (60). In general, we suggest choosing a relatively narrow time window between symptom onset and the beginning of therapy, ideally 72 hours. The time frame also will depend on the local health infrastructure and study logistics and may not represent a viable, real-world clinical strategy. One potential way to limit this challenge is to enroll seronegative pregnant women in high-transmission areas at their first prenatal clinic visit (with instructions to contact the study team if they develop symptoms). A more realistic time frame may be up to 5 days, which might be extended further with the explicit aim of determining the point at which therapy is no longer effective. Large sample sizes would be needed to perform subgroup analyses according to time to therapy.

Women infected in the first and early second trimesters likely would benefit the most, given the course of fetal development (61) and suggestions that the risk for congenital malformations is greatest in early pregnancy (34). Therefore, initial trials should focus on these periods while further epidemiologic data are awaited. Whether virus persists in maternal latent sites (57) raises the question of whether a single course of therapy is sufficient or whether therapy should be repeated. In the case of CMV infection, for example, hyperimmune globulin is administered at monthly intervals throughout pregnancy. This is a critical question that must be addressed. One option may be a randomized 3-group study design: placebo versus single dosing versus multiple dosing. Because of their long half-lives, PSAs may offer an advantage over other therapeutics (62) by obviating the need for daily or weekly dosing. Administering PSAs monthly also might circumvent the theoretical issue of maternal susceptibility to ZIKV reinfection, given the course of immune changes during pregnancy (63). However, multiple PSA doses may increase the risk for antibody-dependent enhancement of ZIKV disease.

STUDY END POINTS

At the very least, primary end points should include miscarriage or stillbirth, presence and degree of IUGR, and presence and degree of microcephaly. The WHO defines microcephaly as an occipitofrontal circumference greater than 2 SDs below the mean (64); however, this definition is somewhat controversial, and other criteria have been used (65). On the basis of the WHO definition, 2% of the general population would be classified as having microcephaly, even though these persons simply are at the low end of the population distribution (66). Furthermore, occipitofrontal circumference is an indirect measure, and microencephaly

(an abnormally small brain) may be present despite normal occipitofrontal circumference. Regional structural changes also likely affect prognosis (67). For these reasons, secondary end points should include magnetic resonance imaging and ultrasonographic assessments in newborns to identify agyria, lissencephaly, ventriculomegaly, intracranial calcifications, and other abnormalities (68). The use of imaging analytics should be standardized among studies, and consensus will be required to define and grade microcephaly and neuroimaging findings.

Additional secondary end points include adverse drug events; degree and duration of maternal viremia (including recurrence); viremia in amniotic fluid; virologic and immunohistologic findings in the placenta; gestational age at delivery; and presence of congenital defects, such as cleft lip, gastroschisis, spina bifida, and heart defects, in newborns. A consistent, consensus-based system for identifying and recording birth defects will be required (69). Fetal ultrasonography should be used to monitor therapeutic efficacy and safety; however, determining whether rare congenital defects are the result of ZIKV or a drug side effect will be challenging. Furthermore, because congenital malformations should be characterized by degree of severity, their identification on ultrasonography despite ongoing therapy would not necessarily imply complete treatment failure. Similarly, detection of ZIKV in amniotic fluid may not necessarily predict treatment failure, because the level of virus in amniotic fluid may determine the risk for complications, as in CMV (70). Thus, a threshold for detectable virus may exist below which therapy likely will still be effective and should continue. Other secondary end points should include epilepsy and neurodevelopmental delays, including vision and hearing defects during the first year of life and ideally throughout childhood. Again, consensus will be required regarding which neurodevelopmental screening tools to use, and their use should be standardized across observational and therapeutic studies. Sampling of neonatal cerebrospinal fluid for ZIKV RNA should be considered.

