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Natural history and epidemiology of Ebola virus disease

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Natural history and epidemiology of Ebola virus disease

by
John Kelly

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of the
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Dedication and Acknowledgement – Thank you, participants! Plenti tenki (Krio for thank you). The journey to complete this PhD degree started during the 2013-2016 Ebola virus disease epidemic in West Africa. In 2014, I took a leave of absence from University of California, San Francisco, to respond to the humanitarian crisis unfolding in Sierra Leone. We knew little about the deadly disease, and modern medicine – vaccines, monoclonal antibodies, and anti-viral therapies – were not available. It was a struggle to ensure that Ebola-infected patients received intravenous fluids and humane care. I felt compelled to pivot from implementation work starting with Wellbody Alliance in 2006 (now Partners In Health – Sierra Leone) to research on Ebola virus disease and other emerging viral infections. This transition was made possible with the enduring support and mentorship of George Rutherford, Sheri Weiser, Paul Farmer, Bailor Barrie, Willi McFarland, Susan Kegeles, Art Reingold, Travis Porco, and others. I want to thank Eugene Richardson for building research community during our collaborative study of Ebola as a pauci-/asymptomatic infection and unrecognized disease. Our community-based cohort in Sierra Leone formed the basis of Chapter 1 and seeded the idea to study clinical sequelae of pauci-/asymptomatic infection and unrecognized Ebola virus disease. I am grateful for the additional support and mentorship of H. Clifford Lane, Michael Sneller, Mosoka Fallah, Cavan Reilly and others in the PREVAIL community who supported this vision. The PREVAIL Ebola Natural History cohort formed the basis of Chapters 2 and 3. While this research was conducted, I spent years in the classroom learning from great teachers, including Maria Glymour, Jeffrey Martin, June Chan, Dave Glidden, Tom Newman, Eric Vittinghoff, Amy Markowitz, and others. With the support of my classmates (Crystal Langlais, Adrienne Epstein, Zara Izadi), I consolidated my epidemiological education through the Qualifying Examination. As I entered the dissertation phase, the COVID-19 pandemic began and again I pivoted, but not without the PhD experience, which is dedicated to the Ebola experience and those who provided unconditional love until the end, including John Kelly (father), Kathleen Kelly (mother), Samantha Kelly (sister), and Elan Guterman (partner).

Contributions

A version of Chapter 1 in this dissertation was accepted in *Open Forum Infectious Diseases* in 2022.[1] A version of Chapter 2 in this dissertation was accepted in *The Lancet Infectious Diseases* in 2022.[2] The Dissertation Committee members supervised the research that forms the basis of these dissertation chapters, the published materials are substantially the product of John Daniel Kelly's period of study at the University of California, San Francisco and were primarily conducted and written by him. The work he completed for each published manuscript is comparable to a standard dissertation chapter.

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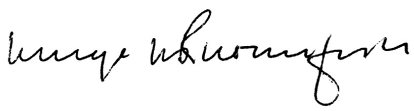

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Epigraph - The Road Not Taken by Robert Frost

Two roads diverged in a yellow wood,

And sorry I could not travel both

And be one traveler, long I stood

And looked down one as far as I could

To where it bent in the undergrowth;

Then took the other, as just as fair,

And having perhaps the better claim,

Because it was grassy and wanted wear;

Though as for that the passing there

Had worn them really about the same,

And both that morning equally lay

In leaves no step had trodden black.

Oh, I kept the first for another day!

Yet knowing how way leads on to way,

I doubted if I should ever come back.

I shall be telling this with a sigh

Somewhere ages and ages hence:

Two roads diverged in a wood, and I—

I took the one less traveled by,

And that has made all the difference.

Natural history and epidemiology of Ebola virus disease

John Daniel Kelly

Abstract

Brief statement of the problem: Although we made significant scientific advances in our understanding of the natural history and epidemiology of Ebola virus during the 2013-2016 Ebola virus disease (EVD) epidemic in West Africa, Ebola virus research has almost exclusively focused on moderate to severe disease rather than asymptomatic and milder infections. Conceptualizing and studying asymptomatically or symptomatically infected individuals as two different groups of unrecognized, Ebola virus-infected individuals has a great potential for paradigm shifts in public health, clinical care, and global policies. There has been a growing body of literature demonstrating a significant burden of pauci-/asymptomatic infection and unrecognized EVD, but the public health and clinical consequences have been unclear, potentially leading to unknown transmission and untreated clinical sequelae. This dissertation has the following objectives: 1) to assess exposure risk in a dose-dependent relationship with severity of illness, 2) examine whether clinical sequelae occur after mild EVD, and 3) identify inflammatory markers that may partially explain how survivors recover from clinical sequelae.

Description of the methods and procedures used to gather data or study the problem: This dissertation leverages two cohorts of Ebola cases and contacts. In Chapter 1, from September 2016 to July 2017, we conducted a cross-sectional, community-based study of Ebola virus disease (EVD) cases and household contacts of several transmission chains in Kono District, Sierra Leone. We used epidemiological surveys and blood samples to define severity of illness as no infection, pauci-/asymptomatic infection, unrecognized EVD, reported EVD cases who survived, or reported EVD decedents. We determine seropositivity with the Filovirus Animal Non-Clinical Group (FANG) EBOV glycoprotein IgG antibody test. We defined levels of exposure risk from eight questions and considered contact with body fluid as maximum

exposure risk. In Chapter 2, from June 2015 through June 2017, we studied a cohort of EVD survivors and their contacts in Liberia. Surveys, current symptoms and physical exam findings, and serology characterized disease status of reported EVD, unrecognized EVD, pauci-/asymptomatic EBOV infection, or no infection. We pre-specified findings known to be differentially prevalent among EVD survivors than contacts. We estimated the prevalence and incidence of selected clinical findings by disease status. In Chapter 3, We used baseline data from a longitudinal cohort of confirmed EVD survivors (seropositive) and their uninfected contacts (seronegative) in Liberia to generate a cytokine profile from stored plasma samples. These data included a sub-cohort of men assessed for Ebola viral shedding in semen. We investigated pre-specified clinical findings previously reported to be differentially prevalent among EVD survivors. Outcomes were self-reported symptoms, physical examination findings, and viral persistence in semen. Using generalized estimating equations, we compared inflammatory markers among survivors and contacts; statistically significant markers ($p \leq 0.01$) were assessed for associations among survivors with and without sequelae.

Summary of the findings: In Chapter 1, this community-based study of EVD cases and contacts provides epidemiological evidence of a dose-dependent relationship between exposure risk and severity of illness, which may partially explain why pauci-/asymptomatic EBOV infection, less severe disease, and unrecognized EVD occurs. In Chapter 2, the findings provide evidence of post-EVD clinical sequelae among contacts with unrecognized EVD but not pauci-/asymptomatic EBOV infection. In Chapter 3, we found evidence of persistent inflammation among survivors, which may be partially explained by ongoing viral shedding. Multiple clinical sequelae were less likely to be associated with two macrophage and pro-inflammatory markers, suggesting that a process of downregulation may be occurring as these survivors experienced clinical recovery.

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List of Abbreviations

AML	AIDS Monitoring Laboratory
AOR	adjusted odds ratio
CI	confidence interval
COVID-19	coronavirus disease 2019
CRP	C-reactive protein
DERC	District Ebola Response Center
EBOV	Ebola virus
EVD	Ebola virus disease
EU	enzyme-linked immunosorbent assay units
FANG	Filovirus Animal Non-Clinical Group
GEE	generalized estimating equations
GRAN-A/B	Granzyme A&B
IQR	interquartile range
IL	interleukin
IL-1RA	IL-1 receptor antagonist
LOD	limit of detection
MIP	macrophage inflammatory protein
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institute of Health
NREB	National Research Ethics Board of Liberia
PREVAIL	Partnership for Research on Ebola Virus in Liberia
RT-PCR	reverse-transcriptase polymerase chain reaction
sTNF-R	soluble receptors for TNFα
TNF-α	tumor necrosis factor-alpha

VHF	Viral Hemorrhagic Fever
WHO	World Health Organization

List of Symbols

R(t)	effective reproduction number

Chapter 1. Association of lower exposure risk with pauci-/asymptomatic infection, less severe disease, and unrecognized Ebola virus disease: a seroepidemiological study

Abstract

Background: It remains unclear if there is a dose-dependent relationship between exposure risk to Ebola virus (EBOV) and severity of illness.

Methods: From September 2016 to July 2017, we conducted a cross-sectional, community-based study of Ebola virus disease (EVD) cases and household contacts of several transmission chains in Kono District, Sierra Leone. We analyzed 154 quarantined households, comprising both reported EVD cases and their close contacts. We used epidemiological surveys and blood samples to define severity of illness as no infection, pauci-/asymptomatic infection, unrecognized EVD, reported EVD cases who survived, or reported EVD decedents. We determine seropositivity with the Filovirus Animal Non-Clinical Group (FANG) EBOV glycoprotein IgG antibody test. We defined levels of exposure risk from eight questions and considered contact with body fluid as maximum exposure risk.

Results: Our analysis included 76 reported EVD cases (both decedents and survivors) and 421 close contacts. Among these contacts, 40 were seropositive (22 pauci-symptomatic and 18 unrecognized EVD), accounting for 34% of the total 116 EBOV infections. Higher exposure risks were associated with having had EBOV infection (maximum risk: adjusted odds ratio [AOR]: 12.1; 95% confidence interval [CI], 5.8-25.4; trend test: $p<0.001$) and more severe illness (maximum risk: AOR: 25.2; 95% CI: 6.2-102.4; trend test: $p<0.001$).

Conclusion: This community-based study of EVD cases and contacts provides epidemiological evidence of a dose-dependent relationship between exposure risk and severity of illness, which may partially explain why pauci-/asymptomatic EBOV infection, less severe disease, and unrecognized EVD occurs.

Introduction

The 2013-2016 Ebola virus disease (EVD) outbreak in West Africa was unprecedented in scale with more than 11,000 deaths and 6,000 survivors reported.[1] After a single zoonotic spillover or human-reservoir relapsing event, Ebola virus (EBOV) can be transmitted from human to human as a result of high-risk exposures, such as direct contact with infected bodily fluids.[2-4] Once infected with EBOV, clinical manifestations ranged from asymptomatic EBOV infection to severe EVD.[5] Emerging evidence suggests that asymptomatic infection and mild illness occur as a substantial proportion of EBOV infections,[6,7] but the pathophysiology of a pauci-/asymptomatic infection remains poorly understood and may result from a combination of nutritional, epidemiological, viral, and immunological host factors.[6,8,9]

The quantity of viral inoculum and its contribution to different infection outcomes has been described in animal models for a number of viruses, including hepatitis B, adenovirus, African swine flu, and influenza.[10-13] Human challenge trials can measure the viable infectious dose of a virus in humans, but in the absence of these trials, epidemiological studies of viral exposures and disease outcomes can act as a surrogate type of investigation to understand who infectious dose impacts humans.[14] Guallar and colleagues reported three clusters of COVID-19 in Madrid, Spain, in which infected persons experienced different disease severity according to distinct magnitudes of reported exposure.[15] A dose-dependent effect of EBOV had been hypothesized after laboratory experiments of aerosolized EBOV showed that viable virus was recovered after 180 minutes and that non-human primates and rhesus monkeys could develop asymptomatic EBOV infection.[16-18] Similarly, in a small study with only 21 seropositive participants, exposure risk to EVD weakly correlated with seropositivity among asymptomatic and symptomatic household contacts.[6] In addition to the unknown role of host and viral determinants of disease severity, we have a limited understanding of how the full spectrum of

disease severity, ranging from asymptomatic and pauci-/asymptomatic infection to severe EVD and death, relates to increasing levels and duration of exposure to EBOV.

In the two years following the EVD outbreak, we sought to explore the relationship between exposure risk and disease severity. We conducted a seroepidemiological investigation of multiple transmission chains in rural communities of Kono District, Sierra Leone. We hypothesized that a dose-dependent relationship occurs between exposure risk and severity of illness.

Methods

Patient consent statement

The study protocol was approved by the Sierra Leone Ethics and Scientific Review Committee and University of California, San Francisco Institutional Review Boards. Written consent was obtained for all participants and permission to access the Viral Hemorrhagic Fever (VHF) database was given by the Kono District Ebola Response Center (DERC), which acted as a coordinating body for Ebola response operations during the outbreak.

Study setting, population, and procedures

We conducted a cross-sectional, community-based study in Kono District, Sierra Leone, from September 2016 to July 2017. This seroepidemiological investigation of transmission chains occurred in the communities of Ngo Town, Ndogboya, Bumpe, and Joe Town within Kono District. The first transmission chain started in late August of 2014 during a burial ceremony in Port Loko District of an individual who had died of EVD. A participant in that burial then returned to her home village of Joe Town, Kono District, and then contracted and unwittingly transmitted EVD which resulted in seven EVD cases (four survivors, three deaths) within the community (see **Supplementary Figure 1a**). The other communities are thought to

be linked through one large transmission chain, starting in mid-October of 2014, and causing outbreaks in Ngo Town (one survivor, four deaths) (see **Supplementary Figure 1b**), Ndogboya (eight survivors, 18 deaths) (see **Supplementary Figure 1c**), and Bumpe (12 survivors, 26 deaths) (see **Supplementary Figure 1d**).

Our study included any reported EVD case and contact who lived in these communities at the time of the local EVD outbreak. Reported EVD cases were identified through the VHF database and were confirmed in interviews with community leaders and healthcare workers, EVD survivors from the communities, the Ebola Survivor Association, and household members. EVD contacts were defined as exposed individuals who lived in a quarantined household (during the Ebola epidemic, all known EVD contacts were placed under mandatory 21-day quarantine within their homes) or someone who lived outside of a quarantine household but who was identified as a close contact in our interviews with EVD survivors, household surrogates of those who died of EVD, or the VHF database.

We obtained a list of households that had been quarantined during the Ebola epidemic from the Kono DERC. In collaboration with community leaders and EVD survivors, our team of local staff corroborated and confirmed all of the quarantined households in each community. These households included all of the reported EVD cases. In 14 households with EVD deaths, we obtained data via a proxy, who was an adult with either the closest relationship or the head of the household. During interviews with EVD survivors, we obtained an additional list of close contacts. We then identified these close contacts, confirmed their exposure history, and enrolled these individuals.

Each study visit included an epidemiological survey, blood draw, and open-ended interview. We collected the exposure risk and other covariate data in the epidemiology survey. At the end of the survey, we conducted an open-ended interview. We asked participants to describe the story of how EVD affected their household with a focus on particular exposure and

transmission events. We held focus groups to corroborate the transmission chain from other informational sources.

The blood samples were transported to a local laboratory for biospecimen processing into plasma aliquots and maintained in a cold chain. These samples were transported to the National Institute of Allergy and Infectious Diseases (NIAID/NIH) in Ft. Detrick, Maryland, USA, where serological testing occurred. After receiving the serology results, we disseminated all of the results to participants. We then re-interviewed participants who were found to be seropositive to further reconstruct possible transmission chains and sources of exposure.

Laboratory measurements

Seropositivity to anti-glycoprotein EBOV-specific IgG antibodies was used to classify the outcome variables and determined through testing of the blood plasma samples. We used the Filovirus Animal Nonclinical Group (FANG) immunoassay, which has 94.4% sensitivity and 96.7% specificity when a cutoff of 548 enzyme-linked immunosorbent assay units (EU) per milliliter was applied to the West African EVD survivor population.[19]

Epidemiological measurements

Primary outcomes were EBOV infection (presence or absence) and severity of EVD illness (five levels). We assumed that participants who were seropositive had an EBOV infection following exposure. Therefore, EBOV infection was defined as those who were seropositive in addition to the reported EVD cases (survivors, decedents). Severity of EVD illness was defined as an ordinal variable with the following progression of disease: no infection, pauci-/asymptomatic infection, unrecognized EVD, reported EVD cases who survived, and reported EVD cases who died. The classification of unrecognized EVD versus reported EVD cases who survived was based on whether an individual had been identified as a case by the Kono DERC

during the outbreak and recorded in the VHF database. The unrecognized EVD cases identified in this study did not receive testing nor medical care during their illness, remained at home through the duration of their infection, and reported on average less symptoms during their post-EVD exposure period.[19] All reported EVD cases who survived and died had at least one laboratory-confirmed PCR-positive test result record in the VHF database, except for two decedents who were probable cases.

To create the disease classifications of pauci-symptomatic infection and unrecognized EVD, we used contact-participants' serostatus and self-reported, post-exposure symptoms of each contact-participant. We created a 16-item symptom checklist from the WHO EVD case definition and asked contact-participants to report the presence or absence of each symptom. Other household members were asked to verify signs and symptoms. We then compiled these responses and classified each contact-participant as either asymptomatic or symptomatic. Participants who were seropositive and asymptomatic (answered no to all 16 questions) were classified as having had a pauci-/asymptomatic infection. We settled on "pauci-/asymptomatic" as the description of individuals who reported being asymptomatic because of the potential for mild symptoms and recall error. Contact-participants who were seropositive and symptomatic were defined as having had unrecognized EVD.

