Post-Ebola Syndrome Presents With Multiple Overlapping Symptom Clusters: Evidence From an Ongoing Cohort Study in Eastern Sierra Leone

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(See the Editorial Commentary by Brett-Major on pages 1055–7.)

Background. Following the 2013–2016 West African Ebola outbreak, distinct, persistent health complaints were recognized in Ebola virus disease (EVD) survivors. Here we provide an in-depth characterization of post-Ebola syndrome >2.5 years after resolution of disease. Additionally, we report subphenotypes of post-Ebola syndrome with overlapping symptom clusters in survivors from Eastern Sierra Leone.

Methods. Participants in Eastern Sierra Leone were identified by the Sierra Leone Association of Ebola survivors. Survivors and their contacts were administered a questionnaire assessing self-reported symptoms and a physical examination. Comparisons between survivors and contacts were conducted using conditional logistic regression. Symptom groupings were identified using hierarchical clustering approaches. Simplified presentation of incredibly complex evaluations (SPICE), correlation analysis, logistic regression, and principal component analysis (PCA) were performed to explore the relationships between symptom clusters.

Results. Three hundred seventy-five EVD survivors and 1040 contacts were enrolled into the study. At enrollment, EVD survivors reported significantly more symptoms than their contacts in all categories ($P < .001$). Symptom clusters representing distinct organ systems were identified. Correlation and logistic regression analysis identified relationships between symptom clusters, including stronger relationships between clusters including musculoskeletal symptoms ($r = 0.63$, $P < .001$; and $P < .001$ for correlation and logistic regression, respectively). SPICE and PCA further highlighted subphenotypes with or without musculoskeletal symptoms.

Conclusions. This study presents an in-depth characterization of post-Ebola syndrome in Sierra Leonean survivors >2.5 years after disease. The interrelationship between symptom clusters indicates that post-Ebola syndrome is a heterogeneous disease. The distinct musculoskeletal and non-musculoskeletal phenotypes identified likely require targeted therapies to optimize long-term treatment for EVD survivors.

Keywords. Ebola; viral hemorrhagic fever; filovirus; survivor; long-term sequelae.

The 2013–2016 West African Ebola outbreak was the largest in history, resulting in >28 000 cases and 11 325 deaths [1]. Sierra Leone had nearly half of all cases and 3956 deaths. Ebola virus disease (EVD) is a severe illness with case fatality rates averaging 50% [2]. The West African Ebola outbreak resulted in a large cohort of EVD survivors who have since presented with distinct, persistent health complaints.

Multiple studies have investigated post-Ebola syndrome in EVD survivors both from the West African outbreak [3–12] and from previous outbreaks in the Democratic Republic of Congo and Uganda [13–19]. Post-Ebola syndrome has been defined variably as a combination of rheumatologic, ophthalmologic, auditory, and more generalized complaints. Psychologic and neurologic conditions have also been reported [13, 15, 16].

Although previous studies have followed long-term EVD survivor sequelae, there have been limitations. Many of these studies were small and anecdotal, and few had a comparison group of healthy controls, with the exception of the Partnership for Research on Ebola Virus (PREVAIL III) [4]. Additionally, few studies have explored symptom complexes in the context of post-Ebola syndrome. Tozay et al described concurrent symptoms in the context of inflammatory markers [3]. However, further study is needed to understand the complex relationship between groups of symptoms. The question remains: Is post-Ebola syndrome a broad constellation of symptoms or do

subgroups of presentations exist? Here we provide an in-depth characterization of post-Ebola syndrome years after resolution of disease. We compared a comprehensive list of symptoms and physical examination findings between survivors and their close household contacts. Additionally, we report subphenotypes of post-Ebola syndrome with overlapping symptom clusters in survivors from Eastern Sierra Leone.

METHODS

Ethics Statement

Ethics approval was obtained through Tulane University and the Sierra Leone Ethics and Scientific Review Committee. Participants provided written informed consent in adults ≥18 years, consent by a parent or guardian and assent in children 12–17 years, and consent by a parent or guardian in children <12 years of age. All study personnel were trained in ethics and research compliance.

Study Design

Potential survivor participants in Eastern Sierra Leone were identified from a national EVD survivor registry maintained by the Sierra Leone Association of Ebola Survivors (SLAES). EVD survivors are eligible for SLAES membership if they have a valid discharge certificate from an Ebola treatment unit (ETU) and provide verified contact information. Household contacts were invited to participate if they resided with at least 1 survivor and were not listed in the EVD survivor registry. Up to 3 household contacts per survivor were selected. Survivors and contacts >6 years of age were enrolled. Children <6 years were excluded because they could not meaningfully answer the survey questions. Study participants were administered a questionnaire assessing self-reported symptoms followed by a physical examination. The symptom questionnaire asked about symptoms experienced before disease, since disease, and symptoms currently experienced. Contacts were instructed to use the survivor's illness as a temporal reference. Current symptom data were collected the same day the physical examination was performed. The questionnaires were designed based on previous reports and through informal discussions with members of SLAES [5]. Questionnaires were administered by trained study personnel in the local language of the participant's choice. Physical examinations were performed by trained healthcare workers using standardized forms.

