Pitfalls in Screening for Ebola Virus Disease: The Variable Febrile and Human Subjective Response!

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Abstract
The Ebola virus outbreak of 2014 in West Africa is unprecedented. As of the situation report from the World Health Organization (WHO) dated November 11, 2014 there were 16,899 suspected, probable and confirmed cases along with 59,870 deaths reported globally in this Public Health Emergency of International Concern. Five cases outside the continent of Africa have been reported. Entry and exit screening for EVD (Ebola Virus Disease) of individuals originating from known outbreak regions currently include the presence of fever or additional symptoms consistent with EVD. Despite these efforts, the rate of transmission \[ R(t) \] remains at 1.4 to 1.7, transnational cases are increasing, and a resurgence of the outbreak in Mali has recently been reported.

This perspective article discusses the pitfalls associated with the determination of fever in the screening process as well as the difficulties with individual, subjective self-reporting of symptoms, and denial of disease as a risk not previously reported.

Additional data is presented with focus upon the mucin-like domain of the Zaire ebola virus glycoprotein with comparison between the current Zaire ebola virus, strain Guekedou, and the Zaire ebola virus, strain Mayinga, 1976. The specific dynamics are illustrated by a multiple sequence alignment and de novo modelling of the Ebola virus, strain Guekedou (August 2014) C-terminus MLD and GP2 translated proteins.

Keywords: Zaire ebola virus; Anosognosia; Ebola Virus Disease; Asymptomatic; WHO

Introduction
The Ebola virus outbreak of 2014 in West Africa is unprecedented. As of the situation report from the World Health Organization (WHO) dated November 11, 2014 there were 16,899 suspected, probable, and confirmed cases, as defined in Table 1, along with 59,870 deaths reported globally in this Public Health Emergency of International Concern (Table 2). Five cases outside the continent of Africa have been reported. Given the overall Case Fatality Rate (CFR) (Guinea) of 62%, the number of actual deaths may be as high as 10,308 (Figure 1, November 28, 2014 WHO situation report (SITREP)).

Five cases outside the continent of Africa have been reported including one asymptomatic traveler from West Africa to the United States. The second case, a nurse involved in the care of the index case in the United States, became ill while travelling within the US, was hospitalized and survived. The concern for Ebola Virus Disease becoming rampant here in the United States is real and complacency cannot be allowed if we are able to contain this outbreak to the West African region.

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Screening Protocol

Entry and exit screening for EVD (Ebola Virus Disease) of individuals originating from known outbreak regions currently include the presence of fever or additional symptoms consistent with EVD. Despite these efforts, the rate of transmission \( R(t) \) remains at 1.4 to 1.7, transnational cases are increasing, and a resurgence of the outbreak in Mali and Sierra Leone have recently been reported.

The purpose of screening is to contain further transmission of the disease. There is no screening checkbox for “not feeling right” also considered as subjective fever.

It is always a good topic as to what constitutes fever. We all remember the days when “normal” was 98.6 degrees F. Now, with digital read outs, Harrison’s textbook of internal medicine defines acute core value (ACV) fever as “a morning oral temperature of > 37.2°C (> 98.9°F) or an afternoon oral temperature of 37.7°C (> 99.9°F) while the normal daily temperature variation is typically 0.5°C (0.9°F).” [1,2].

Additionally, so we are all on the same page, we would really like to have it as close to the “core body temperature” as possible but, in reality, most of us don’t want an esophageal probe every time we feel uneasy, so we do need another way that best approximates our own, individual core body temperature. Only you know what that is.

Also the manner in which temperature is measured and then observed by another is critical in its accuracy. A rectal thermometer is the best, then oral (mouth closed!), temporal artery (not across the forehead, not a strip), then tympanic in that order. Clearly, there is quite a bit of variability. Because of that, my soap-box statement is that I am weary of “check-box” medical history taking, a blanket check box that specifies a single number to fit every single individual just does not work in every situation [2].

In this outbreak, approximately 88% have been reported to demonstrate the presence of “fever” as a symptom during the course of the disease in confirmed cases [3]. Because fever is the predominant symptom, it would be the most reasonable condition to look for in screening for presence of disease.

In a previous outbreak of EBOV, Kikwit (1995), there were 316 probable cases of EVD with only 103 cases with clinical information available (33% total probable EVD); 61% of those were confirmed [4]. 93% had fever during the early phase lasting 1 week. There is no clinical information available for the remainder of those cases.