The explanatory variable was exposure risk, which was adapted from classifications used elsewhere in the EVD literature.[3,6] We asked contact-participants to recall their interactions with EVD case(s) according to 8 types of exposures (see below). Each response to the exposure question was binary (yes/no). We assigned each participant to a single maximal exposure type. We ordered exposure risk to create a 5-level categorical variable according to the questionnaire (from highest to minimal/no exposure) as follows: highest – contact with body fluids through caregiver, tactile burial, or other practices (Q8, Q7); high – direct contact with body fluids (Q6); intermediate – washing an EVD case's clothes (Q5) or sleeping in the same

room (Q4); low – eating from the same dish, or sharing a pot (Q3) or being within two meters of an EVD case or body fluids (Q2); and minimal or no contact (staying greater than two meters from any EVD case or body fluids) (Q1).

Reconstructing the chain of EBOV transmission

We reconstructed temporal and geospatial arrays of EBOV transmission chains inclusive of pauci-symptomatic infection and unrecognized EVD, using methods described elsewhere.[20] In brief, we were able to draft, assess and confirm the transmission chain, and created a classification scheme to describe probabilistic epidemiological links (types 1, 2, 3) between an EVD case and a participant who was EBOV-infected. Type 1 links were considered more likely to be true epidemiological links than type 2, and type 2 more likely than type 3. We used the most probable links to construct the transmission chain.

Data analyses

We described the 5-level exposure risk in the cohort but also presented these data grouped into three levels for its potential simplified public health communication benefit: minimal or no contact (Q1), indirect contact (Q2-3), and direct contact (Q4-8). We assessed the associations of 5-level exposure variable to subsequent EBOV infection and severity of illness. We analyzed this relationship with mixed-effect logistic regression model for the outcome of EBOV infection and with a mixed-effect multinomial logistic regression model for the outcome of severity of EVD illness. Based on evidence from the literature,[3,20] we adjusted for age, sex, educational level, and type of work, and included household as a random effect. We were unable to adjust for viral load (or cycle threshold value) or comorbidities because these data were not collected and/or available. To further evaluate the epidemiologic evidence for a dose-response relationship, we performed Cochran-Armitage test for trend. We repeated these

analyses with the 3-level exposure variable and included them in the **Supplementary Material** as a sensitivity analysis. In analyses of the transmission chain, we estimated the effective reproduction number, R(*t*), by dividing the total number of new EVD cases in each generation by the number of EVD cases in the previous generation.[20] These analyses were performed in R version 3.2.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

The analysis cohort included 497 participants; 76 reported EVD cases and 421 contact-participants were identified from the initial outbreak (see **Figure 1**). Sociodemographic characteristics are presented in **Table 1**. Forty (9.5%) of 421 contact-participants who were seropositive had not previously been identified as EVD cases by either the DERC or community queries. Among the 40 seropositive contact-participants, 18 (45%) reported the presence of symptoms while 22 (65%) reported the absence of symptoms (chi-squared: p=0.39). The seropositive contact-participants, who probably had pauci-/asymptomatic infection or unrecognized EVD, accounted for 34% of 116 EBOV infections (76 reported EVD cases + 40 seropositive contacts) (see **Figure 2**).

We identified that 37 of 40 seropositive participants were in quarantined households and three were outside of the quarantine; all three were symptomatic contact-participants and probably had unrecognized EVD. When we re-interviewed participants who were found to be seropositive, one of the unrecognized EVD cases outside the quarantine traveled to another community while feeling mildly ill and stayed with family who subsequently developed EVD.

Transmission chains

Each community sustained a transmission chain, and we used temporal data of the 76 EVD cases to estimate the average effective reproduction number among communities (**Table**

2). In the first generation of transmission, four individuals transmitted EBOV to 49 individuals (R(1), 12.25; 95%CI, 11.27-13.23). In the second generation of transmission, 49 individuals transmitted EBOV to 37 individuals (R(2), 1.62; 95%CI, 1.21-2.03). In the third generation of transmission, 37 individuals transmitted EBOV to 13 individuals (R(3), 0.42; 95%CI, 0.05-0.79). In the fourth generation of transmission 13 individuals transmitted EBOV to 6 individuals (R(4), 0.51; 95%CI, 0.0-1.07).

Associations of exposure risk with infection and severity of illness

Direct contact was reported in 30.3% of uninfected and 71.2% of infected participants. The majority of pauci-/asymptomatic infection, however, involved minimal or no contact while the majority of those with unrecognized EVD, EVD survivors, and EVD decedents reported direct contact. When we further examined exposure risk patterns, participants with direct contact reported mostly high- and highest-risk exposures (direct contact, or contact with bodily fluids) in contrast to intermediate-risk exposures (eating the same meals, sleeping in the same room) (**Table 3)**.

In adjusted analyses, we observed a dose-dependent relationship based on increasing exposure risk against the outcomes (**Table 4)**. An increasing level of exposure risk was associated with higher odds of infection and severe illness (trend test for infection: $p<0.001$; trend test for severity: $p<0.001$). Highest exposure risk had the strongest magnitude, which was 12.1 times the odds of infection and 25.2 times the odds of severe illness than minimal exposure (infection: 95% CI, 5.7-25.4; severity: 95% CI, 6.2-102.4). High exposure risk was also statistically significant. These associations and its dose-response relationship were replicated with the 3-level exposure risk variable (see **Supplementary Table 1**).

Discussion

This seroepidemiological investigation of EBOV transmission chains in Kono District, Sierra Leone, found a dose-dependent relationship between exposure risk and severity of EVD illness. This finding extends a growing body of EBOV literature,[16-18] suggesting that the size of the initial dose of EBOV that a person is exposed to plays a role in severity of illness. Further, identification of missed cases in the process of reconstructing the transmission chains, including pauci-/asymptomatic infection and unrecognized EVD cases, substantiates the true burden of EBOV infection from the West African outbreak and helps to identify potential patterns of transmission dynamics. Most missed cases were identified within quarantined households, but there were three close contacts with unrecognized EVD who were confirmed to be outside of quarantined households. These non-quarantined and unrecognized EVD cases may have unwittingly propagated the disease to other communities in Kono and elsewhere. Similar observations were made in Guinea and underscore the ongoing surveillance challenges faced by under-resourced and underdeveloped health systems.[21]

We found that 34% of EBOV infections were seropositive and probably had either pauci-/asymptomatic infection or unrecognized EVD. This proportion is consistent with our prior seroepidemiological study in Sukudu, Kono District, and other community-based studies,[6,20] confirming that the public health significance of missed cases is substantial and worthy of consideration as Ebola control and care strategies are revised. Given that some of missed cases in our study were found outside of the quarantine, containment efforts should not only make every effort to identify and isolate people who have been infected with EVD, but also provide those who are infected with aggressive treatment in line with the 2016 revised WHO clinical care guidelines.[22-25] In our study, individuals with pauci-/asymptomatic infection or unrecognized EVD did not require hospitalization, which highlights the spectrum of EVD severity

and the ongoing need to provide clinical care in communities, either through specialized community care centers or appropriately designed clinics.

Although we found that severity of EVD illness was associated with exposure risk, some individuals reporting minimal- or low-risk exposures in our cohort still exhibited a range of symptoms, from unrecognized EVD to death. EVD has been described as a caregivers' disease given that the virus is most frequently propagated via tactile acts of care for the sick, dying and deceased.[26] Some of these tactile acts of care resulted as minimal- or low-risk exposures, and these exposed individuals may be at-risk for severe disease and are difficult to identify in the context of health systems that are under-resourced and underdeveloped due to historical, structural and political-economic causes, and which may quickly be overwhelmed during an Ebola epidemic.[27] Although community resistance, lack of trust, and poor contact identification rate and follow-up have the potential to create additional barriers,[28-30] public health providers should consider more intensive surveillance of contacts as the health system strengthens. If minimal-risk or low-risk exposures were to be tracked, then symptomatic individuals could be expeditiously referred to care. Even if they do not develop severe EVD, this population may still be at risk for prolonged clinical sequelae such as memory loss and joint pains.

In the four communities described in our study, we found that the epidemic curve declined by the third reproductive generation. The timing of epidemic decline was similar to what was found in the few other studies that have described effective reproduction number within single communities.[20] The short timeline within communities emphasizes the need for a rapid response and strong health system to implement control and care interventions. In such a system, we would also be more likely to identify individuals who would otherwise go onto becoming unrecognized EVD cases and this could contribute to rapid epidemic decline.[31,32]

Our study has several limitations. First, we may have missed cases beyond of the identified contact-participants who were identified as exposed, but lack of resources did not

permit us to conduct a serosurvey of entire communities. Second, contact-participants and surrogates for the deceased were asked to recall exposures with the EVD case(s) and symptoms during the post-EVD exposure period; some exposures and mild symptoms may have been forgotten, were under-reported due to lack of trust and misconception, or were unobserved in the case of the surrogates. To mitigate, we disclosed serostatus after the exposure measurements were obtained. Further, we considered participants who reported the absence of symptoms to be pauci-symptomatic, acknowledging that the group probably comprised of asymptomatic and mildly symptomatic individuals. These individuals, however, did not know their serological status when the interview occurred, so this measurement error biased to the null. Third, our cutoff for IgG antibody titers was established through previous studies. Little is known about pauci-/asymptomatic infection; the initial antibody response may be lower or antibody titers may have waned, creating the possibility that we missed additional pauci-/asymptomatic individuals who were part of our study population. Fourth, we were unable to measure potential confounders such as viral load (or cycle threshold value) and comorbidities; given that these covariates were unlikely to change the exposure behaviors, any bias would have been towards the null. Fifth, the generalizability of these communities may be more specific to those that experienced local EVD outbreaks late in the epidemic, when control measures were stronger and missed cases were less likely. Nonetheless, our study was sufficiently powered to demonstrate a dose-dependent relationship with regression models, adjusting for confounders and clustering, in contrast to previous work using more limited statistical approaches.

In conclusion, this study found an association of lower exposure risk with pauci-/asymptomatic infection, unrecognized EVD and less severe disease, which provides impetus for further investigation into the relationship between exposure-risk and severity of illness for EBOV, SARS-CoV-2 and other viral pathogens. Given that our study and others have reported

transmission to individuals without direct contact of EVD cases, we believe the EVD outbreak response community should consider eye protection and masking in the provision of personal protective gear to communities facing EVD outbreaks. Reducing exposure risk among household members unable to quarantine in separate locations or forced into caregiving roles while awaiting ambulances and safe transport to Ebola treatment centers has the potential to prevent severe and deadly EVD illness.

Table 1.1 Sociodemographic characteristics of the community-based cohort in Kono District, Sierra Leone

	All infected[1]	Uninfected	Pauci-/asympto-matic	Unrecognized EVD	Survivor	Decedent
Sex						
Female	42.3% (47)	41.2% (156)	50.0% (11)	44.4% (8)	39.1% (9)	39.6% (19)
Male	57.7 % (64)	58.8% (223)	50.0% (11)	55.6% (10)	60.8% (14)	60.4% (29)
Age						
<19	22.9% (27)	40.9% (155)	27.3% (6)	33.3% (6)	17.4% (4)	22.9% (11)
20-29	19.5% (23)	16.9% (64)	36.6% (5)	16.7% (3)	13.0% (4)	4.2% (2)
30-39	16.9% (20)	15.0% (57)	4.6% (1)	5.6% (1)	17.4% (4)	29.2% (14)
40-49	20.3% (24)	13.5% (51)	18.2% (4)	27.8% (5)	21.7% (5)	20.8% (10)
>50	20.3% (24)	13.7% (52)	13.6% (3)	16.7% (3)	30.4% (7)	22.9% (11)
Education						
None	52.3% (57)	35.1% (133)	40.9% (9)	38.9% (7)	47.8% (11)	62.5% (30)
Primary	18.3% (20)	31.1% (118)	18.2% (4)	16.7% (3)	21.7% (5)	16.7% (8)
Secondary & above	29.4% (32)	32.7% (124)	40.9% (9)	44.4% (8)	30.4% (7)	16.7% (8)
Employment						
Healthcare	13.1% (14)	17.7% (67)	13.6% (3)	22.2% (4)	4.4% (1)	12.5% (6)
Indoor	15.9% (17)	25.9% (98)	13.6% (3)	27.8% (5)	17.4% (4)	10.4% (5)
Outdoor	71.0% (76)	55.9% (212)	68.1% (15)	50.0% (9)	73.9% (17)	72.9% (35)
Head of household						
No	42.2% (46)	67.6% (256)	50.0% (11)	55.6% (10)	34.8% (8)	35.4% (17)
Yes	57.8% (63)	32.2% (122)	50.0% (11)	44.4% (8)	65.2% (15)	60.4% (29)

[1] Cells formatted as percentage (number) (%, N)

Table 1.2: Estimation of the average effective reproduction number among the communities

	Generation 1	Generation 2	Generation 3	Generation 4
	Reproduction No. (incidence)	Reproduction No. (incidence)	Reproduction No. (incidence)	Reproduction No. (incidence)
Communities				
Joe Town	2.0 (2)	3.5 (7)	0.43 (3)	0.67 (2)
Ngo Town	2.0 (2)	1.5 (3)	0.33 (1)	0.00 (0)
Ndogboya	16.0 (16)	1.3 (20)	0.20 (4)	0.25 (1)
Bumpe	29.0 (29)	0.24 (7)	0.71 (5)	0.60 (3)
Overall	12.3 (49)	1.62 (37)	0.42 (13)	0.51 (6)

Table 1.3 Description of exposure risk by EBOV infection and severity of EVD illness

	All infected	Uninfected	Pauci-symptomatic	Unrecognized EVD	Survivor	Decedent
3-Level Exposure Variable						
Minimal or no Contact	17.1% (19)	44.9% (170)	63.6% (14)	5.6% (1)	0% (0)	8.3% (4)
Indirect	11.7 % (13)	24.8% (94)	18.2% (4)	27.8% (5)	0% (0)	8.3% (4)
Direct	71.2% (79)	30.3% (115)	18.2% (4)	66.7% (12)	100.0% (23)	83.3% (40)
5-Level Exposure Variable						
Minimal or no Contact	17.1% (19)	44.9% (170)	63.6% (14)	5.6% (1)	0% (0)	8.3% (4)
Low Risk	11.7 % (13)	24.8% (94)	18.2% (4)	27.8% (5)	0% (0)	8.3% (4)
Intermediate Risk	2.7% (3)	2.11% (8)	4.6% (1)	0.0% (0)	4.4% (1)	2.1% (1)
High Risk	30.6% (34)	17.9% (68)	4.6% (1)	38.9% (7)	39.1% (9)	35.4% (17)
Maximum Risk	37.8% (42)	10.3% (39)	9.1% (2)	27.8% (5)	56.5% (13)	45.8% (22)

Table 1.4 Associations of exposure risk by EBOV infection and severity of EVD illness

Exposure Level	EBOV Infection		Disease severity	
	Adjusted Odds Ratio[1]	P-value	Adjusted Odds Ratio[1]	P-value
Minimal Exposure	Ref	NA	Ref	
Low Risk	1.2	0.06	1.3	0.53
Intermediate Risk	3.4	0.01	3.5	0.08
High Risk	4.5	<0.001	5.1	<0.001
Maximal Risk	9.6	<0.001	11.24	<0.001

[1]Confounding variables included age, sex educational level, and type of work.