Maternal safety monitoring will vary according to the therapeutic agent studied but should, at a minimum, include monitoring of hematologic indices as well as renal and liver function. However, normal pregnancy is associated with several changes in blood indices, including anemia and thrombocytopenia (49), mild increases in alanine and aspartate aminotransferase levels, and marked increases in alkaline phosphatase levels during the third trimester (49), and these changes may complicate monitoring for drug side effects.

CONCLUSIONS

Clear challenges exist in selecting ZIKV therapeutics for CZD prevention, as well as in designing trials. These issues may resurface in a future epidemic of a related or unrelated pathogen. Table 2 summarizes key considerations and recommendations.

Table 2. Key Considerations and Recommendations

Product development should focus on targeting viral replication/survival rather than the host immune response.
Drug repurposing of antiviral small molecules against the Flaviviridae family should be a first approach.
Nonhuman primate models should be used to investigate teratogenic drug risks given the limitations of rodents as models of human CNS development.
Nonengineered PSAs are already used to prevent some infection-related congenital syndromes, have an acceptable safety profile, and may be suitable phase 2 candidates.
The donor pool for anti–ZIKV antibodies should be screened for antibodies against other flaviviruses to minimize the risk for antibody-dependent enhancement.
Multiple regular doses of therapy may be required to prevent congenital malformations given the possibility of viral latent reservoirs, and a 3-group trial design should be considered.
Initial trials should focus on symptomatic pregnant (first-trimester and early second-trimester) women with positive results on blood PCR testing.
Ideally only women who present within 72 h of symptom onset should initially be targeted.
Secondary study end points should include screening for various congenital malformations and neuroimaging assessments.
Secondary study end points should include neurodevelopmental screening assessments, including vision and hearing, during the first few years of life.
The success of therapy should be judged not exclusively by binary outcomes, such as the presence or absence of microcephaly, but also by degree of severity.
The identification of the virus in amniotic fluid or congenital malformations in the context of ongoing therapy should not necessarily imply treatment failure until the degree of disease severity can be characterized.
Consensus and standards therefore are required for disease definitions and classifications, as well as for identifying congenital malformations secondary to therapy.
The target product profile must be low risk to the mother and the fetus and effective in preventing adverse fetal outcomes.

CNS = central nervous system; PCR = polymerase chain reaction; PSAs = pathogen-specific antibodies; ZIKV = Zika virus.

Trials of ZIKV therapeutics in pregnancy raise specific ethical challenges for the fetus and mother. The risk for teratogenicity needs to be balanced against the risk for congenital disease due to ZIKV infection. Defining the fetal populations at greatest risk for severe malformations therefore is critical. Because symptoms of maternal ZIKV infection generally are mild (7), using a drug that poses a substantial risk for serious morbidity or mortality to the mother would be unethical. An ethical framework is needed to address this issue. Ongoing drug discovery and development should be accompanied by preemptive work to produce clinical trial protocols that are resilient to our current knowledge limits and acceptable to the population most at risk. The target product profile should be low risk and acceptable to the mother, low risk to the fetus, highly effective in preventing adverse fetal outcomes, and practical for widespread clinical use in resource-limited settings.

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AD LIBITUM

The Clearing

It came to him
just after his hand left the cold metal rail
and he began to drop.

Before,
a leaden shroud
of sadness
and separation
led him to deem
his agony no longer bearable.

So,
to end his torment,
he jumped off the Golden Gate Bridge.

What came to him as he fell,
tumbling and flailing,
was a thought unexpected–
"The only real problem in your life
is that you just jumped off the Golden Gate Bridge."

He saw the thing that mattered,
that made the rest disperse,
that needed attention utterly.

Having seen,
he fought to right himself,
did,
and lived.

Seeing the thing that mattered–
and righting himself–
he lived.

Lawrence J. Hergott, MD
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Final approval of the article: A.P. Salam, A. Rojek, J. Dunning, P.W. Horby.
Collection and assembly of data: A.P. Salam, A. Rojek.