Data Analysis

Statistical Analysis and Data Management

Data management and statistical analyses were performed in SAS version 9.4 (Cary, North Carolina). The overall study was designed to be able to identify asymptomatic or unrecognized EVD among household contacts of confirmed EVD patients; therefore, the household contacts do not represent an independent control group as seen in a case-control study. Conditional logistic regression was chosen to compare both groups to control for the study design linking survivors with their contacts accounting for the dependence between comparison groups. Age was controlled for by sex and vice versa. Comparisons for all remaining variables were controlled for by age and sex. Pearson correlation and logistic regression were performed in SAS software to explore relationships between symptom clusters.

Hierarchical Clustering

Hierarchical clustering was performed to determine distinct clusters of symptoms and physical examination findings among survivors. Symptoms were included in the hierarchical clustering analysis if they were present in ≥10% of survivors and statistically significantly different between survivors and the household contacts at $P < .010$. We determined symptom clusters by agglomerative clustering using the Ward method in R Studio (version 1.2.5033).

SPICE Analysis

Simplified presentation of incredibly complex evaluations (SPICE) version 6.0 was performed to visualize the complex relationships between symptom clusters identified in the hierarchical clustering analysis according to instructions on the National Institute of Allergy and Infectious Diseases website [20].

Principal Component Analysis

Principal component analysis (PCA) was performed on the same set of variables as the hierarchical clustering analysis in R Studio version 1.2.5033 using the prcomp package. Missing values were imputed according to methods appropriate for each variable. Symptom and age responses were expressed as median values. Missing values in the physical examination were imputed as negative or zero. Outliers were defined as >6 times the standard deviation from the mean and were excluded from analysis. The scree plot method was used to determine number of components and clusters. Groupings were established using k-means clustering. The first 2 principal components (PC1 and PC2) were plotted, with k-means clusters represented by color. Additionally, patient membership in each hierarchically determined symptom cluster was mapped onto the PCA. Contributions to the components were calculated as the variable loading divided by the column total or amount of variance explained by that component.

RESULTS

Participant Characteristics

Between March 2016 and January 2019, 375 EVD survivors and 1040 of their household contacts were enrolled in the study. Mean age was higher in survivors (29.8 ± 14.6 years) vs their contacts (22.8 ± 12.3 years; $P < .001$; Table 1). Survivors and contacts were separated into age categories (<15, 15–40,

>40 years) by sex. There were significantly more female survivors under the age of 15 compared to the contacts ($P = .015$). Overall, after controlling for age, survivors were more likely to be female (56.5%) than their contacts (47.5%), but the difference was not statistically significant ($P = .107$). Survivors were more likely to be widowed than single or married when compared with their contacts ($P = .040$ and $P < .001$, respectively). Survivors were more likely than their contacts to have no formal education. Survivors were hospitalized for a median of 26 days (interquartile range [IQR], 15–38 days) and enrolled a median of 940 days (IQR, 592–1198 days) after discharge from the hospital (Table 2). To provide context for the contact cohort, we looked at the relationship type and duration between contacts and survivors. When defining their relationships with survivors, household contacts were most likely to either live with or be a caretaker of a survivor (85.3% and 67.7%, respectively). Fewer contacts were sexual contacts or listed the relationship as "other" (2.4% and 7%, respectively). Those who replied as "other" were generally a relative, such as mother or sibling. Most contacts reported being in contact with a survivor either before or during their illness (69.9% and 67.7%, respectively). Fewer identified as being in contact with the survivor only after their illness (4.9%).

Self-Reported Symptom Questionnaire and Physical Examination Results
An extensive questionnaire and physical examination were conducted at enrollment to define persistent disease sequelae experienced by EVD survivors (Supplementary Tables 1 and 2). Initially self-reported symptoms and physical examination findings were grouped into 9 categories based on organ system:

Table 1. Patient Demographics

Characteristic	Survivor (n = 375)	Contact (n = 1040)	P Value
Age, y, mean (SD)	29.78 (14.55)	22.84 (12.30)	<.001
Female sex	205 (56.47)	494 (47.50)	.107
Age <15 y	37 (61.67)	112 (43.75)	.015
Age 15–40 y	126 (53.39)	335 (48.27)	.176
Age >40 y	42 (53.16)	47 (52.22)	1
Marital status (>15 y)			
Married	161 (60.70)	266 (66.00)	<.001
Single	62 (23.13)	120 (29.78)	
Widowed	45 (16.79)	17 (4.22)	
Education			
None	84 (31.11)	55 (8.08)	<.001
Some primary	13 (4.81)	102 (14.98)	
Completed primary	46 (17.04)	102 (14.98)	
Some secondary	103 (38.15)	371 (54.48)	
Completed secondary	4 (1.48)	25 (3.67)	
Beyond secondary	20 (7.41)	26 (3.82)	

Data are presented as No. (%) unless otherwise indicated. Age, sex, marital status, and education were assessed in the survivor and contact cohorts. Age was controlled for sex, and sex was controlled for age. Marital status and education were controlled for both age and sex. Statistical analysis comparing survivors to contacts was done using conditional logistic regression in SAS version 9.4 software.

Abbreviation: SD, standard deviation.