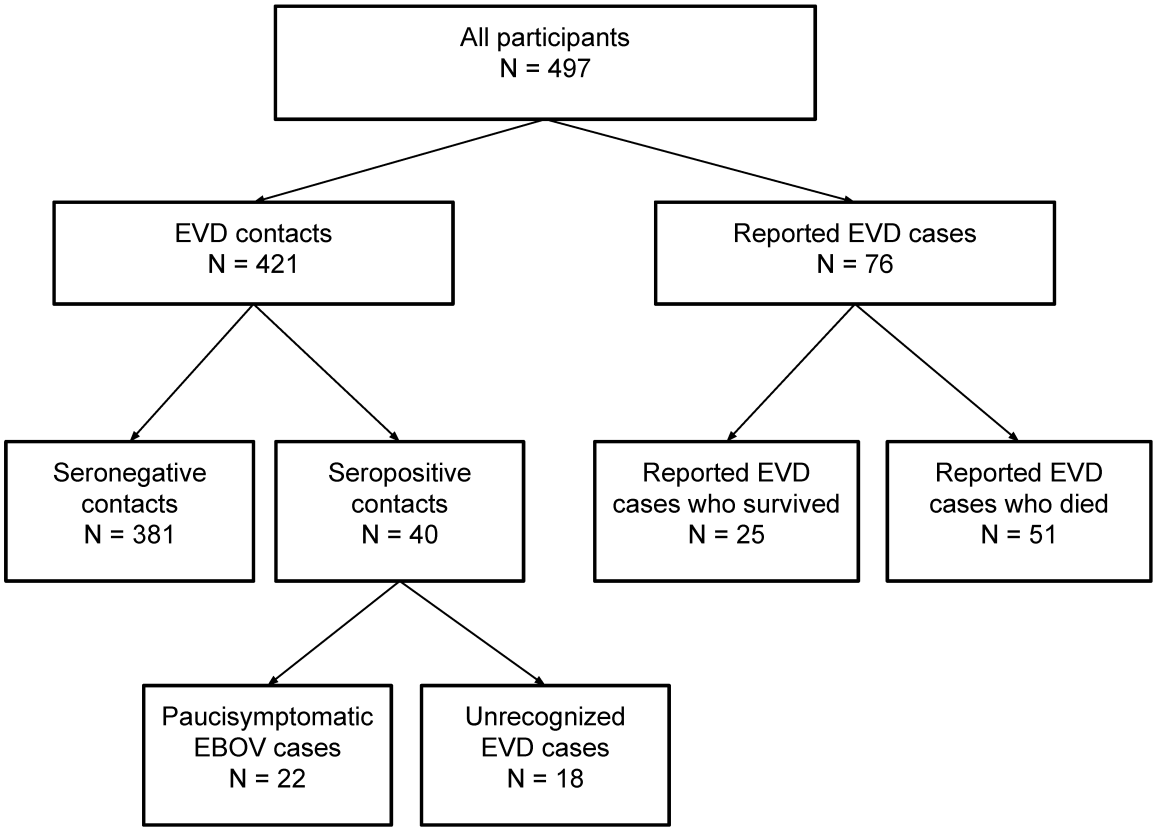


Figure 1.1 Flow diagram of study participants and classification in disease groups

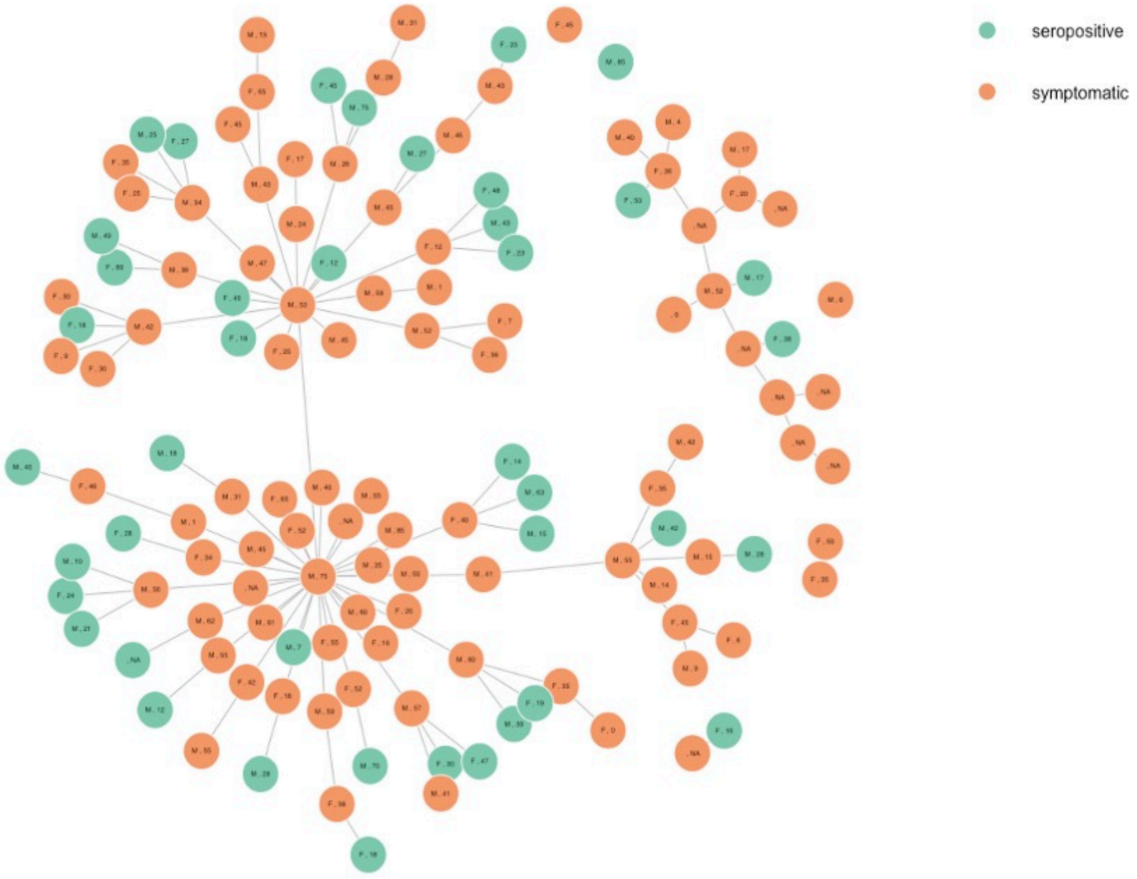
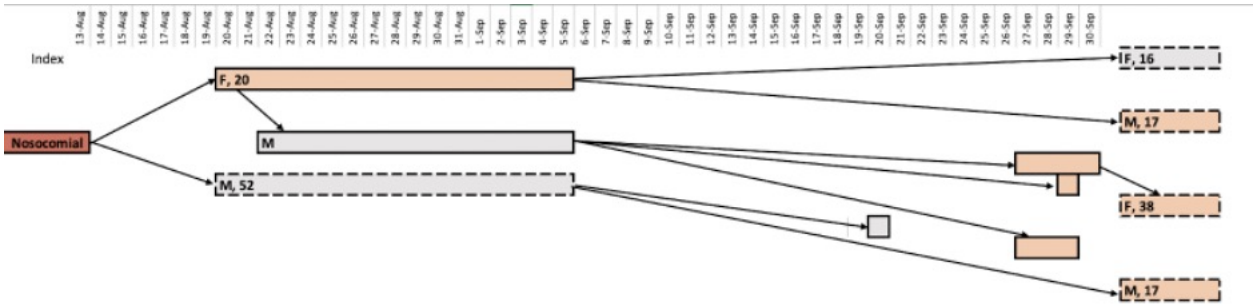


Figure 1.2: Geospatial depiction of the transmission chains inclusive of pauci-/asymptomatic infection and unrecognized EVD. Contact-participants identified through the serosurvey are indicated by green circles while EVD cases are indicated by orange circles.

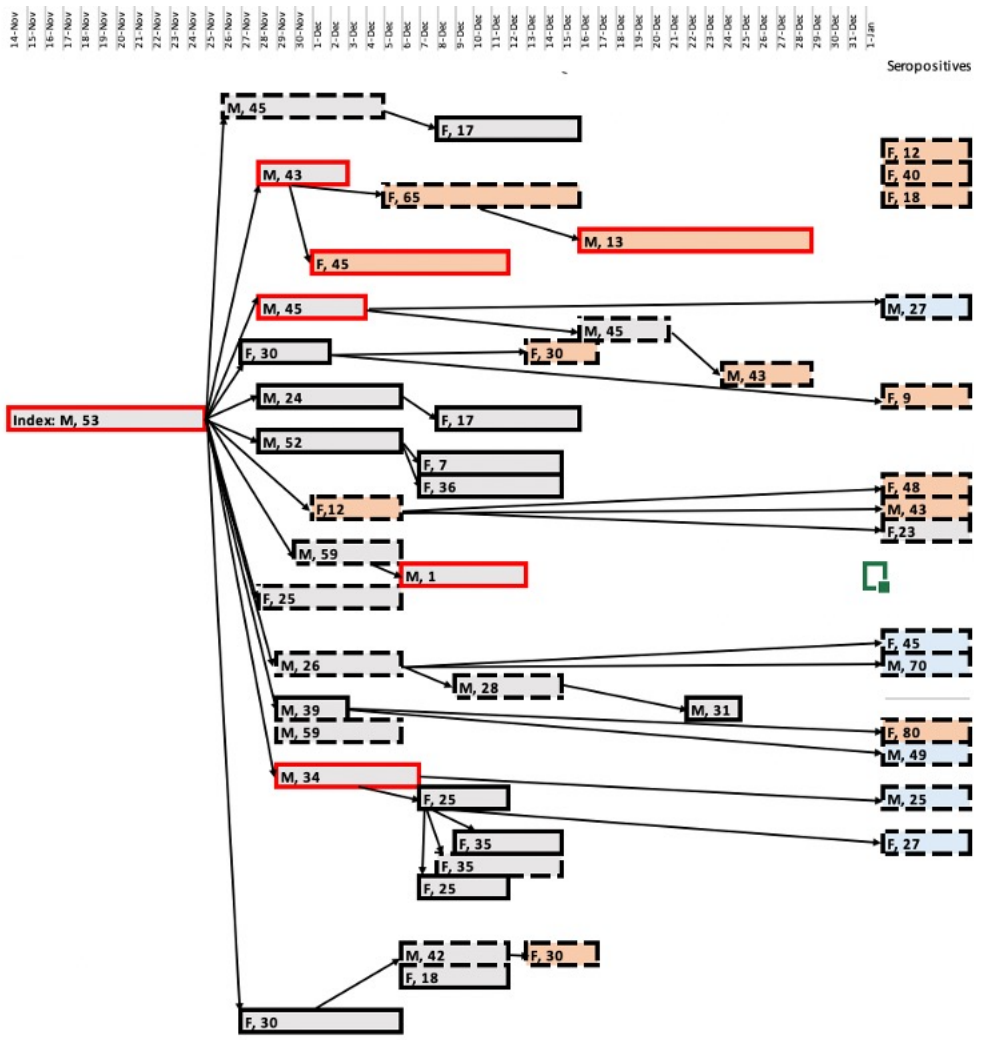
Supplementary Table 1.1 Associations of 3-level exposure risk variable by EBOV infection and severity of EVD illness

Exposure Level	EBOV Infection			Severity of Illness		
	Odds Ratio	95% CI	P-val	Odds Ratio	95% CI	P-val
Minimal Exposure	1.00	NA	NA	1.00	NA	NA
Indirect	1.65	0.69-3.94	0.259	3.56	0.68-16.53	0.14
Direct	8.29	4.42-15.50	<0.001	18.36	5.31-63.49	<0.001

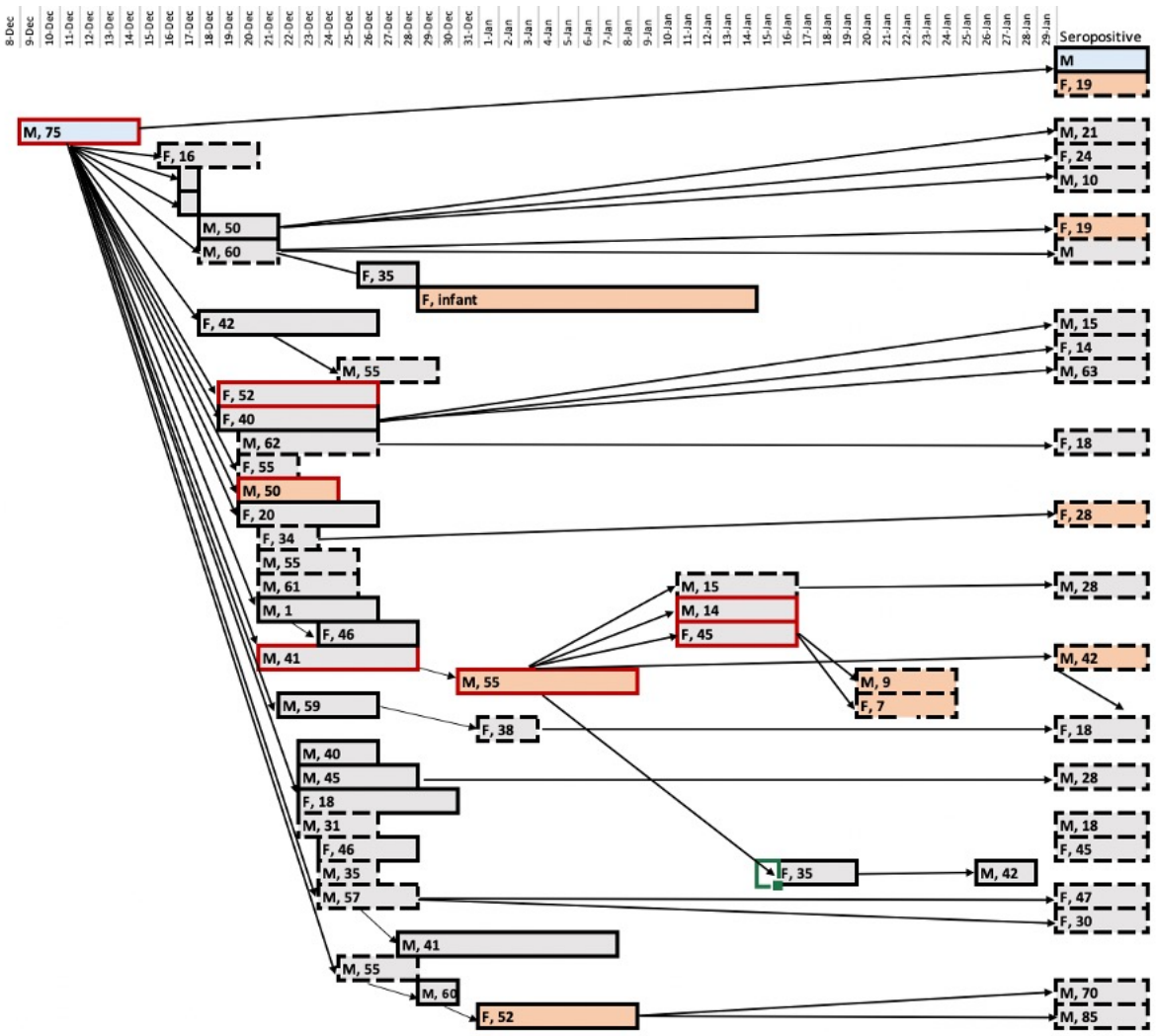


Supplementary Figure 1.1 Epidemiological investigation of Ebola virus disease (EVD) cases in transmission chain in Joe Town, Kono District, Sierra Leone. An EBOV infection is indicated by a box surrounded by a line and filled with a color. Solid lines indicate death and red lines indicate a participant who received a community burial. Dotted lines were participants who survived EBOV infection. Inside the box, grey color describes epidemiological link 1, orange describes link 2, and light blue describes link 3.

Supplementary Figure 1.2 Epidemiological investigation of Ebola virus disease (EVD) cases in transmission chain in NGO Town, Kono District, Sierra Leone. An EBOV infection is indicated by a box surrounded by a line and filled with a color. Solid lines indicate death and red lines indicate a participant who received a community burial. Dotted lines were participants who survived EBOV infection. Inside the box, grey color describes epidemiological link 1, orange describes link 2, and light blue describes link 3.



Supplementary Figure 1.3 Epidemiological investigation of Ebola virus disease (EVD) cases in transmission chain in Ndogboya, Kono District, Sierra Leone. An EBOV infection is indicated by a box surrounded by a line and filled with a color. Solid lines indicate death and red lines indicate a participant who received a community burial. Dotted lines were participants who survived EBOV infection. Inside the box, grey color describes epidemiological link 1, orange describes link 2, and light blue describes link 3.



Supplementary Figure 1.4 Epidemiological investigation of Ebola virus disease (EVD) cases in transmission chain in Bumpe, Kono District, Sierra Leone. An EBOV infection is indicated by a box surrounded by a line and filled with a color. Solid lines indicate death and red lines indicate a participant who received a community burial. Dotted lines were participants who survived EBOV infection. Inside the box, grey color describes epidemiological link 1, orange describes link 2, and light blue describes link 3.

Chapter 2. Clinical sequelae among individuals with pauci-/asymptomatic Ebola virus infection and unrecognized Ebola virus disease in Liberia: a cohort study

Abstract

Background: It is unknown whether individuals with pauci-/asymptomatic Ebola virus (EBOV) infection and unrecognized Ebola virus disease (EVD) develop clinical sequelae. We assessed current symptoms and physical exam findings among individuals with pauci-/asymptomatic infection and unrecognized EVD compared to EVD survivors and uninfected contacts.

Methods: From June 2015 through June 2017, we studied a cohort of EVD survivors and their contacts in Liberia. Surveys, current symptoms and physical exam findings, and serology characterized disease status of reported EVD, unrecognized EVD, pauci-/asymptomatic EBOV infection, or no infection. We pre-specified findings known to be differentially prevalent among EVD survivors than contacts. We estimated the prevalence and incidence of selected clinical findings by disease status.

Findings: Our analytic cohort included 991 reported EVD survivors and 2,688 close contacts. The median time from acute EVD onset to baseline was 317 days (interquartile range, 271 to 366). Among 222 seropositive contacts, 115 had pauci-/asymptomatic EBOV infection and 107 had unrecognized EVD. At baseline, prevalent findings of joint pain, memory loss, muscle pain and fatigue were lowest among those with pauci-/asymptomatic or no infection, increased among unrecognized EVD contacts, and highest among reported EVD survivors. Joint pain was the most prevalent finding: 434/2,466 (18%), no infection; 14/115 (12%), pauci-/asymptomatic infection; 31/107 (29%), unrecognized EVD; and 476/991 (47%), reported EVD. In adjusted analyses, this pattern remained for joint pain and memory loss. Survivors had 2.1 times higher adjusted odds of joint pain compared with unrecognized EVD contacts (95%CI: 1.3-3.4); unrecognized EVD contacts had 1.9 higher adjusted odds of joint pain compared with pauci-/asymptomatic infection and uninfected contacts (95%CI: 1.2-3.0). The adjusted odds of memory loss was 4.5 times higher among survivors than unrecognized EVD contacts (95%CI: 2.4-8.3) and 2.1 times higher among unrecognized EVD contacts than pauci-/asymptomatic

infection and uninfected contacts (95%CI: 1.1-3.8). By 12 months, prevalent findings had decreased in the three infected groups.

Interpretation: Our findings provide evidence of post-EVD clinical sequelae among contacts with unrecognized EVD but not pauci-/asymptomatic EBOV infection.