C

Additional figures for Chapter 4

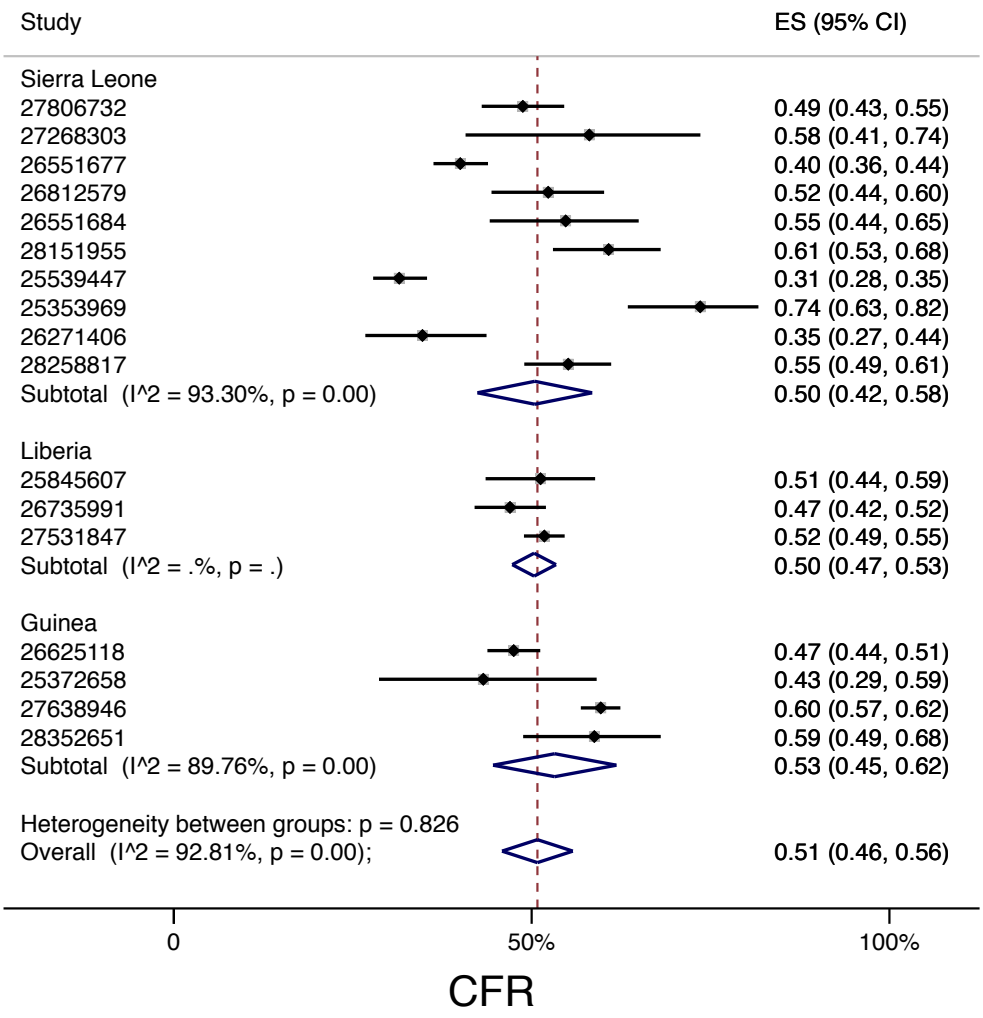
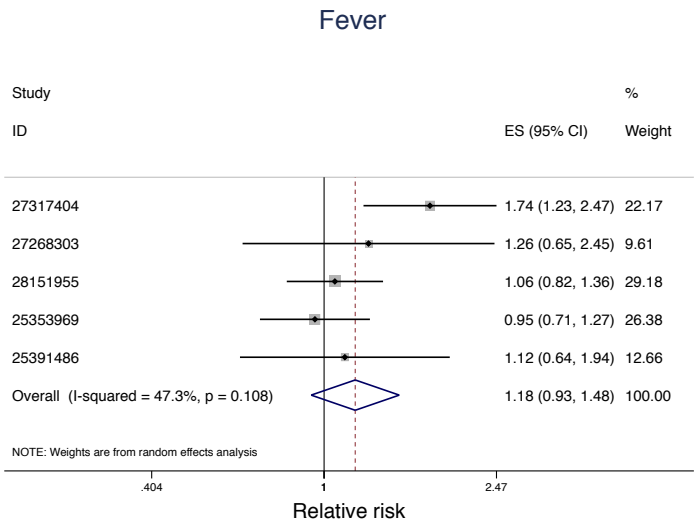