Table 2. Ebola Virus Disease Case and Contact Characteristics

Characteristics	Median (IQR) or No. (%)
Case characteristics	
Days of hospitalization, median (IQR)	26 (15–38)
Time from discharge to enrollment, d, median (IQR)	940 (592–1198)
Household contact characteristics	
Relationship to survivor, No. (%)	
Live with survivor	886 (85.26)
Caretaker of survivor	704 (67.71)
Sexual contact	25 (2.4)
Other	73 (7)
When did you contact the survivor, No. (%)	
Before illness	727 (69.91)
During illness	704 (67.71)
After illness	51 (4.94)

Analysis describing case and contact characteristics was done using SAS version 9.4 software.

Abbreviation: IQR, interquartile range.

musculoskeletal (MSK), neurologic, cardiac, gastrointestinal (GI), ophthalmologic, auditory, psychiatric, constitutional, and miscellaneous. The majority of self-reported symptoms and physical examination findings were significantly more common in survivors than their contacts. There were very high levels of constitutional symptoms such as fever (27.9%) and headache (38.1%) as well as MSK symptoms such as joint (39.1%) and muscle pain (24.5%) in EVD survivors. Ocular symptoms were also highly prevalent in EVD survivors. Interestingly, survivors frequently complained of neurologic symptoms such as numbness/tingling (14.3%) and psychiatric symptoms such as difficulty sleeping (14.2%) and nonsensical vocal outbursts (13.7%).

Multiple physical examination tests examining MSK signs—such as joint tenderness to palpation, decreased range of motion, and joint edema/effusions—were prevalent (>10%), and significantly different between survivors and contacts ($P < .001$ for each variable). At enrollment, EVD survivors reported significantly more symptoms than their contacts in all categories (Figure 1) with >70% of survivors experiencing at least 1 symptom, compared with <50% of their contacts.

Relationships Between Symptoms
Symptom clusters were objectively identified using hierarchical clustering. Six symptom clusters representing distinct organ systems were identified (Figure 2). Generally, each cluster represented 1 or a combination of 2 organ systems. These clusters include MSK, MSK/GI, psychiatric/neurologic, cardiac/GI, ophthalmologic/auditory, and constitutional symptoms.

A SPICE analysis was performed within the original clinically defined organ systems to further validate the clustering of symptoms within organ groups as seen in the hierarchical
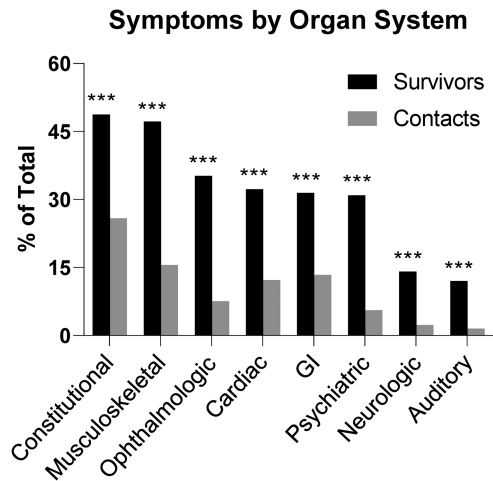
Symptoms by Organ System

Figure 1. Survivors experience significantly more symptoms than their household contacts. Participants experiencing any symptom from the above-defined organ systems are shown. Bar graphs were generated in Prism, and statistics were done in SAS using conditional logistic regression. ***$P < .001$. Abbreviation: GI, gastrointestinal.

clustering (Supplementary Figure 1). The majority of symptoms that cluster in the hierarchical analysis also overlap within each organ system in SPICE. Next, a SPICE analysis was performed using groupings identified by hierarchical clustering analysis to explore relationships between symptom clusters (Figure 3). More than 70% of EVD survivors experienced at least 1 symptom and more than half experienced symptoms from 2 or more clusters compared with approximately 40% and <25% of household contacts, respectively. Symptom clusters containing MSK symptoms often appeared together, while the remaining clusters overlapped with MSK clusters and one another. Interestingly, approximately 30% of survivors experienced no symptoms. SPICE revealed the complexity of relationships between symptom clusters experienced by EVD survivors and contrasted this presentation to their household contacts whose symptom presentation was much less complex.

Correlation and logistic regression analyses were conducted to clarify relationships between symptom clusters. Clusters including MSK symptoms had higher correlations with one another ($r = 0.63$, $P < .001$) but weaker correlations with other clusters ($r < 0.35$, $P < .001$) (Figure 4). Ophthalmologic/auditory symptoms were moderately correlated with the non-MSK clusters ($r > 0.5$, $P < .001$). Interestingly, the psychologic/neurologic, cardiac/GI, and constitutional symptom clusters correlated with one another ($r > 0.6$, $P < .001$). Logistic regression analyses assessing the relationships between symptom groupings revealed several highly significant bivariate associations between symptom groupings (Figure 5). The MSK group was significantly associated with MSK/GI ($P < .001$). Both ophthalmologic/auditory and psychiatric/neurologic groupings

were significantly associated with cardiac/GI and constitutional symptoms ($P < .001$ for each pairing).