Introduction

The 2013-2016 Ebola virus disease (EVD) outbreak in Western Africa resulted in over 28,000 reported cases and prompted intensive study of survivors to understand the clinical complications of their acute illness and create comprehensive care programs.[33-36] EVD survivors experience a wide spectrum of clinical sequelae, ranging from uveitis and memory loss to headache and muscle pain.[37-41] Longer-term study of EVD survivors found that most of these conditions declined in prevalence,[19] but a significant proportion of post-EVD sequelae persisted for as long as 2.5 to 4 years.[42,43]

Individuals with suspected EVD are typically categorized as survivors if they were diagnosed as PCR-positive for Ebola virus (EBOV), discharged alive from a healthcare or Ebola treatment facility, and listed in an EVD registry.[44] In the post-EVD period, EVD survivors have been the focus of international research and programmatic efforts.[45-47] A limited number of studies have also shown a significant burden of pauci-/asymptomatic EBOV infection and unrecognized EVD.[48-51] A significant proportion of the latter never presented to an Ebola treatment facility while infected and were therefore not diagnosed with EVD or recorded as EVD survivors. In addition to those with pauci-/asymptomatic infection, unrecognized EVD survivors may have also had on average, less severe acute EVD than those diagnosed and treated at Ebola treatment facilities.[19] Both groups – those with pauci-/asymptomatic infection and those with unrecognized EVD – may have developed post-infectious clinical sequelae.

There is limited evidence linking the acute EVD illness and its severity to clinical sequelae; however, studies have found certain symptoms and/or higher viremia during acute EVD correlated with subsequent clinical sequelae.[42,52,53] These studies raised the question of whether a dose-response relationship exists between the severity of acute EVD and post-EVD clinical sequela. However, the small size of previous EVD outbreaks and their occurrence mostly in settings with under-resourced health systems prevented systematic study of this

hypothesis among individuals with pauci-/asymptomatic infection and unrecognized EVD. During the 2013-2016 EVD outbreak in Liberia, we constructed a large cohort of EVD survivors and contacts, inclusive of individuals with pauci-/asymptomatic infection and unrecognized EVD who can be assessed for evidence of post-EVD clinical sequelae. Since individuals with pauci-/asymptomatic infection and unrecognized EVD probably had less severe acute disease than reported EVD survivors, we hypothesized that individuals with less severe acute disease experience post-EVD clinical sequelae to a lesser extent than reported EVD survivors, and that a viral-load dependent relationship occurs between severity of acute illness and clinical sequelae.

Methods

Study design, participants, and procedures

We used data from a longitudinal cohort study of EVD survivors and close contacts in Liberia, implemented through a partnership between the Ministry of Health in Libera and NIAID (PREVAIL III; ClinicalTrials.gov number, NCT02431923). A primary objective of PREVAIL III is to determine the sequelae of EBOV infection. Enrollment occurred at three research sites (John F. Kennedy Medical Center and Duport Road Clinic in Monrovia, C.H. Rennie Hospital in Kakata) from June 2015 through June 2017. The methods and findings of the primary study have been published.[19] In brief, PREVAIL III enrolled EVD survivors who were listed in the Liberian Ministry of Health Registry and had a documented diagnosis of EVD. These survivors listed their close contacts, who were then eligible to be enrolled. A close contact was an individual selected by an EVD survivor as someone with whom the survivor had contact during acute EVD or with whom they had sexual contact following acute EVD. Survivors and close contacts underwent study visits every six months that included a symptom checklist, physical

examination, and collection of blood. A subset of participants was referred for a separate eye examination.

The study protocol was approved by the National Research Ethics Board of Liberia (NREB), the University of California, San Francisco Institutional Review Board, and the National Institute of Allergy and Infectious Diseases Institutional Review Board (NIAID IRB) at the United States National Institutes of Health. Written informed consent was obtained from all participants.

Measurements

Serum samples were analyzed for anti-glycoprotein EBOV antibody levels using the Filovirus Animal Nonclinical Group (FANG) assay. A cutoff of 548 enzyme-linked immunosorbent assay units (EU) per milliliter was applied to determine seropositivity with 94.4% sensitivity and 96.7% specificity.[19]

The explanatory variable included four groups: 1) EVD survivors (seropositive), 2) contacts with unrecognized EVD (seropositive), 3) contacts with pauci-/asymptomatic EBOV infection (seropositive), and 4) uninfected contacts (seronegative). We assumed that individuals who were seropositive had an EBOV infection following exposure; we then used the serostatus and self-reported post-exposure symptoms of each participant to determine group membership. Self-reported symptoms were determined with the following question: “Did you develop any of the following symptoms within 21 days of the survivor’s Ebola event?” The checklist of self-reported symptoms included 16 items from the World Health Organization EVD case definition,[54] with responses selected as a binary (yes/no). We compiled these responses and classified each contact-participant as either asymptomatic or symptomatic. Participants who were seropositive and asymptomatic responded no to all 16 questions and were classified as having had a pauci-/asymptomatic infection. We selected “pauci-/asymptomatic” as the description of individuals who reported being asymptomatic because of the potential for

asymptomatic infection or mild symptoms with recall error. Contact-participants who were seropositive and symptomatic were defined as having had unrecognized EVD.

Severity of acute EVD illness was defined by the following categories of disease: no infection, pauci-/asymptomatic infection, unrecognized EVD, and reported EVD cases who survived. The unrecognized EVD cases did not receive testing or medical care during their illness, remained at home through the duration of their infection, and reported, on average, fewer symptoms than reported EVD survivors during their post-EVD exposure period.[19]

Outcomes were defined as current symptoms and physical exam findings reported at each study visit. The current symptoms and physical examination findings reported in this analysis were limited to those found to be significantly more or less prevalent between survivors and close contacts at baseline ($p<0.0001$) in the parent study by Sneller et al.[19] Symptoms included urinary frequency, headache, fatigue, muscle pain, memory loss, and joint pain. Physical examination findings included neurologic findings, chest findings, muscle findings, joint findings, abdominal findings, and uveitis.

Statistical analysis

Categorical baseline factors were compared between groups using chi-squared tests, age at enrollment was compared between groups using one-way ANOVA, and baseline antibody concentrations were compared between survivors and close contact groups using linear regression models with generalized estimating equations (GEE) that adjusted for relationships between survivors and close contacts. Comparisons of the prevalence of self-reported symptoms and abnormal findings on physical examination at the baseline and 12-month study visits were analyzed using GEE logistic regression models. All models were adjusted for age at PREVAIL III enrollment, sex, and enrollment site, except for models comparing uveitis at the 12-month visit, which was only adjusted for age and sex. These GEE

models adjusted for potential correlation of outcomes within groups of survivors and associated close contacts.

We conducted time-to-event analyses for symptoms, physical examination findings, hospitalization, and death within one year of enrollment. Incidence rates per 1000 person-years of symptoms and physical examination findings are reported for those without symptoms or findings at baseline. Time-to-event for symptoms and hospitalization was calculated as the number of days from enrollment to the date of the 6- or 12-month follow-up visit, either the first at which a symptom or hospitalization was reported, or, if neither reported, the last to occur. Survival time was calculated as the number of days from enrollment to death, follow-up discontinuation date, or the one-year anniversary of enrollment, whichever occurred first. The occurrence of symptoms, findings on physical examination, hospitalization, and death within one year of enrollment was compared between groups using Cox proportional hazard models, which adjusted for age, sex, and enrollment site, except the model for uveitis which only adjusted for age and sex. Generalized estimating equations were used to adjust for potential correlation of outcomes within groups of survivors and associated close contacts. We estimated hazard ratios from these Cox models. All analyses were performed using R version 3.3.2 (R Project for Statistical Computing, Vienna, Austria). P-values < 0.05 were considered statistically significant.

Results

Among 3,679 participants, 991 (27%) were reported EVD survivors, 107 (3%) were contacts with unrecognized EVD, 115 (3%) were contacts with pauci-/asymptomatic infection, and 2,466 (67%) were uninfected contacts. The median time from acute EVD onset to baseline was 317 days (interquartile range, 271 to 366). Among all participants, 2,048/3,679 (56%) were female, and the median age was 25 years (interquartile range [IQR]: 15, 36). The baseline characteristics of the analysis cohort are shown in **Table 1**. EVD survivors had higher median

antibody levels than other groups (**Figure 1**). Loss to follow-up at month 12 was 412/3,679 (11%).

We determined prevalence of selected symptoms and examination findings and created a graphical representation for each group at study baseline (**Figure 2**). **Table 2** lists the prevalence of the selected symptoms and exam findings by each group at baseline and month 12.

At baseline, we observed a stepped increase in prevalent findings across groups. Prevalent findings of joint pain, memory loss, muscle pain and fatigue were lowest among those with pauci-/asymptomatic or no infection, increased among contacts with unrecognized EVD, and were highest among EVD survivors. The trend was clearest in the report of joint pain (467/991 [47.1%] EVD survivors; 31/107 [29.0%] contacts with unrecognized EVD; 14/115 [12.2%] pauci-/asymptomatic contacts; 434/2,466 [17.6%] uninfected contacts), memory loss (284/991 [28.7%]; 11/107 [10.3%]; 6/115 [5.2%]; 113/2,466 [4.6%]), muscle pain (227/991 [22.9%]; 16/107 [15.0%]; 10/115 [8.7%]; 242/2,466 [9.8%]), and fatigue (180/991 [18.2%]; 12/107 [11.2%]; 7/115 [6.1%]; 152/2,466 [6.2%]) (**Table 2**).

In adjusted analyses, this pattern remained for joint pain and memory loss. Survivors had 2.1 times higher adjusted odds of joint pain compared with contacts with unrecognized EVD (95% CI: 1.3-3.4) while contacts with unrecognized EVD had 1.9 times higher adjusted odds of joint pain compared with contacts with pauci-/asymptomatic infection and uninfected contacts (95% CI: 1.2-3.0). The largest magnitude of association that followed this pattern was for memory loss. Survivors had 4.5 times higher adjusted odds of memory loss compared with contacts with unrecognized EVD (95% CI: 2.4-8.3) while contacts with unrecognized EVD had 2.1 times higher adjusted odds of memory loss compared to pauci-/asymptomatic contacts and uninfected contacts (95% CI: 1.1-3.8) (**Table 3**).

Other patterns emerged from the results but were not consistently observed across multiple outcomes. For three symptoms (urinary frequency, muscle pain, uveitis), each finding was most prevalent among EVD survivors and observed at similar prevalence among the other three groups. Urinary frequency was observed among 143/991 (14%) of reported EVD survivors but only 3/107 (2.8%) of unrecognized EVD survivors, 5/115 (4.3%) of pauci-/asymptomatic contacts, and 83/2,466 (3.4%) of uninfected contacts. For one symptom (headache), the finding had similarly high prevalence among reported and unrecognized EVD survivors but was equally low among pauci-/asymptomatic and uninfected contacts. In adjusted analyses, these patterns remained for urinary frequency, muscle pain, and headache, but not uveitis (**Table 3**).

From baseline to the 12-month visit, the selected symptoms and clinical findings generally decreased in prevalence. As a result, most of the statistical associations present at baseline were no longer observed at 12 months (**Table 3**).

Over the same 12-month study period, participants reported the new occurrence of selected symptoms and clinical findings that were not reported at baseline. These incident findings occurred among fewer participants than prevalent findings. We compared these incident findings among groups to assess for potential patterns. Three incident findings (headache, memory loss, chest findings) were more likely to occur among EVD survivors or contacts with unrecognized EVD than among contacts with pauci-/asymptomatic infection or uninfected contacts. Survivors had 2.3 times higher adjusted hazard of headache compared with contacts with pauci-/asymptomatic infection (95% CI: 1.3-4.2). Contacts with unrecognized EVD had 9.6 times higher adjusted hazard of memory loss (95% CI: 1.9-49.8) and 2.6 times higher adjusted hazard of chest findings (95% CI: 1.1-6.3) compared with uninfected contacts. We did not find any differences in the rates of hospitalization or mortality among the groups (**Table 4**).

Discussion

In this longitudinal cohort in Liberia following the 2013-2016 Ebola outbreak, we found evidence of post-EVD clinical sequelae in contacts with unrecognized EVD but not in contacts with pauci-/asymptomatic EBOV infection. Prior cohort studies of EVD survivors were smaller in size, so even had they identified a group of contacts with pauci-/asymptomatic infection and unrecognized EVD, they were underpowered, did not use a control group, and were unable to reliably identify the presence or absence of post-EVD symptoms and exam findings considered to be clinical sequelae.[55,56] Our findings were consistent for multiple symptoms (memory loss, headache, and joint pain), which adds strength to the evidence that post-EVD sequelae occur among unrecognized EVD contacts. Once contacts with post-exposure EVD symptoms are identified as seropositive in future outbreaks, the EVD response community should screen this group for post-EVD clinical sequelae and offer clinical care and support services.

We found patterns that more severe acute illness has the potential to cause specific types of post-EVD clinical sequelae. Particularly, memory loss, joint pain, headache, and urinary frequency were observed at higher prevalence across groups (reported EVD >> unrecognized EVD $\geq$ no or pauci-/asymptomatic infection). Other studies have demonstrated that specific features of acute illness (symptoms associated with more severe illness or level of viremia) can lead to post-EVD sequelae, including uveitis and joint pain,[42,53] so our findings support this growing body of evidence. We also extend the evidence for a viral-load dependent association between acute illness and clinical sequelae,[53] by showing its occurrence across the spectrum of clinical manifestations, particularly among those with unrecognized EVD (a group identified as having fewer symptoms than reported EVD survivors during acute illness[19]). Our proof-of-concept study offers insight into the types of post-EVD clinical sequelae potentially observed in the clinical setting; however, we need natural history studies that prospectively enroll individuals

with asymptomatic infection and mild illness during the acute phase and follow them into the convalescence phase in order to confirm our findings.

We recognize several limitations to our study. We lack data (biological, clinical, social, psychological) from the pre-enrollment period, including acute illness, so there is potential unmeasured confounding. Given our lack of within-subject measurements starting from the acute illness, we cannot definitively consider the reported current symptoms or physical examination findings as post-EVD clinical sequelae. Enrollment started nearly a year after survivors were discharged from an Ebola treatment facility, and those who were sicker may have been more likely to participate, which may be a source of selection bias. Our classification of contacts as seropositive or seronegative cannot be used to confirm infection because of potential cross-reactivity and measurement error, but the test performance characteristics of the immunoassay used in this study are highly accurate,[19] robust over time,[57,58] and have been used in several other high-impact studies.[59] In terms of external validity, EBOV vaccines were introduced at the end of this outbreak, so our findings represent the potential for post-EVD clinical sequelae in an unvaccinated population. There were several strengths of our study, including the use of a control group to demonstrate between-group differences and sufficient power to draw reliable conclusions across most groups. We did not have sufficient power to compare unrecognized EVD contacts against contacts with pauci-/asymptomatic infection, even though this was the largest study of unrecognized EVD and pauci-/asymptomatic EBOV infected contacts to date.

This paper emphasizes the public health and clinical care value in identifying contacts with unrecognized EVD, a substantially large population (8.7% in our cohort). Our proof-of-concept study strongly suggests the need for widespread testing of contacts during an EVD outbreak so that unrecognized EVD can be reduced and post-outbreak surveillance of remaining individuals with unrecognized EVD can lead to their identification and linkage to care.

The full clinical spectrum of acute viral infections such as SARS-CoV-2 has become increasingly recognized to cause post-infectious clinical sequelae.[60] In conclusion, contacts with unrecognized EVD can suffer from post-EVD clinical sequelae and are in need of equitable access to care and support services.

Table 2.1 Demographic and follow-up summary. Frequencies and percentages are presented unless specified otherwise. Categorical variables were compared between groups using chi-squared tests and age was compared between groups using one-way ANOVA.