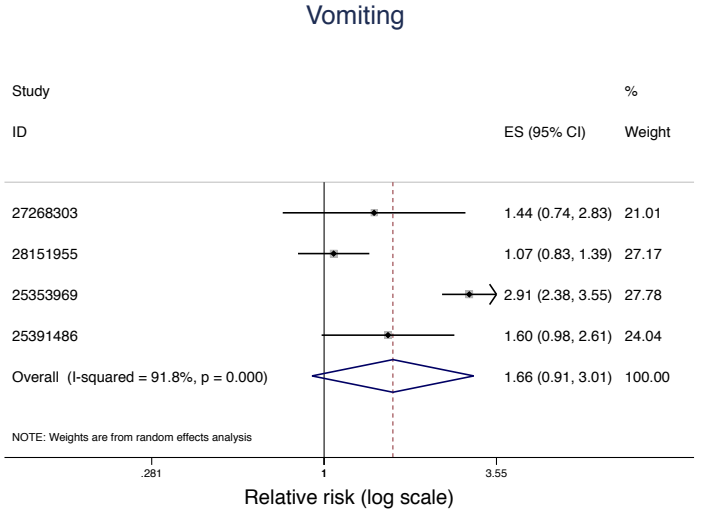
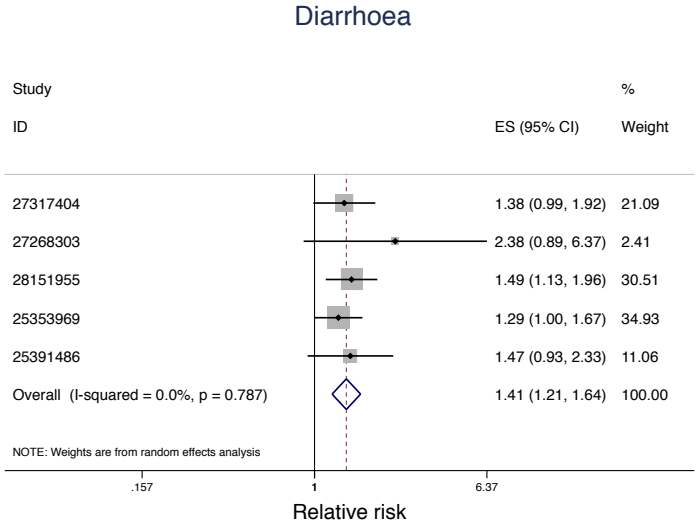