A PCA was performed to further characterize the relationship between symptoms. K-means clustering of the PCA revealed 3 distinct groups of patients, which separated along PC1 and PC2 (Figure 6A). Major contributors to PC1 were generalized or nonspecific symptoms such as dizziness and heart palpitations. The largest contributors to PC2 were physical examination findings related to the musculoskeletal system, which fall into the MSK clusters. Individual membership to the symptom clusters was then mapped onto the plot of PC1 and PC2 (Figure 6B). The 2 clusters that involved MSK complaints separated from the group along PC2, corroborating our findings of a high association between the MSK clusters in our previous analyses. The PCA along with SPICE, correlation analysis, and logistic regression identify 3 general subphenotypes within the survivor cohort: those with MSK symptoms, those without MSK symptoms, and asymptomatic.

DISCUSSION

In this study we provided an in-depth characterization of post-Ebola syndrome several years after discharge from ETUs. To our knowledge, this is the largest controlled study to date studying EVD survivor sequelae >2 years after resolution of disease in Sierra Leone. PREVAIL III, conducted in Liberia, is the only larger study with a control group on this subject. In this study, we built upon the findings of previous studies, both controlled and anecdotal, and present the complex relationships between symptoms experienced by EVD survivors in Eastern Sierra Leone.

Consistent with previously published studies, we found that survivors experienced an elevated incidence of health complaints across multiple organ systems that have persisted for years. More than 70% of survivors had at least 1 symptom and more than half had multiple symptoms concurrently. The high levels of neurologic and psychiatric complaints seen in the survivor cohort were notable and have been less well studied than arthralgias and ocular symptoms in this context.

A major unanswered question in understanding post-Ebola syndrome is how myriad symptoms experienced by survivors are related to one another. Prior to this study, it was unknown whether post-Ebola syndrome consisted of a general spectrum of sequelae or if it can be broken down into specific, definable subphenotypes. We used multiple approaches to look at the relationships between symptom clusters. We objectively defined symptom clusters using hierarchical analysis visualized by a dendrogram (Figure 2) and used the resulting symptom clusters for further analysis. In the hierarchical analysis, symptoms clustered intuitively, roughly within organ system. Tozay et al investigated symptoms in the context of long-term follow-up of post-Ebola syndrome and reported that pairings of join
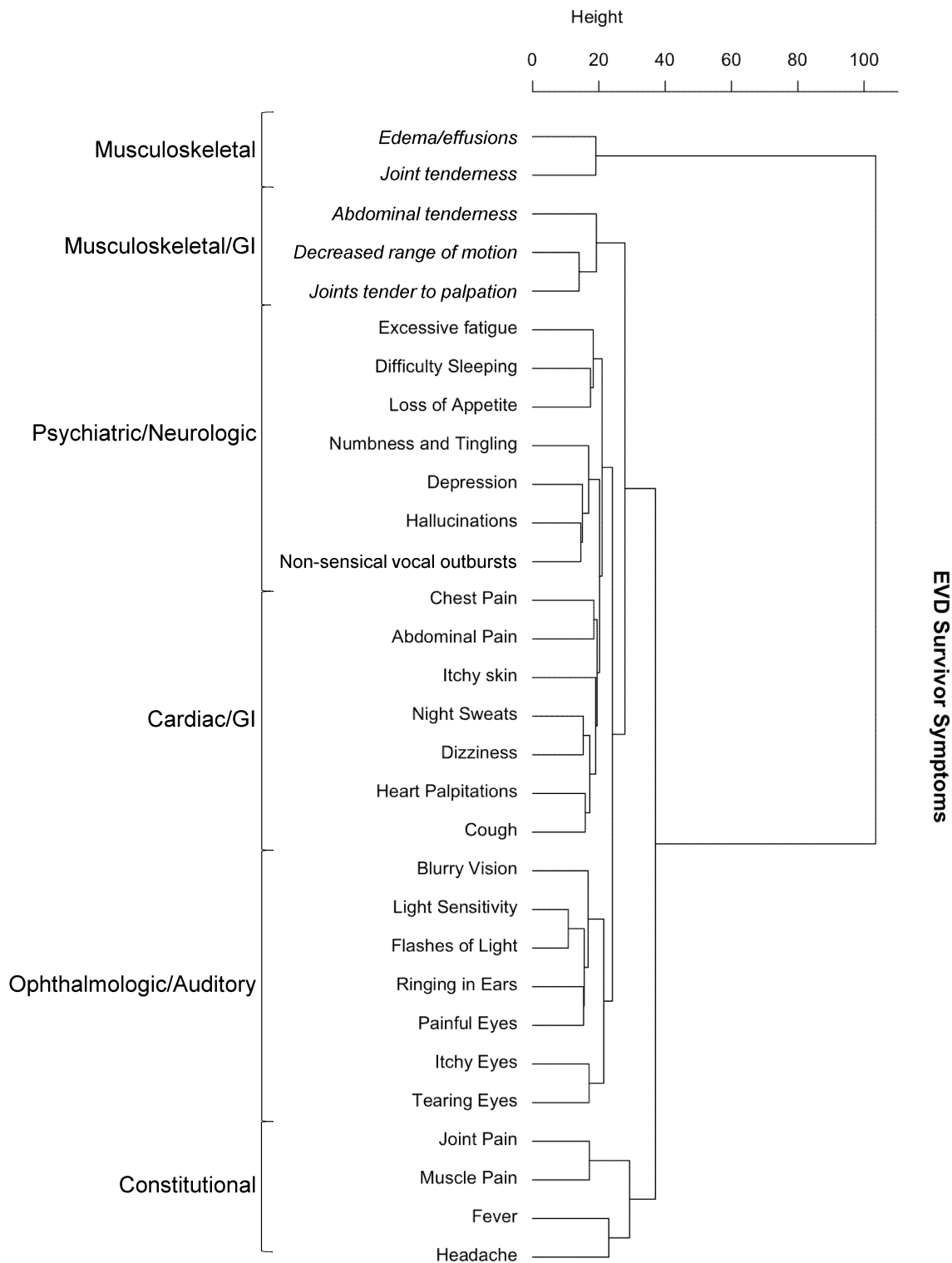
Figure 2. Symptoms experienced by Ebola virus disease survivors cluster into distinct groups. The dendrogram was constructed in R Studio using agglomerative clustering with the Ward method and includes the symptoms and physical examination findings with >10% frequency in survivors and $P < .01$. Physical examination findings are italicized. Abbreviations: EVD, Ebola virus disease; GI, gastrointestinal.