	Survivors (N=991)	Unrecognized EVD[1] (N=107)	Pauci- or asymptomatic (N=115)	Uninfected (N=2466)	Overall (N=3679)	*p*-value for difference between groups
Female	545 (55)[2]	66 (61.7)	63 (54.8)	1374 (55.7)	2048 (55.7)	0.62
Age in years at enrollment	29 (19, 40)	25 (16, 37)	23 (14, 34)	23 (14, 35)	25 (15, 36)	<0.0001
log_{10} antibody concentration (EU/ml)	4.28 (4.1, 4.52)	4.19 (3.64, 4.45)	3.44 (2.92, 3.92)	1.93 (1.69, 2.2)	2.2 (1.81, 4.01)	see Figure 1
Days from Ebola treatment unit discharge to enrollment	317 (271, 366)	NA	NA	NA	NA	NA
Ebola treatment unit discharge date unknown	212 (21.4)	NA	NA	NA	NA	NA
Enrollment site						< 0.0001
JFK	618 (62.4)	48 (44.9)	51 (44.3)	1144 (46.4)	1861 (50.6)	
C.H. Rennie	161 (16.2)	28 (26.2)	34 (29.6)	678 (27.5)	901 (24.5)	
Duport Road	212 (21.4)	31 (29)	30 (26.1)	644 (26.1)	917 (24.9)	
Completed 12-month follow-up visit	881 (88.9)	97 (90.7)	101 (87.8)	2188 (88.7)	3267 (88.8)	0.92
Baseline ophthalmic exam	889 (89.7)	57 (53.3)	39 (33.9)	972 (39.4)	1957 (53.2)	<0.0001
12-month ophthalmic exam	544 (54.9)	33 (30.8)	24 (20.9)	595 (24.1)	1196 (32.5)	<0.0001

[1] EVD = Ebola Virus Disease
[2] Cells formatted as number (percentage), N(%) or median (IQR)

Table 2.2 Prevalence of selected symptoms and findings on physical examination at baseline and 12 months

	Survivors	Unrecognized EVD	Pauci-/asymptomatic	Uninfected
Urinary frequency				
Baseline visit	143 (14.4)	3 (2.8)	5 (4.3)	83 (3.4)
12-month visit	18 (2)	1 (1)	0 (0)	21 (1)
Headache				
Baseline visit	474 (47.8)	54 (50.5)	37 (32.2)	879 (35.6)
12-month visit	283 (32.1)	12 (12.4)	8 (7.9)	275 (12.6)
Fatigue				
Baseline visit	180 (18.2)	12 (11.2)	7 (6.1)	152 (6.2)
12-month visit	45 (5.1)	1 (1)	1 (1)	24 (1.1)
Muscle pain				
Baseline visit	227 (22.9)	16 (15)	10 (8.7)	242 (9.8)
12-month visit	110 (12.5)	11 (11.3)	8 (7.9)	161 (7.4)
Memory loss				
Baseline visit	284 (28.7)	11 (10.3)	6 (5.2)	113 (4.6)
12-month visit	41 (4.7)	1 (1)	0 (0)	3 (0.1)
Joint pain				
Baseline visit	467 (47.1)	31 (29)	14 (12.2)	434 (17.6)
12-month visit	237 (26.9)	16 (16.5)	12 (11.9)	189 (8.6)
Chest findings				
Baseline visit	57 (5.8)	1 (0.9)	10 (8.7)	69 (2.8)
12-month visit	15 (1.7)	1 (1)	4 (4)	20 (0.9)
Joint findings				
Baseline visit	50 (5.2)	2 (1.9)	1 (0.9)	50 (2)
12-month visit	22 (2.5)	2 (2.1)	2 (2)	25 (1.1)
Neurologic findings				
Baseline visit	54 (5.4)	4 (3.7)	2 (1.7)	35 (1.4)
12-month visit	14 (1.6)	1 (1)	0 (0)	13 (0.6)
Muscle findings				
Baseline visit	49 (5.1)	3 (2.9)	0 (0)	30 (1.2)
12-month visit	9 (1)	0 (0)	0 (0)	9 (0.4)
Abdominal findings				
Baseline visit	181 (18.3)	16 (15)	19 (16.5)	278 (11.3)
12-month visit	111 (12.6)	12 (12.4)	10 (9.9)	205 (9.4)
Uveitis				
Baseline visit	239 (26.9)	10 (17.5)	6 (15.4)	118 (12.1)

12-month visit	181 (33.3)	6 (18.2)	5 (20.8)	94 (15.8)

Table 2.3 Odds ratios (95% CI) for selected symptoms and findings on physical examination. All odds ratios were estimated using logistic regression models that adjusted for age, sex, and enrollment site (except for the odds ratios for uveitis, which are adjusted for age and sex only) and used GEE to adjust for relationships between survivors and close contacts.

	Survivors vs. Unrecognized EVD		**Survivors vs. Pauci-/asymptomatic**		**Unrecognized EVD vs. Uninfected**		**Pauci- or asymptomatic vs. Uninfected**	
	Baseline visit	12-month visit	Baseline visit	12-month visit	Baseline visit	12-month visit	Baseline visit	12-month visit
Urinary frequency	8.14 (2.73, 24.29)	2.4 (0.3, 19.1)	4.42 (1.79, 10.91)	NA	0.73 (0.24, 2.17)	0.94 (0.12, 7.52)	1.34 (0.53, 3.4)	NA
Headache	0.88 (0.58, 1.34)	2.99 (1.6, 5.59)	1.82 (1.17, 2.86)	4.99 (2.37, 10.53)	1.78 (1.18, 2.69)	0.97 (0.52, 1.8)	0.86 (0.55, 1.33)	0.58 (0.27, 1.23)
Fatigue	1.79 (0.88, 3.62)	4.82 (0.66, 35.1)	3.21 (1.45, 7.11)	5.02 (0.69, 36.61)	1.78 (0.87, 3.61)	0.93 (0.12, 7.01)	0.99 (0.46, 2.15)	0.89 (0.12, 6.65)
Muscle pain	2.02 (1.14, 3.57)	1.4 (0.69, 2.84)	3.53 (1.73, 7.18)	2.37 (1.15, 4.87)	1.53 (0.87, 2.69)	1.81 (0.91, 3.61)	0.88 (0.43, 1.77)	1.07 (0.53, 2.17)
Memory loss	4.47 (2.41, 8.3)	3.56 (0.49, 25.78)	8 (3.47, 18.47)	NA	2.06 (1.1, 3.84)	7.5 (0.8, 70.47)	1.15 (0.5, 2.65)	NA
Joint pain	2.13 (1.34, 3.39)	1.61 (0.93, 2.79)	6.69 (3.7, 12.1)	2.24 (1.15, 4.36)	1.89 (1.21, 2.97)	2.14 (1.23, 3.75)	0.6 (0.34, 1.09)	1.54 (0.78, 3.03)
Chest findings	6.91 (0.95, 50.27)	1.91 (0.24, 14.92)	0.65 (0.32, 1.35)	0.43 (0.14, 1.34)	0.31 (0.04, 2.28)	1.02 (0.13, 7.97)	3.32 (1.63, 6.76)	4.55 (1.51, 13.75)
Joint findings	3.59 (0.78, 16.51)	1.01 (0.22, 4.55)	7.1 (0.93, 54)	0.9 (0.2, 4.1)	0.79 (0.17, 3.57)	1.73 (0.39, 7.65)	0.4 (0.05, 3.01)	1.94 (0.44, 8.53)
Neurologic findings	1.64 (0.62, 4.37)	1.21 (0.17, 8.4)	3.21 (0.7, 14.66)	NA	2.31 (0.87, 6.15)	1.71 (0.24, 12.13)	1.18 (0.26, 5.36)	NA
Muscle findings	3 (0.79, 11.33)	NA	NA	NA	1.75 (0.45, 6.74)	NA	NA	NA
Abdominal findings	1.59 (0.93, 2.72)	1.17 (0.58, 2.34)	1.12 (0.66, 1.91)	1.2 (0.58, 2.48)	1.17 (0.7, 1.95)	1.1 (0.56, 2.18)	1.65 (0.99, 2.76)	1.07 (0.53, 2.16)
Uveitis	1.72 (0.85, 3.48)	2.12 (0.76, 5.95)	2.02 (0.82, 4.93)	1.77 (0.63, 4.92)	1.5 (0.74, 3.05)	1.15 (0.41, 3.26)	1.29 (0.52, 3.16)	1.39 (0.49, 3.9)

Table 2.4 Incidence of selected symptoms and findings on physical examination, hospitalization since last follow-up visit, and death within 1 year after enrollment. Hazard ratios are from Cox proportional hazard models and are adjusted for age, sex, and enrollment site unless noted otherwise. The models used GEE to adjust for relationships between survivors and close contacts. Time-to-event for symptoms and hospitalization was calculated as the number of days from enrollment to the date of the 6- or 12-month follow-up visit, either the first at which the symptom was reported, or, if no symptom reported, the last that took place. Survival time was calculated as the number of days from enrollment to death, follow-up discontinuation date, or the 1-year anniversary of enrollment, whichever occurred first.

	N new cases (Cases / 1000 person-yr)				Hazard Ratios (95% CI)			
	Survivors	Unrecognized EVD	Pauci- or asymptomatic	Uninfected	Survivors vs. Unrecognized EVD	Survivors vs. Pauci- or asymptomatic EVD	Unrecognized EVD vs. Uninfected	Pauci-symptomatic vs. Uninfected
Urinary frequency	14 (19'.25)	2 (21.4)	2 (20.49)	48 (22.64)	1.09 (0.25, 4.76)	0.96 (0.22, 4.28)	0.85 (0.21, 3.45)	0.96 (0.23, 3.99)
Headache	142 (368.24)	15 (340.63)	10 (141.87)	302 (227.71)	0.94 (0.54, 1.61)	2.33 (1.29, 4.22)	1.5 (0.9, 2.52)	0.6 (0.34, 1.08)
Fatigue	68 (99.96)	5 (58.84)	6 (62.22)	71 (34.84)	1.56 (0.62, 3.91)	1.38 (0.59, 3.26)	1.61 (0.65, 4.04)	1.82 (0.78, 4.25)
Muscle pain	122 (194.88)	15 (190.38)	11 (121.02)	244 (127.42)	0.94 (0.56, 1.57)[1]	1.35 (0.72, 2.53)[1]	1.33 (0.8, 2.19)[1]	0.93 (0.51, 1.71)[1]
Memory loss	50 (85.07)	2 (22.89)	0 (0)	5 (2.38)	3.57 (0.85, 14.9)[1]	NA	9.61 (1.86, 49.75)[1]	NA
Joint pain	112 (277.49)	8 (120.21)	12 (135.34)	209 (119.58)	1.82 (0.94, 3.53)	1.64 (0.93, 2.91)	1.01 (0.53, 1.91)	1.12 (0.64, 1.96)
Chest findings	23 (28.37)	6 (63.84)	3 (31.78)	49 (23.11)	0.47 (0.18, 1.18)	0.85 (0.26, 2.85)	2.62 (1.09, 6.29)	1.43 (0.45, 4.52)
Joint findings	26 (33.26)	1 (10.72)	2 (19.71)	19 (8.91)	2.1 (0.31, 14.4)	1.05 (0.25, 4.54)	1.14 (0.17, 7.83)	2.27 (0.53, 9.7)
Neurologic findings	21 (26.19)	0 (0)	0 (0)	9 (4.15)	NA	NA	NA	NA
Muscle findings	13 (16.74)	0 (0)	0 (0)	9 (4.23)	NA	NA	NA	NA
Abdominal findings	81 (120.16)	13 (163.12)	6 (70.87)	187 (97.97)	0.71 (0.39, 1.27)	1.56 (0.67, 3.67)	1.56 (0.89, 2.74)	0.7 (0.3, 1.64)
Uveitis	91 (188.9)	3 (99.68)	3 (148.48)	55 (97.33)	NA	NA	0.86 (0.28, 2.7)[1]	2.23 (0.64, 7.74)[1]
Hospitalization	53 (62.57)	3 (31.31)	4 (39.45)	54 (24.7)	2.16 (0.68, 6.79)	1.52 (0.54, 4.27)	1.16 (0.36, 3.72)	1.64 (0.61, 4.42)
Death ≤1 year after enrollment	4 (4)	2 (18.8)	1 (8.7)	11 (4.5)	0.21 (0.04, 1.21)	0.42 (0.05, 3.86)	3.9 (0.79, 19.4)	1.94 (0.25, 15.3)

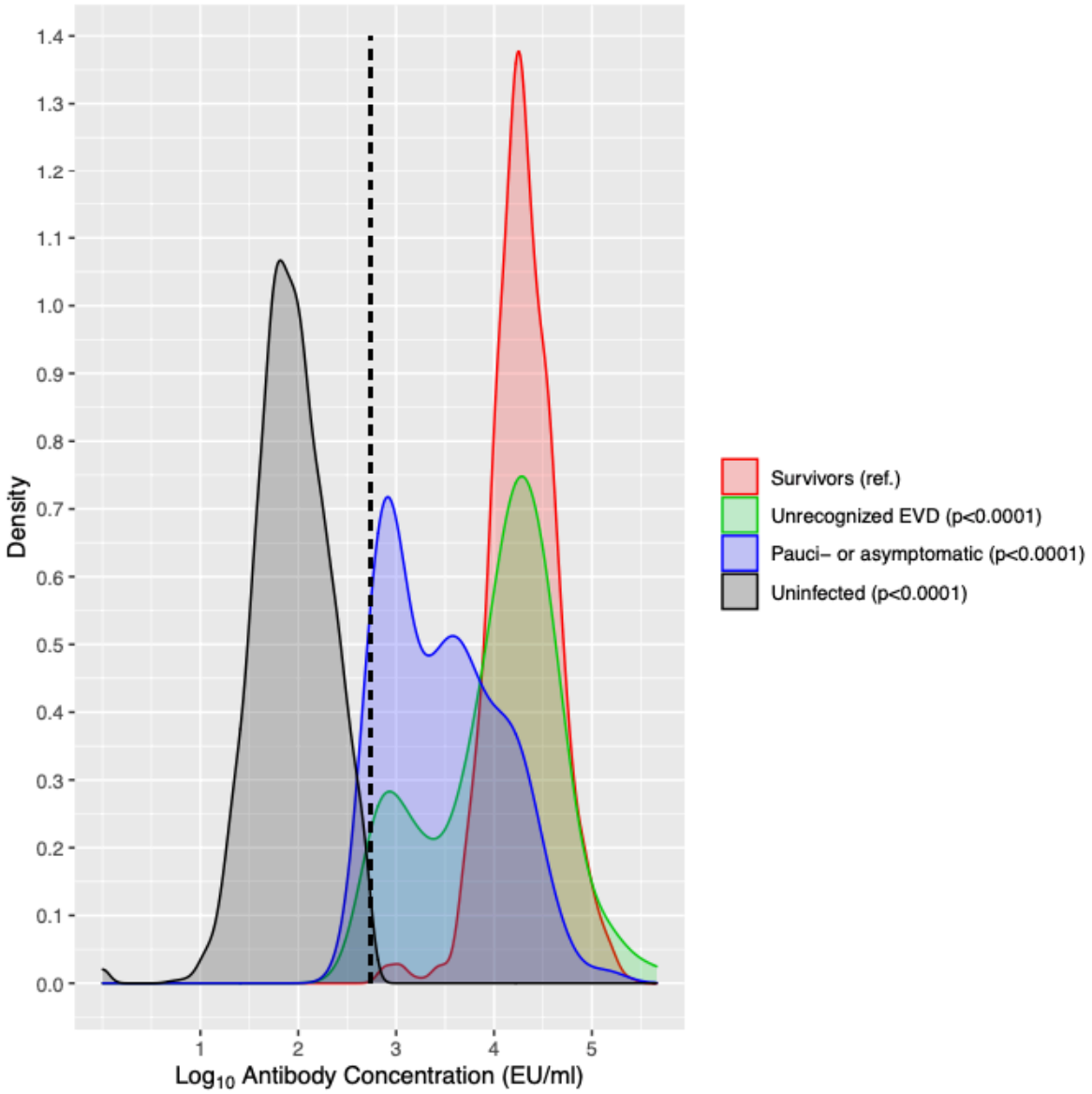


Figure 2.1 Antibody concentrations by group and p-values for tests comparing concentrations in the close contact groups to survivors. Concentrations were compared using GEE linear regression models that adjusted for relationships between survivors and close contacts. The cutoff for seropositivity is indicated by a vertical line.

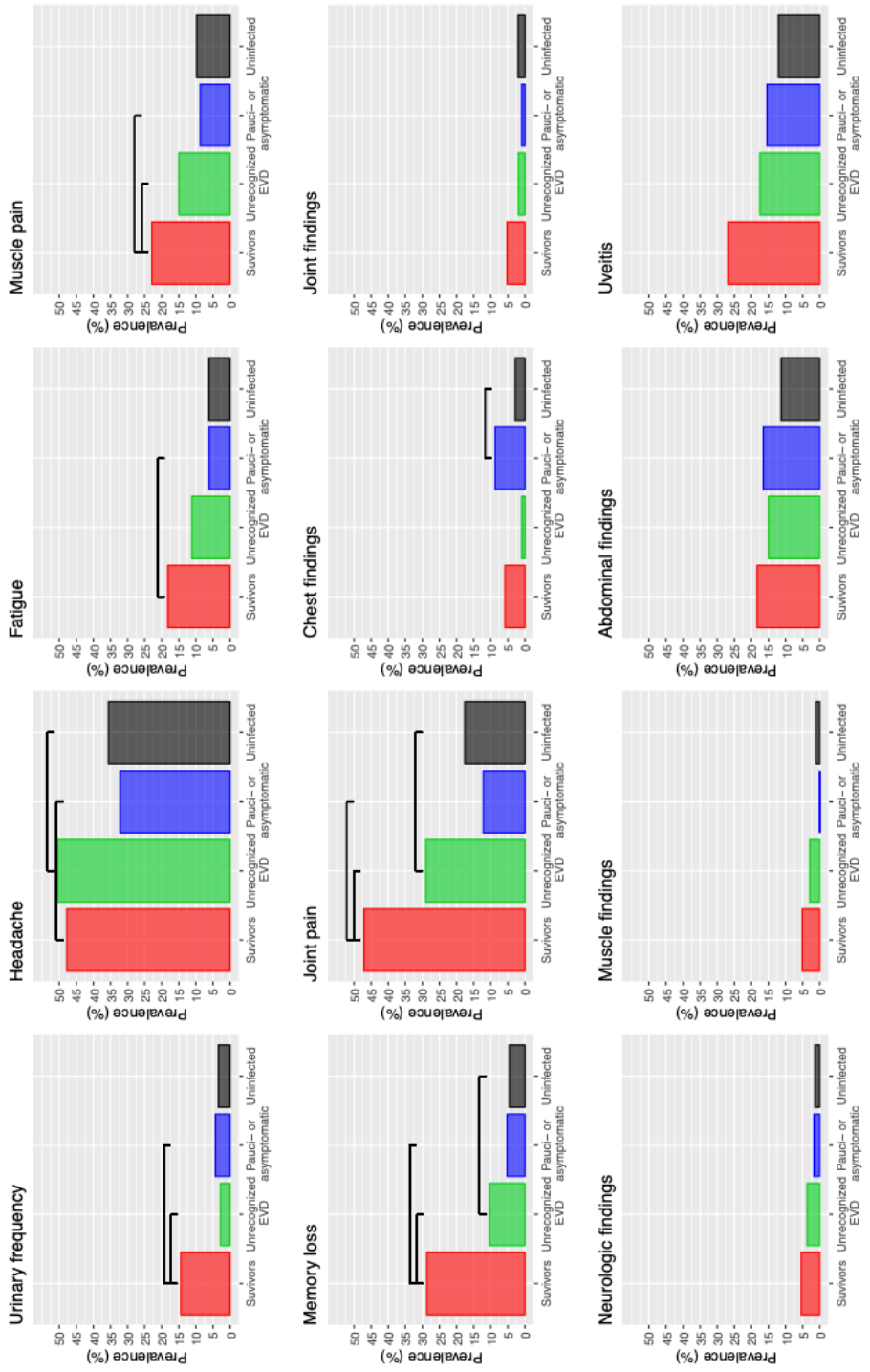


Figure 2.2 Prevalence of selected symptoms and findings on physical examination at baseline. Statistically significant comparisons (alpha=0.05) are indicated by horizontal brackets. Survivors were compared to the unrecognized EVD and pauci-/asymptomatic groups and the unrecognized EVD and pauci-/asymptomatic groups were compared to the uninfected group. Comparisons were made using logistic regression models that adjusted for age, sex, and

enrollment site (except the model for uveitis, which adjusted for age and sex only) and used GEE to adjust for relationships between survivors and close contacts.