Figure C.1: Meta-analysis for case fatality rate, stratified by country. Analysis includes the primary data-set only. ES is effect size.







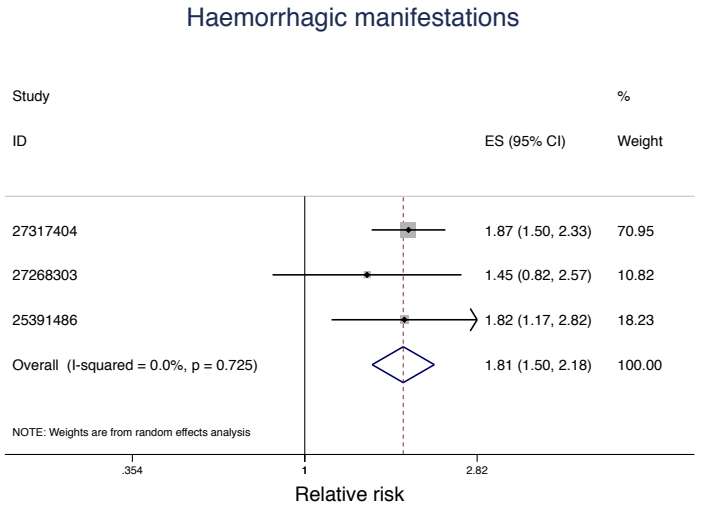
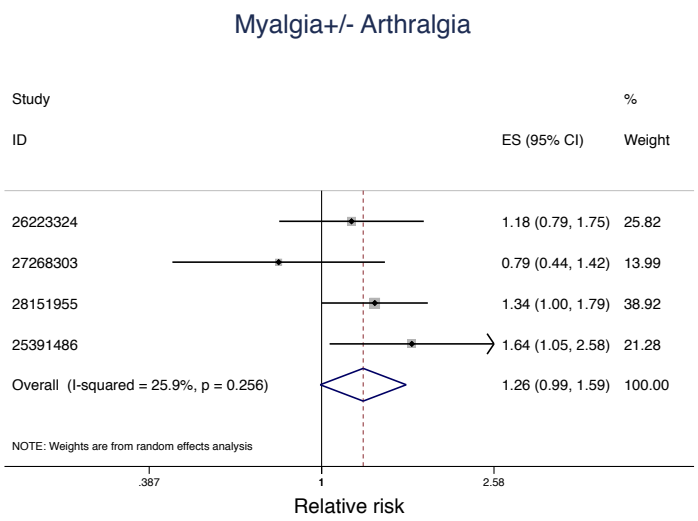
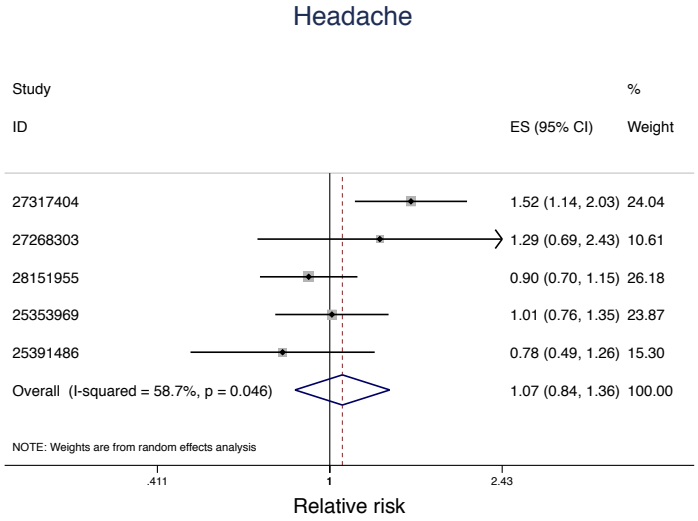
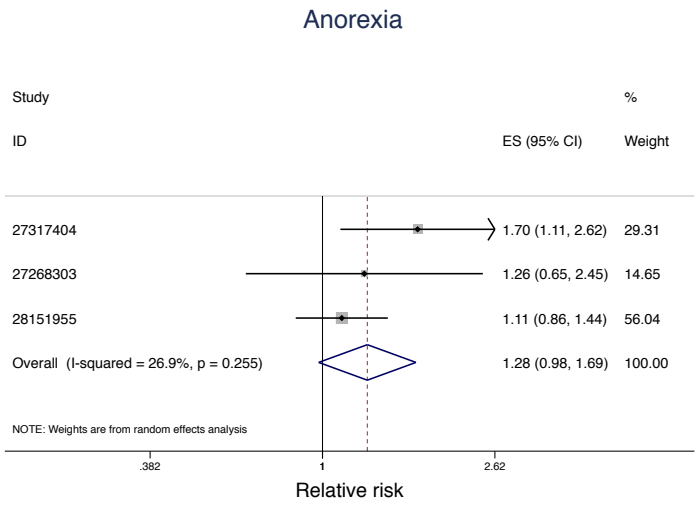


