H41 A Molecular Diagnostic Determination of Human vs. Bovine Origin of Viral-Associated Aggressive Natural Killer (NK) Cell Leukemia

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Learning Overview: After attending this presentation, attendees will have: (1) gained familiarity with diagnostic features of aggressive NK aggressive cell leukemia, a fulminant malignancy that may remain undetected until the time of postmortem examination; (2) developed an understanding of the critical role viruses can play in the development of such malignancies; and (3) appreciation for the utility of molecular diagnostic testing in elucidating the nature/source of viral infections.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the importance of collaborating with pathology specialties, such as molecular and hematopathology, in order to more effectively investigate and accurately certify deaths in cases of suspected viral-associated hematologic malignancies.

A 51-year-old Hispanic male with no known medical history who worked on a dairy farm presented with acute hepatic failure of unknown origin three days prior to his death. Laboratory testing in process at the time of death ultimately revealed quantitative Epstein-Barr Virus (EBV; *human gammaherpesvirus 4*) DNA copies at >1,000,000IU/mL. At autopsy, external and internal examination was notable for diffuse jaundice, anasarca, and evidence of disseminated intravascular coagulation. The liver revealed a background of micronodular cirrhosis with superimposed acute necrosis. There was marked splenomegaly (1,290g) with extensive geographic necrosis. Hepatosplenic histologic sections showed atypical lymphocytes that were positive for EBV-encoded RNA (EBER) and CD56, consistent with EBV-infected NK cells. Circulating EBV-infected NK cells were also seen in sections of vessels. Overall these findings—in conjunction with the patient's fulminant clinical presentation—appeared consistent with EBV-associated aggressive NK cell leukemia.

The state Occupational Safety and Health Administration (OSHA) was subsequently contacted due to a third parties' concern that this represented a work place-acquired viral infection and death. While there is a *bovine gammaherpesvirus 4* (BHV-4) linked to respiratory and reproductive clinical manifestations in dairy cattle, it is not known to commonly infect and/or cause disease in humans. Due to presumed genetic similarity between the human (EBV) and bovine (BHV-4) herpesviruses, there was concern that the Polymerase Chain Reaction (PCR) primer used for the antemortem EBV test could have amplified BHV-4, and thus been erroneously reported as an EBV DNA PCR copy number. To address this concern, real-time PCR (rtPCR) was repeated on a patient blood sample obtained at the time of autopsy, and the amplicon generated from rtPCR was Sanger sequenced. Sequence comparison of the amplification product to various nucleotide sequence databases using Basic Local Alignment Search Tool confirmed the viral infection to be EBV (human origin) rather than BHV-4 (bovine origin).

EBV is one of the most common human viruses in the world. Antibodies to EBV have been demonstrated in all population groups with a worldwide distribution; approximately 90%–95% of adults are EBV-seropositive. EBV infection has been associated with the development of an array of hematologic malignancies, including NK cell leukemia. Aggressive NK cell leukemia is a rare hematologic malignancy that is most commonly seen in Asia, Central America, and South America. The disease has a highly aggressive course with a median survival of <2 months. High levels of circulating plasma EBV at the time of diagnosis (as were found in the current case) have been suggested to portend a worse prognosis. Hepatosplenomegaly and acute hepatic failure have been reported in conjunction with aggressive NK cell leukemia. In conclusion, the cause of death in this case was fulminant acute hepatic failure due to EBV-associated aggressive NK cell leukemia. This case serves to illustrate the utility of molecular diagnostic testing in distinguishing human vs. bovine viral origin in cases in which there is concern for occupational exposure to the infectious agent.

Molecular Diagnostics, EBV, Aggressive NK Cell Leukemia