Chapter 3. Association of inflammatory markers with Ebola viral persistence and clinical sequelae

Abstract

Background: A high proportion of Ebola virus disease (EVD) survivors experience clinical sequelae, but the possible contributory role(s) of viral persistence and persistent inflammation in post-acute disease pathogenesis is poorly understood.

Methods: We used baseline data from a longitudinal cohort of confirmed EVD survivors (seropositive) and their uninfected contacts (seronegative) in Liberia to generate a cytokine profile from stored plasma samples. These data included a sub-cohort of men assessed for Ebola viral shedding in semen. We investigated pre-specified clinical findings previously reported to be differentially prevalent among EVD survivors. Outcomes were self-reported symptoms, physical examination findings, and viral persistence in semen. Using generalized estimating equations, we compared inflammatory markers among survivors and contacts; statistically significant markers ($p \leq 0.01$) were assessed for associations among survivors with and without sequelae.

Results: Our analysis cohort consisted of 1,044 participants (EVD survivors: n=594; uninfected contacts: n=450); the sub-cohort of 243 male survivors included 81 (33%) shedders. Median time from acute EVD to baseline was 317 days (interquartile range, 271-366). EVD survivors showed a pattern of elevated inflammatory markers indicative of macrophage (MCP-1, TNF-α, MIP1B, MCSF, CD14) and angiogenic factor activation (VEGFA) compared with contacts. MCP-1 was associated with a lower odds of memory loss (AOR: 0.66; 95%CI: 0.47, 0.91), urinary frequency (AOR: 0.62; 95%CI: 0.41, 0.95), and musculoskeletal abnormalities (AOR: 0.47; 95%CI: 0.26, 0.84); MCSF was associated with joint pain (AOR: 0.77; 95% CI 0.59, 0.99). Among the male sub-cohort, MCP-1 (AOR: 1.8; 95%CI: 1.04, 2.9) and VEGFA (AOR: 1.5; 95% CI: 1.2, 1.9) were elevated among viral shedders compared with non-shedders.

Conclusion: In a longitudinal cohort sampled approximately one year after acute EVD, we found evidence of persistent inflammation among survivors, which may be partially explained by

ongoing viral shedding. Multiple clinical sequelae were less likely to be associated with two macrophage and pro-inflammatory markers, suggesting that a process of downregulation may be occurring as these survivors experienced clinical recovery.

Introduction

A sizable proportion of Ebola virus disease (EVD) survivors experience post-acute clinical sequelae, including joint pain, uveitis, and memory loss, among others.[19,53] Viral persistence and persistent inflammation are two mechanisms that are known to explain specific clinical sequelae, such as uveitis.[58,61] These mechanisms are also hypothesized to cause other clinical sequelae, including symptoms (e.g., joint pain) and physical examination findings (e.g., musculoskeletal abnormalities).[43]

Acute and chronic inflammation has been described among survivors who complete recovery without clinical sequelae,[62] but few studies have explored patterns of immune activation in those with clinical sequelae.[3] Early in acute EVD, survivors are reported to experience a transient release of interleukin-1β (IL-1β), IL-6, tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-1α (MIP-1α) and MIP-1β,[63] followed by an uncomplicated recovery phase (without clinical sequelae) during which IL-1 receptor antagonist (IL-1RA) and soluble receptors for TNFα (sTNF-R) and IL-6 (sIL-6R) persist until symptoms resolve.[62] In a small sub-study of the Postebogui cohort in Guinea, established toward the end of the 2013-2016 EVD outbreak in West Africa, some of these previously identified inflammatory markers were detected at higher levels among survivors than healthy controls up to two years post-EVD.[58] This study also correlated persistent inflammation with the presence of symptoms,[58] suggesting that immune dysregulation, e.g., activation of chemoattractant non-specific immune cells, may partially explain post-acute symptoms. Such dysregulation and post-acute disease processes (after antiretroviral therapy for HIV), specifically in upregulation of macrophages,[64] has been reported in other infectious diseases (e.g., HIV, SARS-CoV-2).[65-67]

Viral persistence is a second biological mechanism that causes post-acute clinical sequelae of EVD.[38,61] Although EBOV clears from the blood relatively quickly,[68] viral persistence continues in immune-privileged sites such as the eyes, central nervous system, and testes, and

contributes to the disease pathogenesis observed among EVD survivors with uveitis and meningoencephalitis.[38,61] Following the 2013-2016 EVD outbreak, surveillance efforts found prolonged periods of intermittent viral shedding in the semen (up to three years), which cleared over time.[68-70] Viral sequencing of survivors from the 2021 EVD outbreak in Guinea, however, provided strong evidence of a survivor serving as a human EBOV reservoir from the 2013-2016 EVD outbreak rather than a novel spillover event from an animal reservoir.[71] Although viral persistence and reservoirs have been demonstrated to varying degrees across a series of studies,[70,72] the confirmed existence and precise site(s) of a hypothesized EBOV human reservoir remain unsettled. Further, these earlier studies were not designed to assess the presence of systemic chronic inflammation among those with and without EBOV viral shedding. Viral persistence, however, has been described as a driver of ongoing inflammation in other infectious diseases, particularly HIV.[73]

To inform the design and deployment of targeted treatments, there is a need to determine if clinical sequelae observed at least one-year post-EVD are associated with persistent inflammation, and the extent to which ongoing viral shedding is a driver of inflammation. The U.S. Partnership for Research on Ebola Virus in Liberia (PREVAIL) created a cohort of EVD survivors and their close contacts toward the end of the 2013-2016 West Africa EVD epidemic and identified a set of symptoms and physical examination findings that were more prevalent among survivors than contacts.[19] Over the first year of follow-up, the clinical sequelae of many survivors resolved. In this report, we characterize the cytokine profile of a sub-group of EVD survivors and contacts to investigate the association of inflammatory markers with post-acute clinical sequelae and viral persistence, starting at the baseline visit from the PREVAIL III cohort (median 317 days with interquartile range of 271 to 366). The implications of this investigation may deepen insights explicating the pathogenesis of resolving and/or

persistent post-infectious sequelae for EVD and other viral diseases, leading to more rational and efficient development of anti-viral and/or anti-inflammatory agents.

Methods

Ethics statement

The study protocol was approved by the National Research Ethics Board of Liberia, University of California, San Francisco, Institutional Review Board, and the National Institute of Allergy and Infectious Diseases Institutional Review Board (NIAID IRB) at the United States National Institutes of Health. Written informed consent was obtained from all participants.

Overall Design

This was a sub-cohort of the longitudinal cohort of EVD survivors and contacts implemented through a partnership between the Liberian Ministry of Health and NIAID.

Study Population

In the parent cohort, described in detail elsewhere,[19] EVD survivors were enrolled about 12 months following acute EVD (study baseline) if they had a documented diagnosis of EVD and were listed in the EVD registry created by the Liberian Ministry of Health. EVD survivors provided information regarding their close contacts while acutely ill or sexual contacts after recovery. Contacts with no history of EVD were invited to enroll as controls. EVD survivors and close contacts underwent similar study procedures during proximate time periods, which involved a medical examination (symptom checklist, physical examination, and collection of blood) at study baseline and every 6 months thereafter, for up to 5 years. A subset of participants received detailed eye examinations by multiple trained ophthalmologists at which time the diagnosis of uveitis[19] was made using the Standardization of the Uveitis

Nomenclature.[74] A subset of male participants provided semen samples, which were subsequently test for EBOV RNA.

In this study, we included participants aged ≥18 years at baseline, with baseline plasma available for analysis. Among EVD survivors, we included those confirmed to be seropositive by anti-glycoprotein EBOV IgG serological testing with Filovirus Animal Nonclinical Group (FANG) assay. Among contacts, we included those confirmed to be seronegative by the FANG immunoassay. We drew a sex- and survivor-stratified random sample of the parent cohort to create a sub-cohort reflecting the prevalence of symptoms and clinical findings in the parent cohort. The sample was enriched for two sub-groups: 1) EVD survivors and contacts with uveitis, and 2) EVD survivors who were male and part of the semen cohort.

Measurement of serum cytokines

Our explanatory variables were plasma cytokine levels at baseline visit. We used stored plasma samples to create a cytokine profile with the Ella *Protein Simpleplex* platform at the AIDS Monitoring Laboratory (AML) within the Frederick National Laboratory for Cancer Research (Frederick, MD, USA). The cytokine profile was composed of the following biological analytes: CRP, IFN-β, IL-1RA/IL-1F3, IL1-α and IL-1 β, IL-6, TNF, TNFR1, TNFRII, ICAM-1, MCP1, MCP2, M-CSF, sCD14, Granzyme A&B, IL-10, IL-5, IL-8, IL-2, IL-2RA, MIP-1α, MIP-1β, and VEGFA&B.

Measurements of clinical findings and semen viral RNA

Outcomes were self-reported symptoms, physical examination abnormalities, and viral RNA shedding. To reduce the likelihood of Type 1 errors, we examined previously reported clinical findings associated with post-EVD clinical sequelae. As reported in the one-year assessment of PREVAIL III,[19] EVD survivors had a higher prevalence than did close contacts of:

fatigue, headache, muscle pain, joint pain, urinary frequency, memory loss, musculoskeletal findings, neurological findings, and uveitis (acute and inactive) (all with P<0.0001). We used the modified GeneXpert Ebola reverse-transcriptase polymerase chain reaction (RT-PCR) to test for RNA-positivity in the semen. Male survivors provided multiple specimens, and a survivor was classified as a shedder if he had one or more positive tests.

Statistical analysis

In cross-sectional analyses, we assessed the association of inflammatory markers at baseline with clinical findings (symptoms, examination abnormalities) at baseline and viral RNA shedding in semen (shedders). Symptoms, physical examination findings, and viral RNA shedding were dichotomous outcomes. Cytokine levels were log-2 transformed and treated as continuous predictor variables. We determined associations of inflammatory markers comparing survivors with contacts. We used generalized estimating equations with an independence working correlation structure to estimate odds ratios, adjusting for age, sex, survivor status, and clustering for survivor-contact relationships. We then restricted analyses to survivors and examined inflammatory markers, which had strong, statistical significance ($p \leq 0.01$), for associations with and without sequelae (clinical findings and ongoing viral shedding). We considered associations with and without sequelae to be statistically significant with a p-value of less than 0.05. All statistical analyses were performed with R software, version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Table 1 presents complete demographic results of the analytic cohort. Of 1,044 participants, 594 (57%) were positive EVD survivors and 450 (43.1%) were seronegative contacts (controls). Among survivors, 307 (52%) were male, and there were data on viral

shedding for 243 (79%) of them. Ophthalmic data were available for 1,041 (99.7%) participants. The median time from acute EVD onset to baseline study visit was 317 days (interquartile range, 271 to 366). Most inflammatory markers reached detectable levels among all or nearly all participants. Notable exceptions were IL-1A, IL-1B, IL-2, and INF-β, which were below the limit of detection (LOD) for more than 85% of participants (**Supplementary Table 1**).

Table 2 presents reported symptoms and physical examination findings. Of 594 survivors, 524 (88%) had at least one clinical finding. About half reported headache (47%) and joint pain (55%). Uveitis was the most common objective finding (35%). Among the 243 men sampled for RNA viral shedding in semen, 81 (33%) had EBOV RNA detected in at least one sample.

Macrophage and immune activation among EVD survivors versus controls

EVD survivors had a pattern of elevated inflammatory markers indicative of macrophage and angiogenic factor activation compared with controls (**Table 3**). In particular, higher median plasma levels of MCP-1, MCP-2, TNF-α, MIP1B, MCSF, CD14, VEGFA, and CRP were associated with survivors as compared with controls (**Figure 1**). Of these eight markers, MCP-1, TNF-α, MIP1B, MCSF, and VEGFA had the strongest associations with survivors, which were reported as follows ($p \leq 0.01$): for MCP1, adjusted odds ratio [AOR]: 1.6; 95% confidence interval [CI]: 1.2, 2.0; for TNF-α, AOR: 1.3; 95% CI: 1.1, 1.5; for MIP1B, AOR: 1.3; 95% CI: 1.1, 1.5; for MCSF, AOR: 1.4; 95% CI: 1.1, 1.7; and for VEGFA, AOR: 1.3; 95% CI: 1.2, 1.4. Other markers of immune activation were not associated with survivors as compared with controls.

Macrophage and pro-inflammatory markers were associated with multiple clinical sequelae

Among EVD survivors, we found that a pattern of plasma levels of MCP-1 and MCSF (macrophage and pro-inflammatory markers) inversely associated with multiple clinical

sequelae, including urinary frequency, memory loss, joint pain, and musculoskeletal findings (**Table 4**). This pattern was strongest between MCP-1 and clinical sequelae. Higher median plasma levels of MCP-1 had a lower adjusted odds of being detected among survivors with urinary frequency (AOR: 0.62; 95% CI: 0.41, 0.95; p=0.03), memory loss (AOR: 0.66; 95% CI: 0.47, 0.91; p=0.01), and musculoskeletal abnormalities (AOR: 0.47; 95% CI: 0.26, 0.84; p=0.01) than among those without these sequelae (**Figures 2A-C**). In addition, higher median plasma levels of MCSF had a lower adjusted odds of being detected among survivors with joint pain (AOR: 0.77; 95% CI 0.59, 0.992; p=0.04; **Figure 2D**).

Ongoing viral RNA shedding in semen correlated with activation of macrophages and vascular endothelial growth factor

Among the male sub-cohort evaluated for viral RNA shedding, we found a pattern of inflammatory markers (MCP-1, VEGFA) conversely correlating with ongoing viral RNA shedding (**Table 4**). Higher median plasma levels of MCP-1 (macrophage and pro-inflammatory marker) had a higher adjusted odds of being detected among shedders than non-shedders (AOR: 1.75; 95% CI: 1.04, 2.93; p=0.04). Other markers of macrophage activation were not associated with RNA viral shedding, but higher median plasma levels of the angiogenic factor VEGFA was associated with RNA viral shedding (AOR: 1.52; 95% CI: 1.2, 1.9; p<0.01) (**Figure 3**). We did not find evidence of any additional sources of immune activation comparing RNA viral shedders with non-shedders (**Supplementary Table 2**).

Discussion

In this large longitudinal cohort of EVD survivors compared with controls, we observed a set of associations that offer insight into potentially causative mechanisms for inflammation and post-acute clinical sequelae. We found that survivors had evidence of systemic chronic

inflammation, particularly macrophage markers and angiogenic factors, which was partially driven by ongoing viral RNA shedding. After selecting five inflammatory markers with the strongest statistical significance among survivors, we found an inverse relationship between plasma levels and clinical sequelae, suggesting that a process of downregulation may be occurring. These findings are consistent with the observation from the PREVAIL parent cohort that a large proportion of EVD survivors experienced clinical recovery over the first year of follow-up. Such an anti-inflammatory effect can be further substantiated because this pattern was consistent observed for two inflammatory markers with a similar function and associated with multiple clinical sequelae. These findings implicate the dysregulation of inflammatory markers in post-acute disease pathogenesis as far out as one-year post-EVD while re-enforcing the need to study the relationship between inflammatory markers and clinical sequelae at earlier points in disease course.

Our study leveraged its large sample size and one-year post-EVD timepoint to robustly evaluate the relationships of 25 inflammatory markers to survivors and identify a series of highly significant plasma markers. These findings confirmed the presence of systemic chronic inflammation among survivors in the Guinean cohort but not a second Liberian cohort. In the latter cohort, half of the inflammatory markers were below the LOD,[75] which may have been partially due to the timing of biospecimen collection and partially due to the disease burden. Among our survivor cohort, we had significant group of males who had ongoing EBOV RNA shedding and found that two of five highly statistically significant inflammatory markers were also elevated among shedders. As a result, we were able to identify a potentially causal relationship between viral persistence and the inflammatory markers MCP-1 and VEGFA. An intriguing hypothesis is that ongoing viral replication in the testes may have caused cellular damage, induced chemoattractants for macrophages, and thereby contributed to these persistently elevated inflammatory markers.