Figure C.2: Relative risk of death in EVD patients presenting with a given symptom - panel A







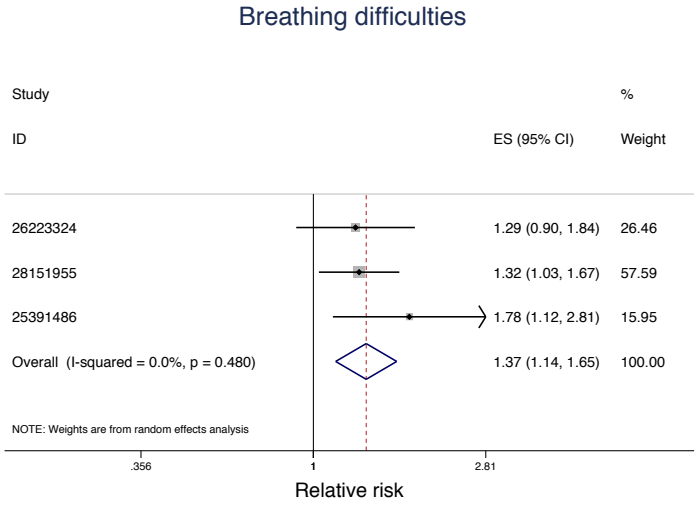
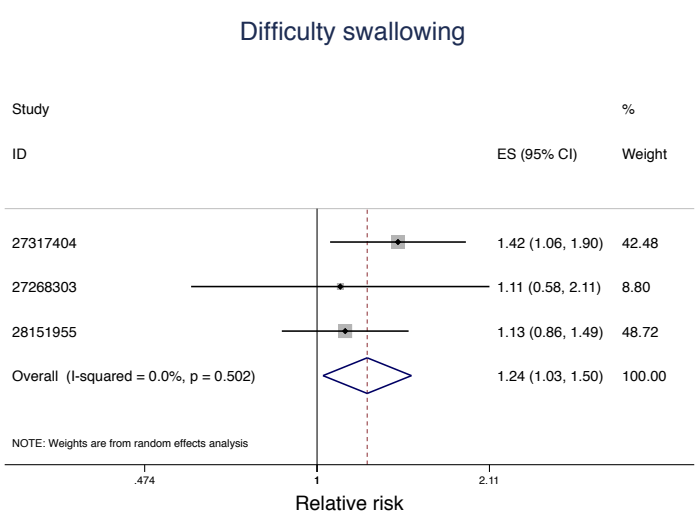
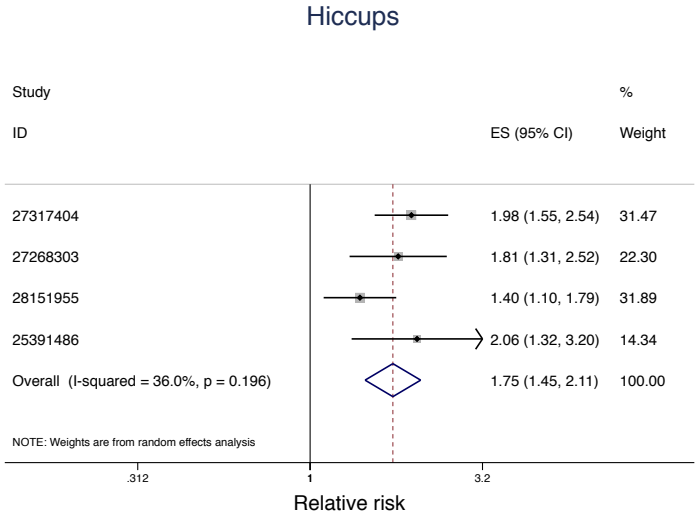
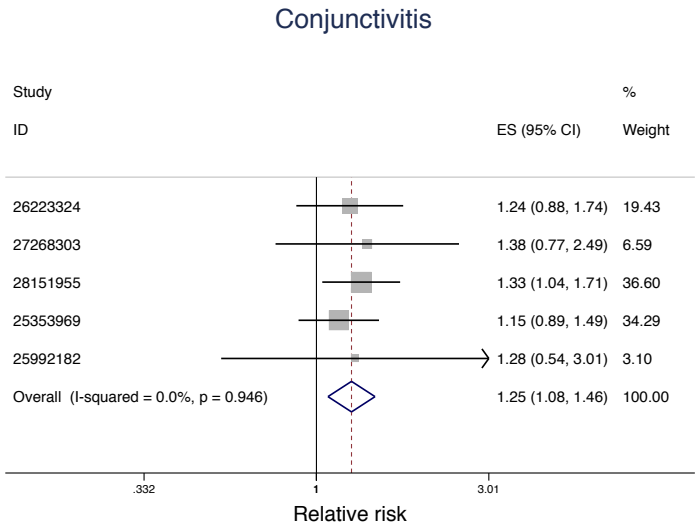