pain, headache, and fatigue were the most common [3]. To our knowledge this is the only previous study specifically looking at concurrent symptoms in EVD survivors. We also saw high levels of joint pain, headache, and fatigue in our survivor cohort, and similarly joint pain and headache clustered together in our analysis.
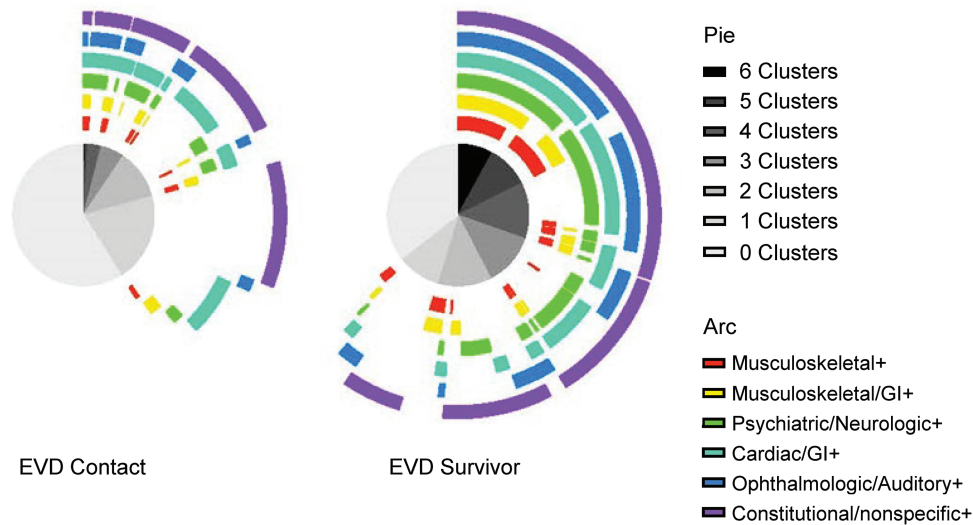
Figure 3. Ebola virus disease survivors present with clusters of symptoms that represent subphenotypes of post-Ebola syndrome. Simplified presentation of incredibly complex evaluations (SPICE) analysis was conducted using SPICE version 6. Pie graphs represent the number of symptom clusters experienced by participants, and arcs indicate which symptom cluster participants are experiencing. Overlapping symptom clusters in both survivors and contacts are presented. Abbreviations: EVD, Ebola virus disease; GI, gastrointestinal.

SPICE analysis allowed an exploratory visualization of patterns between symptom clusters. We noted an overlap between clusters containing MSK symptoms, symptomatic individuals without MSK symptoms, and approximately 30% of the survivors who experienced no sequelae. While general subgroups are apparent through SPICE, the approach reveals constellations of symptom pairings but does not define relationships. Pearson correlation and logistic regression analyses were used to investigate how symptom clusters relate to one another both linearly and nonlinearly, revealing 2 main phenotypes among symptomatic survivors: MSK symptoms and remaining symptom clusters. Logistic regression upheld the relationships identified in the correlation analysis. Together, these analyses revealed both linear and nonlinear relationships between symptoms, which largely coincide and can be visualized in the SPICE plots. The PCA provided further validation of these results through

visualization of the underlying structure of the variation within the data. K-means clustering again showed 3 distinct groups roughly defined by MSK involvement: MSK, without MSK symptoms, and asymptomatic.

The underlying process leading to persistent symptoms in some but not all EVD survivors remains unknown. Hypotheses including ongoing inflammation due to persistent infection vs autoimmune phenomena have been proposed [21]. However, to date, no definitive data have been published. By increasing our understanding of post-Ebola syndrome, it may be possible to identify a root cause in some EVD survivors. Our analyses have identified 3 subphenotypes among EVD survivors. Further study is needed to determine if those with and without MSK symptoms have different inflammatory profiles. Such an analysis would be the first step in determining if these different phenotypes are due to persistent or postinfectious inflammation.