Our findings build on the prior work of Wiedemann et al. in the EVD cohort in Guinea, which identified systemic chronic inflammation and links to clinical sequelae in a small cohort of 35 survivors (after 2 years).[58] The Guinean survivors displayed elevated IL-8, TNF-α, IL-1RA, soluble CD40L and CCL5; our cytokine panel overlapped with most of the inflammatory markers evaluated in the latter cohort, including IL-8, TNF-α, and IL-1RA. However, Wiedemann et al. only found elevated levels of TNF-α among survivors. We found that elevated levels of MCP-1 and MCSF were significantly correlated with multiple post-acute clinical sequelae, including urinary frequency, memory loss, joint pain and musculoskeletal findings. These as yet mechanistically unexplained relationships are hypothesis-generating for the concept that differing disease mechanisms, e.g., immune dysregulation, contribute to different clinical phenotypes.[43]

Although our analyses with viral shedding and clinical sequelae demonstrated opposing relationships with MCP-1, it is possible that our survivor cohort consists of multiple phenotypes, including those with persistent symptoms, ongoing recovery, and prior recovery, which could explain our observation. We did not have the sample size to evaluate these sub-groups, but it is possible that earlier in the post-acute disease process, viral replication may be linked to urinary frequency, memory loss, and musculoskeletal abnormalities, either directly or indirectly through MCP-1. In a small, randomized trial of remdesivir vs placebo in the PREVAIL parent cohort,[76] remdesivir accelerated Ebola viral clearance from the semen starting two months after administration. In addition to possible benefits from earlier anti-viral therapy, earlier studies of clinical sequelae should include MCP-1 and MCSF as inflammatory markers in the cytokine panel because evidence of these markers driving clinical sequelae may lead to development of anti-inflammatory agents.

We recognize several limitations. Most survivors were enrolled in this cohort nearly one-year after acute illness, so we did not have repeat measurements with which to evaluate viral

RNA persistence, inflammation, and disease mechanisms within the immediate first months following illness, nor could we link observations to acute illness. We also did not look at cytokines at multiple future timelines to determine the relationship over longer period of time. This study only included a single assessment of 25 inflammatory markers; there may have been unmeasured cytokines with relationships to viral persistence and clinical findings among survivors. Although we did not evaluate immunological parameters other than inflammatory markers, we performed multiple comparisons, which opens the possibility of type-1 errors. Our series of observations were consistent internally as well as with the general trajectory of the cohort and its clinical recovery. Given the lack of data about severity of acute illness, this and other missing measurements may have contributed to unmeasured confounding.

In conclusion, survivors known to be recovering from clinical sequelae of Ebola disease one-year post infection displayed evidence of anti-inflammatory processes, specifically lower levels of macrophage and pro-inflammatory markers. Regardless of clinical sequelae, our evidence of persistent inflammation, which correlated with viral persistence in this well-characterized cohort, could suggest that early anti-viral and/or anti-inflammatory therapy may shorten time to recovery. This suggests there will be utility in exploring the relationship of systemic inflammation with viral persistence and post-acute clinical sequelae earlier in recovery. Further work capturing these markers and clinical findings in the post-acute phase hold potential to inform the development of next-generation therapeutics that could prevent or treat select clinical sequelae.

Table 3.1 Demographic summary of Ebola virus disease (EVD) survivors and contacts status (survivors: N=594; contacts: N=450)

	Close contacts (N = 450)	Survivors (N = 594)	Overall (N = 1044)
N (%) female	228 (50.7)	287 (48.3)	515 (49.3)
Age in years at enrollment, median (IQR)	33(25,44)	33 (26,42)	33 (25, 43)
Months of follow-up, median (IQR)	42 (36,42)	42 (42,48)	42 (38, 44)
Individuals with ophthalmic data, N (%)	450 (100)	591 (99.5)	1041 (99.7)
Individuals with viral persistence data N (%)	NA	243 (40.9)	NA

Table 3.2 Description of clinical findings and viral persistence among EVD survivors at baseline visit (N=594)

Outcome	Survivors reporting finding N=594 (%)
Fatigue	112 (19)
Headache	281 (47)
Muscle pain	150 (25)
Joint pain	325 (55)
Urinary frequency	93 (16)
Memory loss	185 (31)
Musculoskeletal findings	47 (8)
Neurological findings	35 (6)
Any uveitis (N=591)	207 (35)
Acute uveitis (N=591)	39 (7)
Viral persistence in the semen (N=243)	81 (33)

Table 3.3 Associations of inflammatory markers with EVD survivor status (survivors: N=594; contacts: N=450)

	Survivor Status log2-transformed Med. (Q1,Q3) [% < LOD]			
	Contact (N = 450)	**Survivor (N = 594)**	**Odds Ratio (95% CI)**	**p-value**
Macrophage-specific cytokines				
MCP1	6.81 (6.492, 7.155) [0%]	6.971 (6.552, 7.326) [0%]	1571 (1.206, 2.047)	< 0.001
MCP2	4.299 (3.95, 4.77) [0%]	4.427 (4.024, 4.918) [0.2%]	1.217 (1.013, 1.462)	0.036
TNFA	2.291 (1.971, 2.685) [1.8%]	2.385 (2.043, 2.779) [1.2%]	1.261 (1.053, 1.511)	0.012
TNFR1	9.796 (9.598, 10.06) [0%]	9.862 (9.618, 10.1) [0%]	1.347 (0.988, 1.838)	0.06
TNFR2	11.14 (10.84, 11.47) 0%]	11.16 (10.85, 11.55) [0%]	1.2 (0.945, 1.524)	0.135
MIP1A	4.725 (4.28, 5.226) [0%]	4.822 (4.371, 5.411) [0%]	1.07 (0.983, 1.164)	0.119
MIP1B	5.706 (5.288, 6.007) [2.7%]	5.816 (5.416, 6.254) [1.9%]	1271 (1.086, 1.488)	0.003
MCSF	8.5 (8.126, 8.854) [0%]	8.59 (8.196, 9.021) [0%]	1.301 (1143, 1.693)	< 0.001
Non-macrophage specific cytokines				
VEGFA	5.172 (4.473, 5.99) [0.7%]	5.485 (4.78, 6.452) [0.3%]	1.278 (1157, 1.413)	< 0.001
VEGFB	5.383 (5.024, 5.735) [0.2%]	5.354 (5.014, 5.785) [0%]	1.178 (0.046, 1.466)	0.143
GRANA	7.154 (6.827, 7.485) [0%]	7.216 (6.857, 7.62) [0%]	1.189 (0.968, 1.461)	0.099
GRANB	3.703 (3.111, 4.351) [0%]	3.699 (3.147, 4.38) [0.2%]	0.969 (0.873, 1.076)	0.557
1L10	2.537 (1.894, 3.258) [0.7%]	2.414 (1.795, 3.507) [0.2%]	1.03 (0.931, 1.139)	0.57
IL2RA	10.48 (10.17, 10.82) [0%]	10.48 (10.17, 10.83) [0%]	1.019 (0.809, 1.284)	0.873
ILIRA	7.899 (7.565, 8.355) [0%]	7.866 (7.477, 8.394) [0%]	1.04 (0.882,1.227)	0.639
IL8	2.287 (1.791, 2.799) [0.4%]	2.374 (1.916, 2.941) [0.8%]	1.112 (0.968,1.277)	0.132
ICAM1	18.61 (18.32, 18.88) [0%]	18.6 (18.24, 18.91) [0%]	0.816 (0.641, 1.038)	0.098
CD14	20.15 (19.83, 20.42) [0%]	20.2 (19.89, 20.55) [0%]	1.332 (1.031, 1.722)	0.028
CRP	20.22 (18.88, 21.75) [0%]	20.41 (19.05, 22.07) [0%]	1.075 (1.009, 1.147)	0.026

Table 3.4 Associations of inflammatory markers with post-acute sequelae of clinical findings and viral RNA shedding (survivors: N=594)

		MCP-1	TNF-alpha	MIP-1B	VEGF-A	MCSF
Total Cohort (N = 594)						
Fatigue	112 (19)	0.85 (0.58, 1.24)	0.92 (0.69, 1,24)	1.08 (0.85, 1.39)	0.87 (0.74, 1.02)	0.92 (0.67, 1.27)
Headache	281 (47)	1.00 (0.74, 1.35)	1.2 (0.94, 1.53)	1.21 (0.99, 1.48)	0.96 (0.84, 1.08)	0.79 (0.61, 1.02)
Muscle pain	150 (25)	1.02 (0.73, 1.43)	0.95 (0.72, 1.24)	1.00 (0.80, 1.25)	0.87 (0.75, 1.00)	0.84 (0.63, 1.12)
Joint pain	325 (55)	1.03 (0.76, 1.39)	1.01 (0.79, 1.27)	0.84 (0.69, 1.04)	0.95 (0.84, 1.08)	**0.77 (0.59, 0.99)**
Urinary frequency	93 (16)	**0.62 (0.41, 0.95)**	1.13 (0.81, 1.59)	1.05 (0.80, 1.36)	1.00 (0.84, 1.19)	0.88 (0.62, 1.25)
Memory loss	185 (31)	**0.66 (0.47, 0.91)**	0.81 (0.63, 1.04)	0.97 (0.79, 1.19)	0.94 (0.82, 1.08)	0.78 (0.59, 1.03)
MSK[1] findings	47 (8)	**0.47 (0.26, 0.84)**	1.31 (0.82, 2.08)	1.18 (0.81, 1.71)	1.05 (0.84, 1.32)	0.81 (0.51, 1.29)
Neurological findings	35 (6)	0.85 (0.45, 1.60)	1.05 (0.64, 1.72)	0.80 (0.56, 1.16)	0.76 (0.57, 1.01)	0.67 (0.39, 1.16)
Uveitis Data (N = 591)						
Acute uveitis	39 (7)	0.96 (0.53, 1.75)	0.75 (0.48, 1.19)	0.80 (0.57, 1.11)	1.06 (0.83, 1.35)	0.92 (0.56, 1.53)
Non-acute uveitis	207 (35)	0.99 (0.73, 1.36)	1.03 (0.8, 1.31)	0.96 (0.79, 1.18)	1.12 (0.98, 1.27)	1.13 (0.87, 1.48)
Viral Data (N = 243)						
Viral shedding	81 (33)	**1.75 (1.04, 2.93)**	0.96 (0.67, 1.36)	0.91 (0.61, 1.37)	**1.52 (1.21, 1.91)**	1.19 (0.74, 1.90)

[1] MSK = musculoskeletal

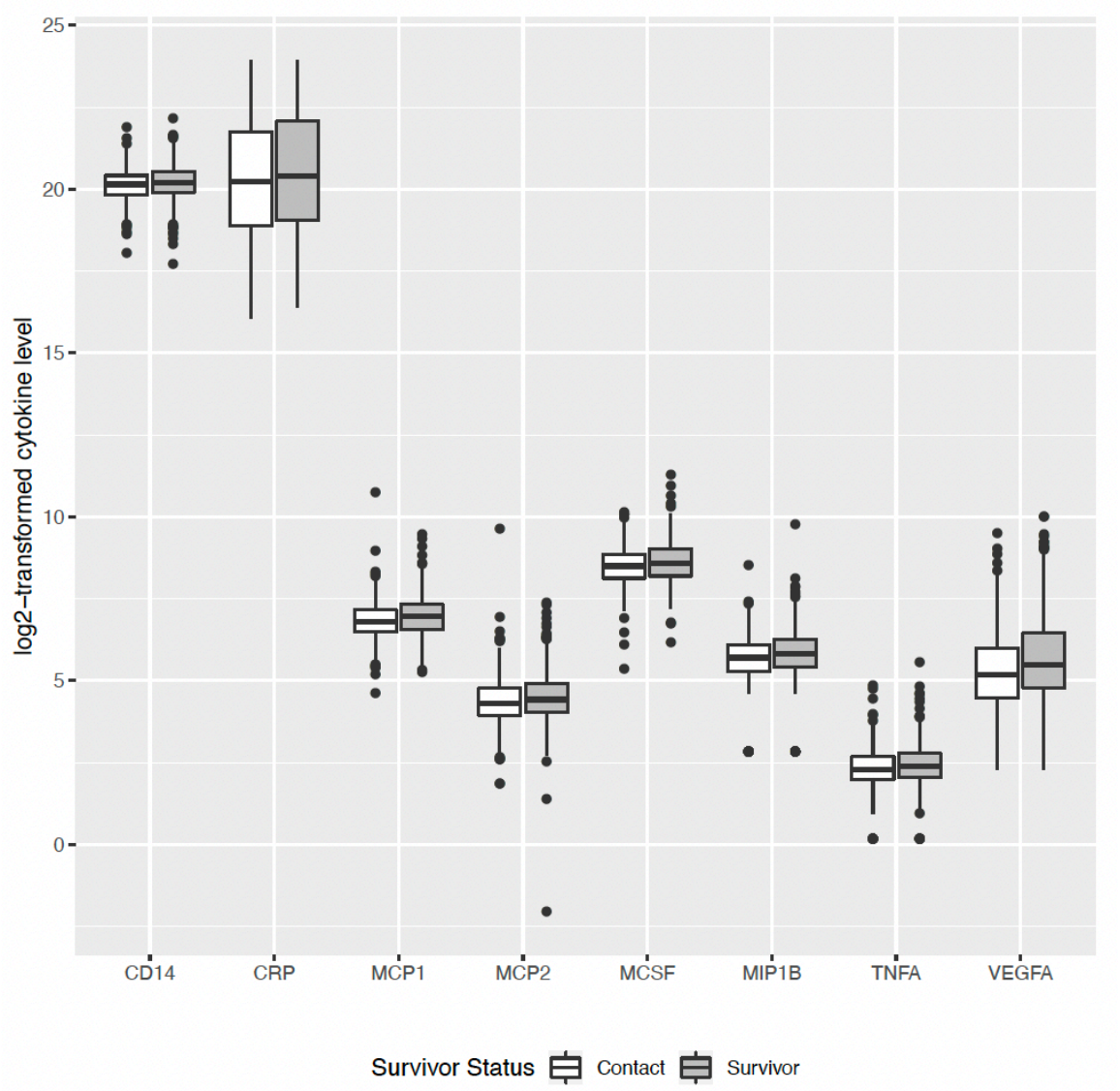


Figure 3.1 Associations between inflammatory markers and survivor status (EVD survivors: n=594; uninfected contacts: n=450). The following inflammatory markers had statistically significant associations: CD14, CRP, MCP1, MCP2, MCSF, MIP1B, TNFA, and VEGFA.

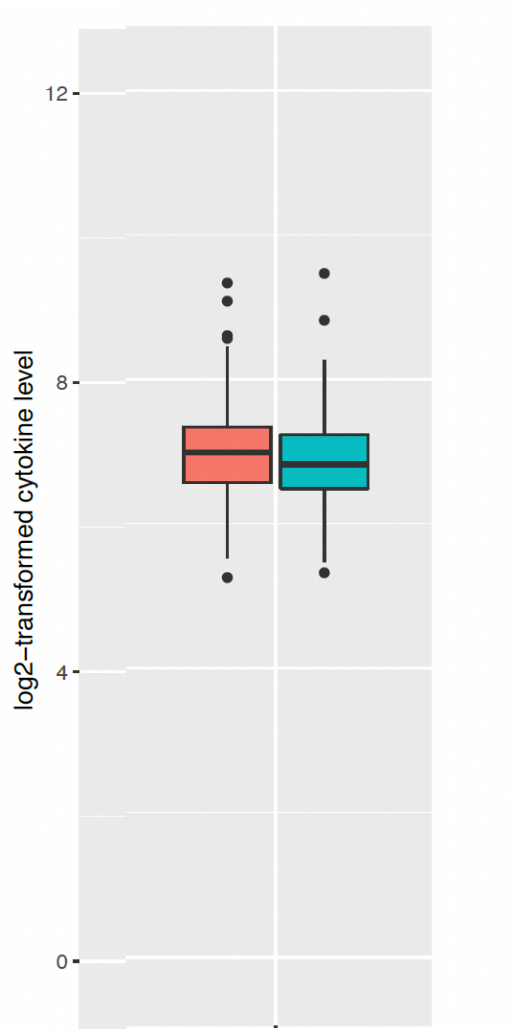


Figure 3.2 Association of urinary frequency and MCP-1 among EVD survivors (N=594). Median plasma levels of the inflammatory marker were indicated in red colored boxes among survivors without urinary frequency and were indicated in blue colored boxes among those with urinary frequency.