The typical early phase and late phase symptoms are shown in Figures 2 and 3 below. In the Kikwit outbreak, this information could represent the observations that 21 cases (7%) were without the symptom of fever during the course of the disease and would have gone undetected. This being a rural population, the consequences of this oversight, given a total population of 400,000 in 1995, would appear to be minimal but the actual number of individuals with the presentation are not known with any accuracy.

In this propagated epidemic that has spread to urban areas in Guinea, Liberia, Mali, and Sierra Leone of West Africa, and with transnational risk of transmission by various routes, there have been over 21,369 suspected, probable and confirmed cases as of January 21, 2015. Assuming 12% of those cases do not present with fever that could represent over 2500 cases that would potentially transmit the virus asymptomatically during the entire course of the infectious period.

It has been the observation in several of the past outbreaks that asymptomatic cases occurred. Even in the first reported outbreaks in 1976-1979. Studies from cases of asymptomatic Ebola disease during the Gabon outbreak in 1996 revealed asymptomatic and replicative Ebola infections in 7 people which were confirmed by results of lab testing of those individuals for virus and immunoglobulin at intervals. Interval testing was done to prevent assumptions that cases had prior exposures to Ebola which might confound results [5,6]. Asymptomatic manifestations of Ebola Virus Disease in humans might be related to the immune response because of the notable observation of IL-1B, IL-6, and TNF that appeared early in the disease process of asymptomatic cases and the absence of those in cases presenting symptoms [7].

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**Figure 1:** Clinical symptoms West Africa outbreak 2014-2015.

**Figure 2:** Early Symptoms of Ebola Virus Disease (EVD) (World Health Organization).

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Figure 3: Late clinical features of Ebola Virus Disease (World Health Organization).

Figure 4: Graph of subclinical and clinical course on Ebola Virus Disease (EVD).

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Clinical Scenario and Pathogenesis

The latent period, symptom delay, and duration of infectious period are shown below the bar graph of the entire course of disease in EVD (incubation through resolution of disease) in Figure 4.

For an example, consider the following general scenario of infection.

On day 1, you are infected by an Ebola virus by whatever cause. The virus then passes into human tissue. It then must specifically recognize a receptor on a cell (dendritic cell, macrophage) and attaches itself to that specific receptor on that specific cell to interact in essentially a key and lock formation (Figures 5 and 10). This is not a random event as there are many folds in the viral glycoprotein (GP spike is much smaller) that must bind with and interact with the 3d structure of its receptor. Structure of the protein is the most important aspect.

The initial virus then injects its RNA and, to greatly simplify the rest of the process makes several viral proteins after it converts its antisense (“backward”), single strand, RNA from 3’ → 5’ to 5’ → 3’. It then also must make multiple copies of its original antisense genome, reassemble the virus together and then “bud” along with taking part of the host cell to form its envelope. Budding is the process by which the newly formed viruses leave the infected cell and incorporate some of the host cell’s membrane into its own capsule before going on to infect new cells. The person it infects reacts to this invasion by the production of cytokines, resulting in a fever (febrile response).

Figure 5: Pathway of filovirus entry mediated by the viral proteins GP₁ and GP₂.
This obviously is not “abrupt” and this prodromal phase where symptoms begin gradually before the full manifestation of the disease occurs can be where inadvertent transmission can occur. People then take Tylenol, Aspirin, Ibuprofen, etc, drink water; pass it off as anxiety, and walk on. The febrile response is masked by exogenous anti-inflammatories. As mentioned above, this occurred in the initial case of the traveler from Liberia to the United States (T.D) and is documented in a clinical course of a single patient infected from a single case in infection by Tai Forest ebola virus (TAFV) [8]. In a previous report on experimental infection on rhesus monkeys [9], a fluctuating febrile to a febrile course is shown below (Figure 7).

**Bioinformatic Data**

**Alignment of EBOV, strain Guekedou**

The dynamics of this transmission may be different from past outbreaks of EBOV. To evaluate this further, the translated amino acid sequence of the Zaire ebola virus glycoprotein segment (GP1, GP2 1676 aa; gi|8479501|sp|O111457.1|VGP_EBOG4; NCBI) was submitted as the query “DELTA BLAST” (DELTA-BLAST constructs a PSSM using the results of a Conserved Domain Database search and searches a sequence database) at the National Center for Biotechnology Institute (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) and the sequences obtained from strain GuekedouAHX24667.2|EBOV_8.2014_GUEKODOU/1676 EBOV AUGUST 2014] and Kissedougou [AHX24649.2|EBOV_Kissidougou_GUI_2014/1676 KISSIDOUGOU 2014]. Figure 8 shows the actual translated sequences.

