Title: Joint West Africa Group (JWARG) RV 466/EID 005 Protocol- Severe Infectious Disease: Surveillance, Detection, Risks and Consequences in West Africa.

Introduction

The Joint West African Research Group (JWARG) was established as a global research platform for military and civilian entities to detect, prevent and mitigate emerging infectious diseases of epidemic/ pandemic potential perform disease surveillance in West Africa. The main goals of the program are to determine etiologies and outcomes of acute febrile illness in West Africa, as well as build capacity in resource-limited settings to enable local response to pathogens with outbreak potential.

Since 2017, JWARG's RV466/EID 005 research study has been enrolling acutely ill adult participants (≥ 18 years) with undifferentiated fever or significant perturbations in other vital signs with a suspected infectious etiology, and healthy controls across military and civilian hospitals in Nigeria, Ghana, and Liberia. At enrollment and follow-up (which occurs within 28 ± 7 days), demographic, exposure and clinical data were obtained and specimens were collected for a wide-range of laboratory assays (depending on local capabilities), including routine chemistry and hematology, rapid tests for HIV and malaria, blood smear microscopy, and blood cultures. Multiplex antigen and molecular-based assays were also employed to detect a panel of viruses. Specifically, MAGPIX IgG antibody detection was performed for major hemorrhagic fevers, including Rift Valley Fever Virus (RVFV), Marburg Virus (MARV), Ebola Virus (EBOV), Lassa Virus (LASV), and Crimean-Congo Hemorrhagic Fever virus (CCHF). A pan-alphavirus, pan-flavivirus panel was also employed Luminex bead-based technology was used to create an antibody capture/reporter sandwich assay. This system was used instead of ELISA as it has higher analyte sensitivity and specificity, and is a high-throughput assay. Individual samples were selected for equipoise in the final clinical diagnosis and were further analyzed by Real-time PCR (RT- PCR) with primers designed for identical targets to those used by the MAGPIX, where possible. Synthetic RNA were used as positive controls.

As of February 2022, 756 participants were enrolled from three countries: (Nigeria = 552 (73%); Ghana = 165 (22%); Liberia = 39 (5%)). 358 (47%) participants were male and 398 (53%) female. The median age at enrollment across all sites was 35 (IQR=26 - 45) years (median (IQR) was 34 (26-45) in Nigeria, 36 (28-51) in Ghana and 37 (26-42) in Liberia. A total of 522 blood cultures were performed, 263 across all Nigeria sites, 228 in Ghana. Liberia did not implement bacterial cultures. The most common pathogens isolated were *Staphylococcus* and *Streptococcus* species (8/27, 32%). Of 102 samples tested from Ghana, 15 (15%) were IgG reactive to RVFV, 36 (35%) to the pan-alphavirus panel, 93

(93%) to pan-flavivirus panel, 7 (7%) to MARV, 24 (24%) to EBOV, 7 (7%) to LASV, and 8 (8%) to CCHF. All 102 samples were also tested for antigen capture, and all found to be negative. In Nigeria, of 154 samples tested by MAGPIX IgG 64 (42%) were reactive to RVFV, 58 (38%) to the pan-alphavirus panel, 110 (71%) to the pan-flavivirus panel, 1 (<1%) to MARV, 14 (9%) to EBOV, 29 (19%) to LASV, and 13 (8%) to CCHF. Likewise, antigen detection on all 154 Nigerian samples was negative. Three hundred eighty-four (384) samples were tested by real-time RT-PCR for the aforementioned hemorrhagic fever viruses, and none tested positive. Agnostic genomic sequencing of samples from five (5) participants with typical clinical features of a viral syndrome, but negative by RT-PCR, yielded no viral sequences of interest.

Our laboratory interrogations yielded a limited number of pathogen-centric findings in this cohort of acutely ill West African adults. There were no detected cases of flavivirus, alphavirus or other hemorrhagic fevers despite the relatively high endemicity of some of these infections in the sub-region. Initial eligibility criteria were broader in an effort to capture more participants; however we found the acuity of illness among participants to be mild, thus potentially accounting for the absence of circulating high-threat pathogens. Additionally, we found that local populations show a high degree of exposure to several outbreak-prone diseases such as Ebola and flaviviruses, as evidenced by the IgG Magpix results. This may potentially challenge our existing knowledge of the transmission dynamics and clinical presentations of these viral infections within the population. A more targeted approach toward more acutely ill populations, coupled with enhanced bacteriology and next-generation sequencing capabilities will be critical to improved yield of platforms for detection of etiologic agents of undifferentiated severe acute illness of infectious origin.

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