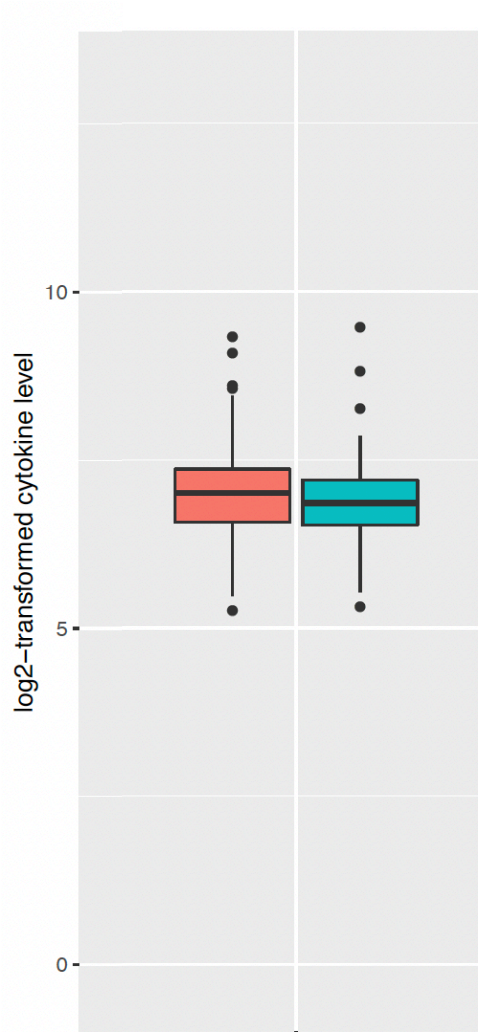


Figure 3.3 Association of memory loss and MCP-1 among EVD survivors (N=594). Median plasma levels of MCP-1 were indicated in red colored boxes among survivors without memory loss and were indicated in blue colored boxes among those with memory loss.

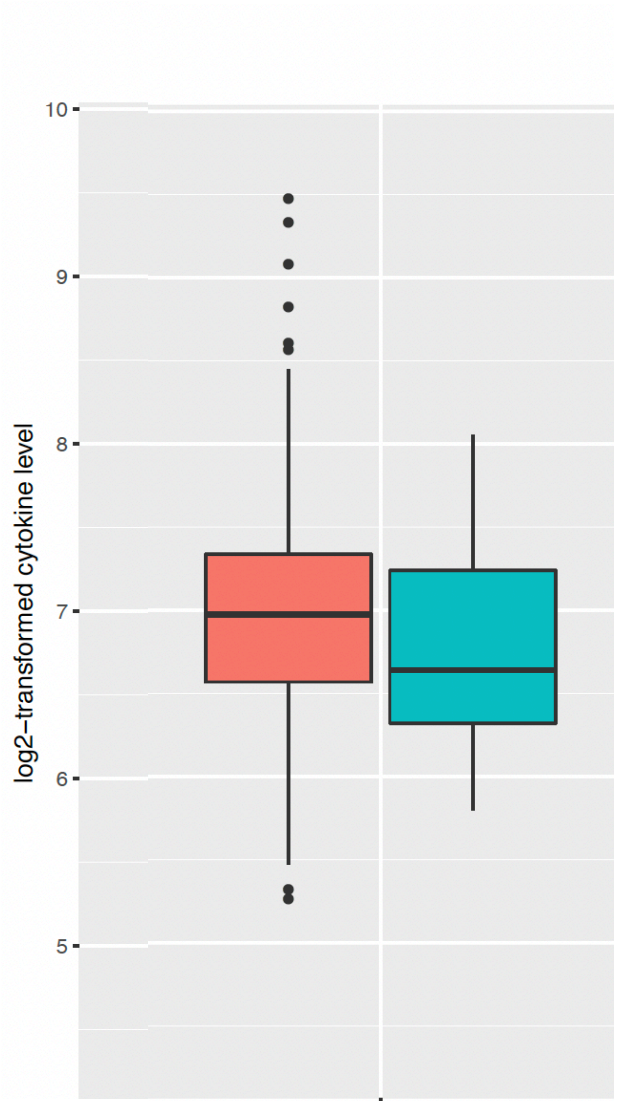


Figure 3.4 Association of musculoskeletal abnormalities and MCP-1 among EVD survivors (N=594). Median plasma levels of MCP-1 were indicated in red colored boxes among survivors without musculoskeletal abnormalities and were indicated in blue colored boxes among those with musculoskeletal abnormalities.

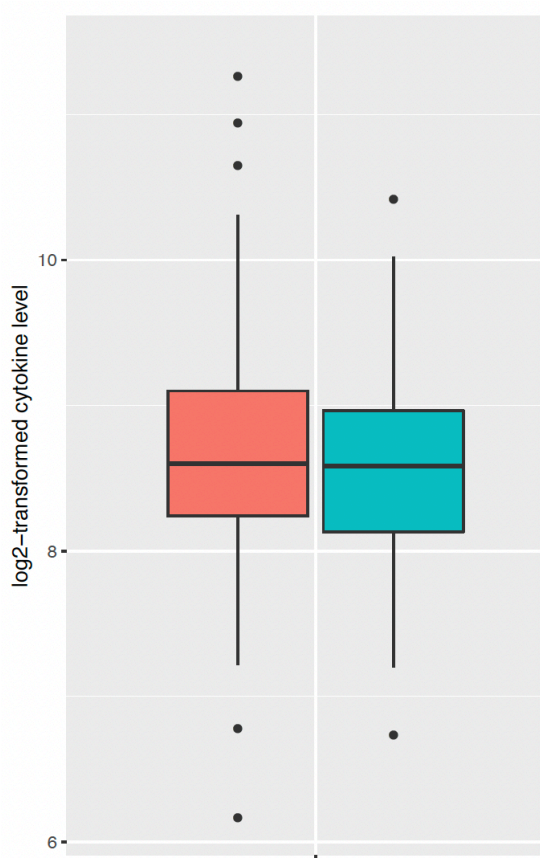


Figure 3.5 Association of joint pain and MCP-1 among EVD survivors (N=594). Median plasma levels of MCP-1 were indicated in red colored boxes among survivors without joint pain and were indicated in blue colored boxes among those with joint pain

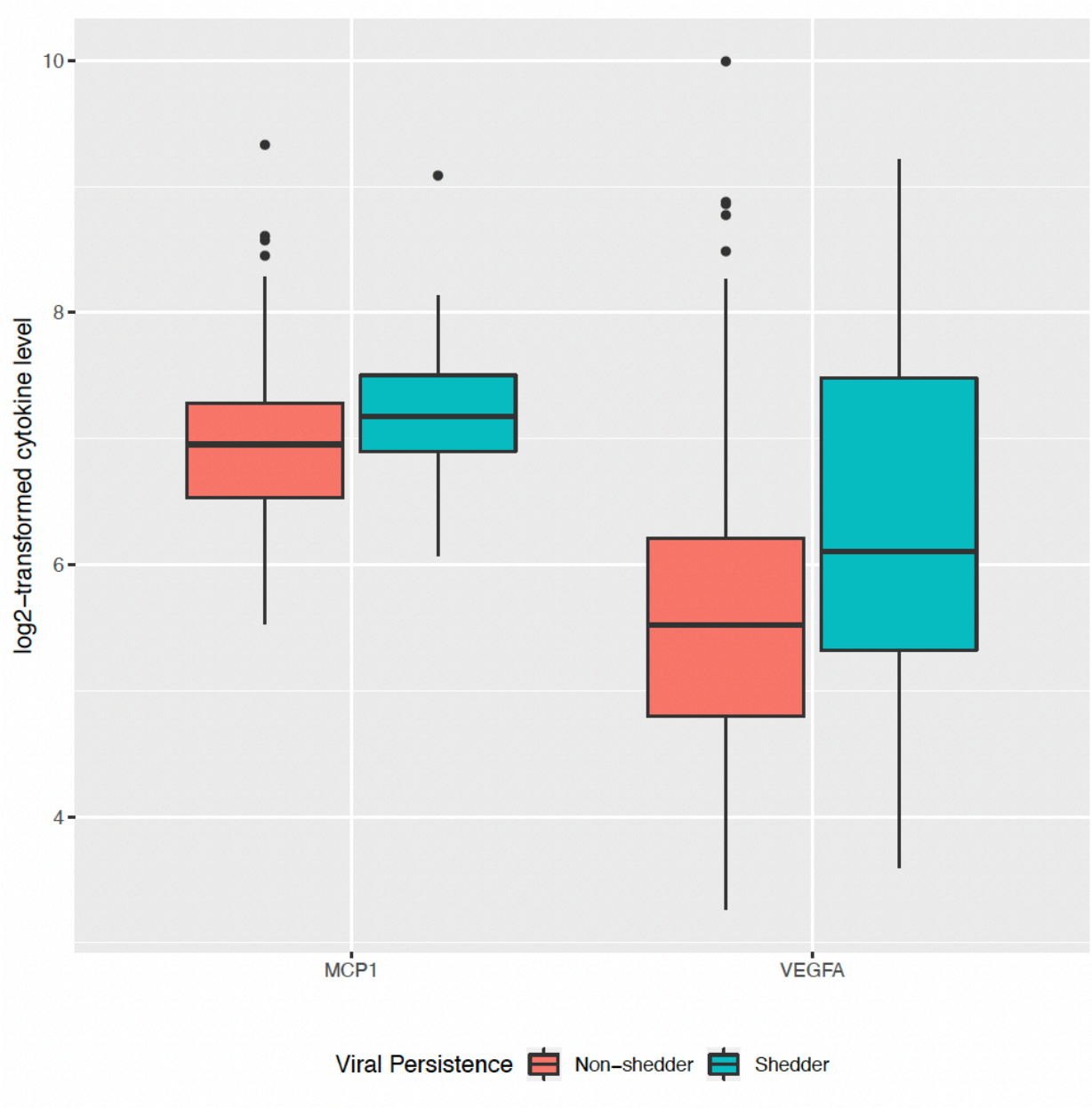


Figure 3.6 Associations of MCP1 and VEGFA with Ebola viral RNA shedding in the semen in survivors (shedder: N=81; non-shedder: N=162).

Supplementary Table 3.1 Summary of inflammatory markers among Ebola virus disease survivors and contacts

	N uncensored measurements	Med. (Q1, Q3)	log2-transformed Med. (Q1, Q3)	Coefficient of Variation of log2-transformed variable	N (%) below LOD	N (%) above LOD
MCP1	1044	119.5 (91.78, 153.1)	6.901 (6.52, 7.258)	10.98	0 (0%)	0 (0%)
IL1A	1	0.416 (0.416, 0.416)	-1.267 (-1.267, -1.267)	-0.02	1043 (99.9%)	0 (0%)
IL1B	138	0.105 (0.105, 0.105)	-3.252 (-3.252, -3.252)	-0.12	906 (86.8%)	0 (0%)
TNFA	1029	5.145 (4.01, 6.762)	2.363 (2.004, 2.758)	1.34	15 (1.4%)	0 (0%)
MIP1A	1037	27.26 (20.3, 40.35)	4.769 (4.344, 5.334)	58.09	0 (0%)	1 (0.1%)
MIP1B	1016	54.2 (40.2, 73.06)	5.76 (5.329, 6.191)	7.14	23 (2.2%)	0 (0%)
GRANA	1038	145.9 (114.9, 187.8)	7.189 (6.844, 7.553)	11.11	0 (0%)	0 (0%)
GRANB	1037	13.01 (8.772, 20.64)	3.702 (3.133, 4.367)	31.94	1 (0.1%)	0 (0%)
IL10	1040	5.545 (3.58, 10.4)	2.471 (1.84, 3.379)	6.37	4 (0.4%)	0 (0%)
IL2	6	0.32 (0.32, 0.32)	-1.644 (-1.644, -1.644)	-0.12	1038 (99.4%)	0 (0%)
IL2RA	1043	1426 (1155, 1817)	10.48 (10.17, 10.83)	65.8	0 (0%)	1 (0.1%)
IL5	724	0.49 (0.034, 0.972)	-1.031 (-4.857, -0.041)	-0.78	320 (30.7%)	0 (0%)
VEGFA	1039	40.15 (25.26, 77.73)	5.327 (4.658, 6.28)	18.05	5 (0.5%)	0 (0%)
VEGFB	1043	41.18 (32.34, 54.14)	5.364 (5.015, 5.758)	7.53	1 (0.1%)	0 (0%)
INFBETA	8	3.08 (3.08, 3.08)	1.623 (1.623, 1.623)	6.43	1035 (99.2%)	0 (0%)
IL1RA	1042	236 (181.8, 331.4)	7.883 (7.506, 8.373)	37.69	0 (0%)	0 (0%)
IL6	497	0.205 (0.205, 2.165)	-2.286 (-2.286, 1.114)	-7.53	546 (52.3%)	0 (0%)
IL8	1036	5.07 (3.595, 7.425)	2.342 (1.846, 2.892)	21.64	7 (0.7%)	0 (0%)
MCP2	1042	20.87 (15.88, 29.3)	4.383 (3.989, 4.873)	6.43	1 (0.1%)	0 (0%)
MCSF	1043	375.1 (284, 492)	8.551 (8.15, 8.942)	23.79	0 (0%)	0 (0%)
TNFR1	1043	912.9 (779, 1082)	9.834 (9.605, 10.08)	29.72	0 (0%)	0 (0%)
TNFR2	1043	2256 (1836, 2948)	11.14 (10.84, 11.53)	99.04	0 (0%)	0 (0%)
ICAM1	1043	398200 (319400, 489700)	18.6 (18.29, 18.9)	7765.69	0 (0%)	0 (0%)
CD14	1043	1175000 (943300, 1475000)	20.16 (19.85, 20.49)	22460.76	0 (0%)	0 (0%)
CRP	976	1310000 (516800, 4049000)	20.32 (18.98, 21.95)	218860.69	0 (0%)	66 (6.3%)

Supplementary Table 3.2 Associations of inflammatory markers with viral persistence in the semen among survivors

	Viral Persistence log2-transformed Med. (Q1, Q3) [% < LOD]				
	Non-shedder (N=162)	**Shedder (N=81)**	**Odds Ratio (95% CI)**	**p**	**p***
MCP1	6.954 (6.53, 7.283) [0%]	7.175 (6.9, 7.502) [0%]	1.745 (1.038, 2.032)	0.035	
IL1A	-1.267 (-1.267, -1.267) [99.4%]	-1.267 (-1.267, -1.267) [100%]	> 10% censored		1
IL1B	-3.252 (-3.252, -1.525) [72.8%]	-3.252 (-3.252, -3.252) [77.8%]	> 10% censored		0.439
TNFA	2.467 (2,142, 2.815 [3.1%]	2.376 (2.081, 2.742) [1.2%]	0.957 (0.674,1.36)	0.808	
MIP1A	4.865 (4.454, 5.425) [0%]	4.953 (4.616, 5.507) [0%]	1.043 (0.873, 1.247	0.642	
MIP1B	5.976 (5.589, 6.385) [0%]	6.012 (5.588, 6.349) [1.2%]	0.911 (0.606,1.37)	0.656	
GRANA	7.175 (6.866, 7.553) [0%]	7.246 (6.856, 7.503) [0%]	1.063 (0.658, 1.717)	0.804	
GRANB	4.087 (3.623, 4.864) [0%]	4.107 (3.383, 4.893 [0%]	0.848 (0.672, 1.072	0.168	
IL10	2.602 (1.862, 3.377) [0.6%]	2.269 (1.753, 3.237 [0%]	0.88 (0.699, 1.107	0.274	
IL2	-1.644 (-1.644, -1.644) [99.4%]	-1.644 (-1.644, -1.644) [100%]	> 10% censored		1
IL2RA	10.49 (10.22, 10.89 [0%]	10.48 (10.12,10.91) [0%]	0.862 (0.508, 1.464)	0.583	
IL5	-0.771 (-1.877, 0.065) [24.7%]	-0.971 (-4.857, -0.086) [34.6%]	> 10% censored		0.129
VEGFA	5.521 (4.796, 6.207) [0%]	6.105 (5.322, 7.48) [0%]	1.52 (1.211, 1.907)	<0.001	
VEGFB	5.345 (4.987, 5.804) [0%]	5.271 (5.013, 5.677) [0%]	0.863 (0.559, 1.331)	0.504	
INFBETA	1.623 (1.623, 1.623 [99.4%]	1.623 (1.623, 1.623 [100%]	> 10% censored		1
ILIRA	7.758 (7.391, 8.175) [0%]	7.626 (7.341, 7.986) [0%]	0.989 (0.666, 1.468)	0.956	
IL6	-2.286 (-2.286, 0.718) [63.6%]	-2.28 (-2.286, 1.036) [55.6%]	> 10% censored		0.265
IL8	2.438 (2.04, 3.092) [0.6%]	2.646 (2.35, 3.029) [0%]	1.231 (0.902, 1.688)	0.189	
MCP2	4.488 (4.072, 4.986) [0%]	4.613 (4.201, 5.206) [0%]	1.269 (0.823, 1.957)	0.281	
MCSF	8.661 (8.252, 8.961) [0%]	8.588 (8.239, 8.986) [0%]	1.186 (0.743, 1.896)	0.475	
TNFR1	9.876 (9.614, 10.13) [0%]	9.869 (9.589, 10.13) [0%]	0.913 (0.469, 1.777)	0.789	
TNFR2	11.22 (10.84, 11.56) [0%]	11.12 (10.86, 11.56) [0%]	0.908 (0.543, 1.519)	0.713	
TCAML	18.6 (18.25, 18.94) [0%]	18.6 (18.21, 18.97) [0%]	0.944 (0.619, 1.439)	0.789	
D14	20.22 (19.91, 20.54) [0%]	20.2 (19.95, 20.62) [0%]	1.193 (0.706, 2.017)	0.509	
CRP	19.87 (18.39, 21.22) [0%]	19.72 (18.82, 21.24) [0%]	1.002 (0.863, 1.164)	0.978	

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