The Guekedou sequence was then submitted as a BLASTp query filtered to return acceptable protein databank templates. 3CSY (Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor:Lee, J.E., Fusco, M.L., Hessell, A.J., Oswald, W.B.,...

**Figure 7:** Initial febrile response in rhesus monkeys [8] with mean core body temperature changes in serial sampling group on macaques. At days 5-6 of the viremia, an initial febrile response is shown. Additionally, clear fluctuations are present with a hypothermic response on days 7 and 8 post-exposure. A similar response is seen in Figure 6 above (human) and correlation with rising qPCR.


**Figure 8:** Amino acid sequence of the Glycoprotein (GP1 and GP2) from the 2014 outbreak.

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Figure 9: Amino acid alignment of EBOV, strain Guekogou (GUI) with 3CSY (Zaire ebola virus GP1) and 1EBO (GP2 with mucin domain). The fusion peptide sequence GKLGLITNTIAGVAGLI, is in blue square rectangle.
Figure 10: Schematic Overview of the Zaire ebola virus conserved domains with interacting residues. The hydrophobic mucin-like domain (MLD) has only a partial (12%) coverage with a PDB template.

Figure 11: Global multi sequence alignment (Clustal Omega) [MSA] of the Zaire ebola virus GP2 and mucin domain (1EBO) with secondary structure. The poorly conserved residues of EBOV, strain Guekedou, are within the mucin domain (red rectangle). Secondary structure generated with Ipred and visualized within Jalview [Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25: 1189-1191. doi:10.1093/bioinformatics/btp033]. The α-helices are in red bars, whereas the β-strands are depicted as a green arrow.
The aligned sequences were then submitted into Modeller within UCSF Chimera using 3CSY|Chain A, for the GP1 template and 1EBO|Chain K for the mucin-like domain/GP2 segment (Figures 12 and 13). 97% homology was noted throughout GP1 and 99% through GP2.

The mucin-like domain, however, was poorly conserved. The Ebola virus glycoprotein mucin-like domain (MLD) is implicated in Ebola virus cell entry, inflammation, and immune evasion [10]. Further investigation is required to evaluate clinical implications, such as onset of fever due to these changes in the mucin-like domain.

Figure 12: 3D model of the mucin-GP2 domain with superimposition of EBOV, strain Guekedou [GenPep AHX24667.2 (NCBI)]. The poorly conserved mucin domain is colored red and the conserved GP2 residues are in blue. The template, 1EBO_Chain A (PDB) was downloaded from the PDB databank and colored tan. Modelling performed with Modele in UCSF Chimera, v. 1.10 [UCSF Chimera--a visualization system for exploratory research and analysis; Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. J Comput Chem. 2004 Oct;25(13):1605-12.] Note the charged residues are opposite in direction.

Denial of Disease

Denial of disease is significant, especially when the disease can be fatal [11,12]. We have all observed patients with cancer deny having it until it has metastasized. Human behavior cannot fit in a check box nor does it necessarily respond to rules, human declared regulations, and legislations (Anosognosia).

Denial of Disease [13] is characterized by:

a. Refusing to acknowledge a stressful problem or situation,
b. Avoiding facing the facts of the situation, and
c. Minimizing the consequences of the situation

A rise in temperature from the individual normal to the elevated stage (fever) that is maintained through the virus’s life cycle [14-19] commands attention. Any point on this elevation timeline can indicate contagion. It just so happens that in EVD, that rise is quick. There is still the pre-prodromal and prodromal stage of fever, headache, myalgia, and malaise that really could be indicative of a number of other diseases. Throw in denial and, well, I think we see the consequences [12,13] and in particular to reference the cases here in the United States.

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**Conclusion**

The Ebola virus outbreak of 2014 in West Africa is unprecedented as to the severity and the dynamics of transmission in this outbreak. It is clear we need further evaluation into the incubation period of this strain with respect to aerosol transmission and delayed febrile response given the genetic changes, particularly with respect to the changes identified within the mucin-like domain of glycoprotein in the EBOV Guekedou strain of this outbreak in 2014. As such, avoiding denial of symptoms, avoiding complacency, and overcoming anosognosia is of high importance if this disease is to be contained. The risk of Ebola virus becoming an endemic disease in West Africa is becoming extremely likely in this propagated outbreak.

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