There were limitations that must be considered when interpreting these results. First, there is potential for both ascertainment and misclassification biases due to the enrollment criteria. EVD survivors were eligible for enrollment if they were part of a national registry of EVD survivors discharged from an ETU and in possession of a valid discharge certificate. It is possible, though unlikely, that those classified as survivors were not in fact survivors. It is also possible that some contacts were indeed survivors with unrecognized infection. In the absence of serological data this cannot be ruled out. Additionally, it is possible that those with greater symptoms were more likely to participate in these studies. Finally, this was not a randomized study and thus is not directly generalizable to the greater population of EVD survivors in other areas. However, these data do provide a starting point for understanding post-EVD symptom complexes in other populations.

Pearson's r	Musculoskeletal	Musculoskeletal/GI	Psych/Neuro	Cardiac/GI	Ophth./Auditory	Constitutional
Musculoskeletal	1	0.628	0.280	0.351	0.339	0.332
Musculoskeletal/GI	0.628	1	0.286	0.332	0.249	0.335
Psych/Neuro	0.280	0.286	1	0.664	0.529	0.650
Cardiac/GI	0.351	0.332	0.664	1	0.554	0.604
Ophthalmologic/Auditory	0.339	0.249	0.529	0.554	1	0.527
Constitutional	0.332	0.335	0.650	0.604	0.527	1

Figure 4. Correlation analysis. Pearson correlations were conducted between each symptom cluster to show linear relationships between symptom clusters. Correlation coefficient is shown. $P < .001$ for all correlations. Abbreviation: GI, gastrointestinal.
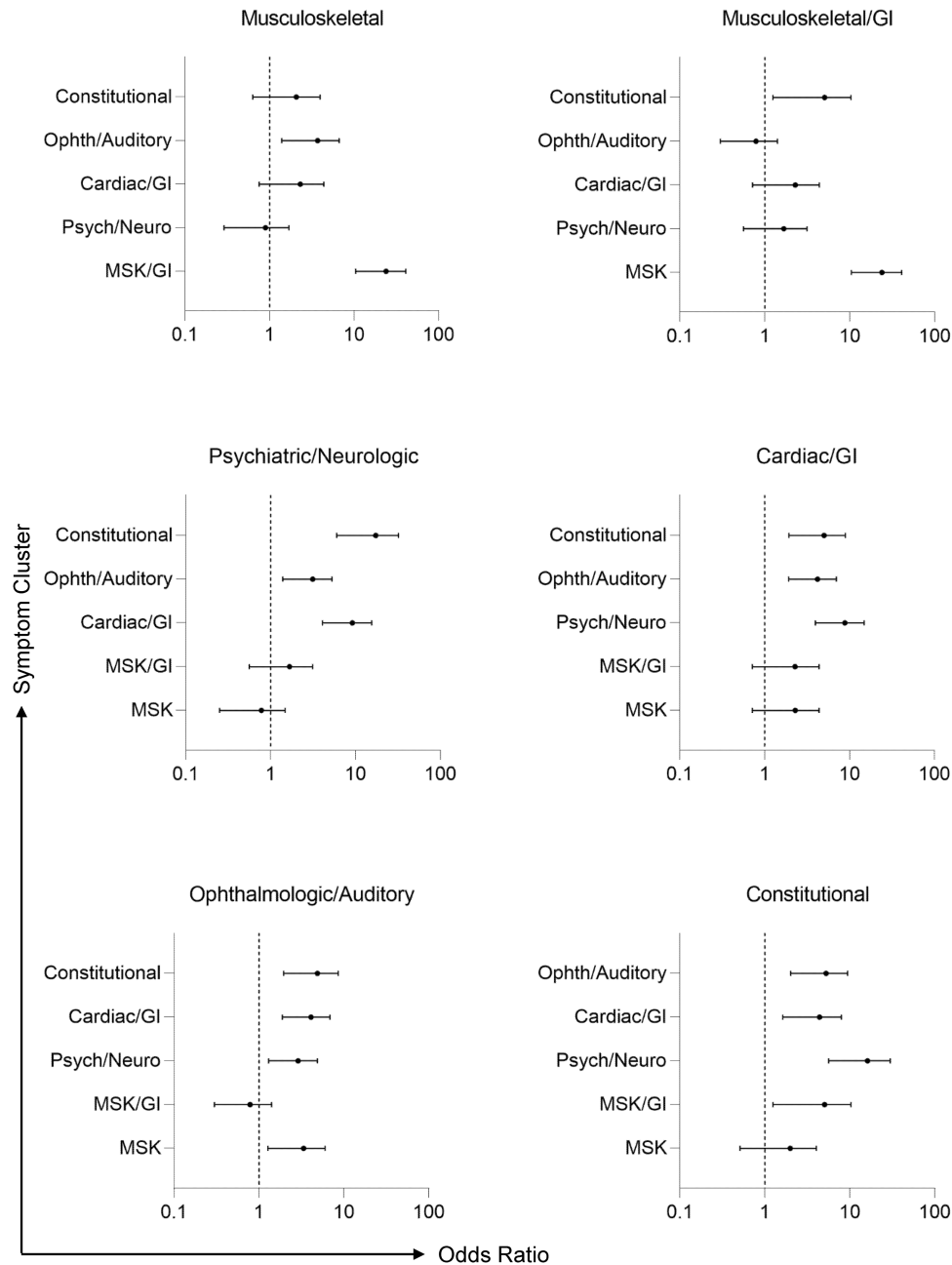
Figure 5. Logistic regression analysis. Logistic regression modeling each symptom cluster by the other clusters. Forest plots with the odds ratio and 95% confidence intervals are shown. Vertical line indicates an odds ratio of 1. Abbreviations: GI, gastrointestinal; MSK, musculoskeletal.