Figure C.3: Relative risk of death in EVD patients presenting with a given symptom - panel B







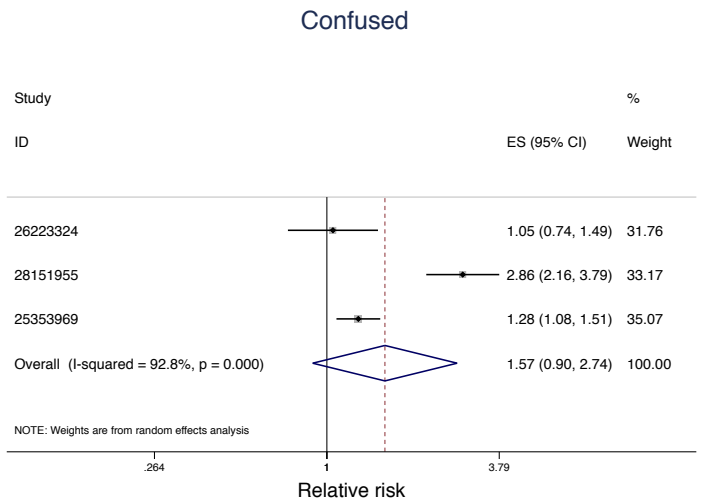


Figure C.4: Relative risk of death in EVD patient presenting with a given symptom - panel D.

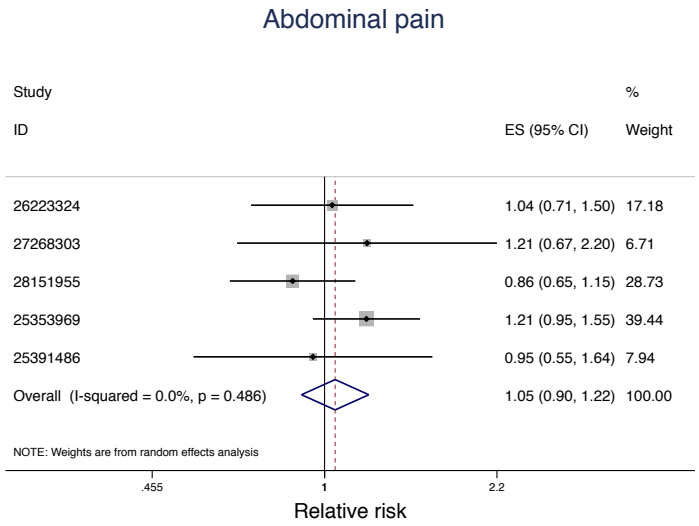


Figure C.5: Relative risk of death in EVD patient presenting with a given symptom - panel D. Forest plots are only shown for symptoms with three or more articles reporting data.

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