REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Pathogen-derived Circulating Nucleic Acids As Biomarker Candidates For Assessment Of Countermeasures Against Aerosol-transmitted Viral Pathogens

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Background: Acutely infectious viruses such as Venezuelan Equine Encephalitis Virus (VEEV) constitute an important threat to the warfighter because of the lack of approved prophylactic and therapeutic countermeasures. VEEV is also highly stable and infectious as an aerosol, which greatly increases the risk to the warfighter. When VEEV is acquired by the inhalational route, there is an increased possibility for CNS manifestations, higher potential for mortality and neurological sequelae in survivors. Early diagnostics that can accurately identify exposure and host viral load will be instrumental to the deployment of supportive therapies early, which will contribute to better symptom management, as well as, to evaluate countermeasure effectiveness at the molecular level.

Objective: To establish the dynamics of pathogen-derived nucleic acid as circulating biomarkers, with and without intervention (vaccination), in the context of different routes of exposure to VEEV in the mouse model. We quantified circulating biomarker abundance by polymerase chain reaction (PCR) with virus genome-specific primers, using primers against representative regions of the early expressed nonstructural genes (nsP1) and the subsequently expressed structural genes (capsid). We included serum, bronchioalveolar lavage (BAL) and urine as source samples for circulating biomarkers, as well as, target organs (brain, lung) to draw correlative relationships between infected organs and biomarker dynamics in circulation. Finally, we have included extracellular vesicles (EVs) derived from the urine as another sample source for biomarkers.

Results: Our results revealed that both nsP1 and capsid PCRs are positive in the lung and brain samples on days 2, 4, 6 and 8 post infection, with about 9X more viral nucleic acid in the brain sample than the lung at the 2- and 4-day time frame, which increases to >10X at the later time points. BAL samples showed the presence of virus nucleic acid on day 2 post infection, which decreased steadily at later time points. The urine/stool samples showed the presence of viral RNA on all time points analyzed, but at lower levels than those detected in the BAL. The viral nucleic acid load in the lungs steadily decreased from day 2 to day 8, while the brain nucleic acid load showed a steady increase. Ongoing studies are focused on 1) evaluating the presence of viral nucleic acid in the circulating EVs and their correlation with urine and serum biomarkers, and 2) in vivo biomarker dynamics in vaccinated animals

Outlook: Our results so far have revealed the dynamic changes in biomarker abundance based on the nature of the sample that is analyzed, and the route of exposure. We envision that the outcomes of these studies will be highly pertinent to evaluate effectiveness of candidate vaccines and therapeutics that are currently in preclinical development. Similar challenges/strategies also apply for several other viral pathogens including other alphaviruses, bunyaviruses, arenaviruses and filoviruses that are highly relevant to the DTRA mission and to the warfighter. Respiratory pathogens such as coronaviruses also pose the similar challenges to the civilian population.

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