The nature of a self-reported questionnaire could result in recall bias among survivors compared with controls. Having experienced a severe illness, survivors may be more acutely aware of their health status than their household contacts. However, while not having experienced the illness themselves, household contacts may have a heightened awareness of their health having witnessed a severe illness at close hand. Additionally, the question order on the questionnaire (based on a questionnaire from a previously published study) was not random, nor validated, which could lead to biases in

the hierarchical clustering. However, we did see some MSK symptoms and physical examination findings—recorded at different parts of the study visit—cluster together, which can provide some evidence that the intuitive clustering we observed was not entirely based on questionnaire design. Finally, the healthcare professionals administering the physical examination were not blinded; thus, their assessment could have been subconsciously biased. The impact of these potential biases must be considered when interpreting the results of this study.
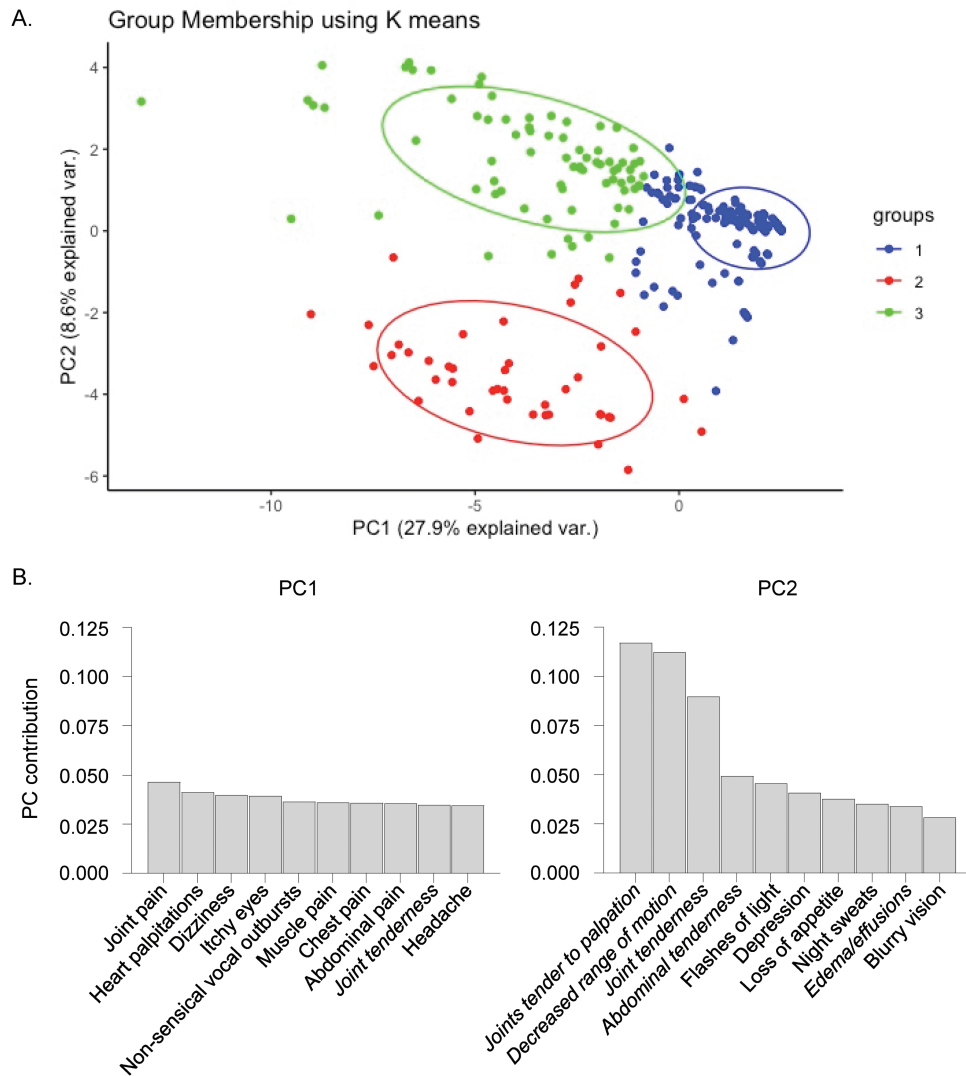
Figure 6. Principal component analysis (PCA) shows distinct groupings based on symptoms and physical examination findings in Ebola virus disease survivors. *A*, PCA was conducted in R Studio using the symptoms and physical examination findings present in >10% of the survivors and statistically significantly different between survivors and contacts with *P* < .01. Groupings were established using k-means clustering. Outliers were determined using the Tukey method and were excluded from the analysis. The first 2 principal components (PC1 and PC2) were plotted, with k-means clusters represented by color. *B*, Variables, including symptoms and physical examination findings, with the highest contribution to PC1 and PC2 are shown. Physical examination findings are italicized.

CONCLUSIONS

This study presents an in-depth characterization of post-Ebola syndrome in Sierra Leonean survivors >2 years after disease. Our findings corroborate results from previous studies on post-Ebola syndrome while building a better understanding of the complexity of sequelae in EVD survivors. To our knowledge, this is the first study describing the interrelationship between different groups of symptoms presenting in post-Ebola syndrome. The relationships between symptom clusters indicate that this syndrome is a heterogeneous disease. Our results suggest that EVD survivors can be classified into 3 groups based on musculoskeletal involvement and those who experience no sequelae. Future studies are needed to further understand changes in sequelae

over time, the mechanisms driving differential presentations of post-Ebola syndrome, and the impact of concurrent symptom complexes on EVD survivors.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

developing diagnostics, therapeutics, and vaccines for Lassa fever and other severe diseases. Tulane University and various industry partners have filed United States and foreign patent applications on behalf of the VHFC for several of these technologies. If commercial products are developed, VHFC members may receive royalties or profits. R. F. G. is currently an affiliate and co-founder of Zalgen Labs, LLC. This does not alter our adherence to journal policies on sharing data and materials. All other authors report no potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Centers for Disease Control and Prevention. Ebola: 2014–2016 Ebola outbreak in West Africa. Available at: https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/index.html. Accessed 7 May 2020.
2. World Health Organization. Ebola virus disease: key facts 2020. Available at: https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease. Accessed 12 June 2020.
3. Tozay S, Fischer WA, Wohl DA, et al. Long-term complications of Ebola virus disease: prevalence and predictors of major symptoms and the role of inflammation. Clin Infect Dis **2020**; 71:1749–55.
4. Sneller MC, Reilly C, Badio M, et al. A longitudinal study of Ebola sequelae in Liberia. N Engl J Med **2019**; 380:924–34.
5. Mattia JG, Vandy MJ, Chang JC, et al. Early clinical sequelae of Ebola virus disease in Sierra Leone: a cross-sectional study. Lancet Infect Dis **2016**; 16:331–8.
6. Mohammed H, Vandy AO, Stretch R, et al. Sequelae and other conditions in Ebola virus disease survivors, Sierra Leone, 2015. Emerg Infect Dis **2017**; 23:66–73.
7. Nanyonga M, Saidu J, Ramsay A, Shindo N, Bausch DG. Sequelae of Ebola virus disease, Kenema District, Sierra Leone. Clin Infect Dis **2016**; 62:125–6.
8. Qureshi AI, Chughtai M, Loua TO, et al. Study of Ebola virus disease survivors in Guinea. Clin Infect Dis **2015**; 61:1035–42.
9. Tiffany A, Vetter P, Mattia J, et al. Ebola virus disease complications as experienced by survivors in Sierra Leone. Clin Infect Dis **2016**; 62:1360–6.
10. Wilson HW, Amo-Addae M, Kenu E, Ilesanmi OS, Ameme DK, Sackey SO. Post-Ebola syndrome among Ebola virus disease survivors in Montserrado County, Liberia 2016. Biomed Res Int **2018**; 2018:1909410.
11. de St Maurice A, Ervin E, Orone R, et al. Care of Ebola survivors and factors associated with clinical sequelae—Monrovia, Liberia. Open Forum Infect Dis **2018**; 5:ofy239.
12. Bausch DG. Sequelae after Ebola virus disease: even when it's over it's not over. Lancet Infect Dis **2015**; 15:865–6.
13. Clark DV, Kibuuka H, Millard M, et al. Long-term sequelae after Ebola virus disease in Bundibugyo, Uganda: a retrospective cohort study. Lancet Infect Dis **2015**; 15:905–12.
14. Rowe AK, Bertolli J, Khan AS, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidémies à Kikwit. J Infect Dis **1999**; 179:S28–35.
15. Kelly JD, Hoff NA, Spencer D, et al. Neurological, cognitive, and psychological findings among survivors of Ebola virus disease from the 1995 Ebola outbreak in Kikwit, Democratic Republic of Congo: a cross-sectional study. Clin Infect Dis **2019**; 68:1388–93.
16. Wendo C. Caring for the survivors of Uganda's Ebola epidemic one year on. Lancet **2001**; 358:1350.
17. Rodriguez LL, De Roo A, Guimard Y, et al. Persistence and genetic stability of Ebola virus during the outbreak in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis **1999**;179:S170–6.
18. Bwaka MA, Bonnet MJ, Calain P, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J Infect Dis **1999**; 179:S1–7.
19. Kibadi K, Mupapa K, Kuvula K, et al. Late ophthalmologic manifestations in survivors of the 1995 Ebola virus epidemic in Kikwit, Democratic Republic of the Congo. J Infect Dis **1999**;179:S13–4.
20. Roederer M, Nozzi JL, Nason MC. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry A **2011**; 79:167–74.
21. Wiedemann A, Foucat E, Hocini H, et al; PostEboGui Study Group. Long-lasting severe immune dysfunction in Ebola virus disease survivors. Nat Commun **2020**; 11:3730.