Biomarker Correlates of Survival in Pediatric Patients with Ebola Virus Disease

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

Outbreaks of Ebola virus disease occur sporadically in sub-Saharan Africa and are associated with exceptionally high case-fatality rates. The disease onset is nonspecific and is characterized by abrupt onset of fever, fatigue, headache, myalgia, and gastrointestinal distress 3 to 13 days after exposure to the virus. The *Ebolavirus* genus includes 5 different viruses that result in different case-fatality rates: Ebola virus, Sudan virus, and Bundibugyo virus cause fatal infections, but neither Tai Forest virus nor Reston virus has been associated with human fatalities.

Pediatric patients have been underrepresented in Ebola virus disease studies because total numbers of affected children in any given Ebola virus disease outbreak, whether associated with Ebola virus or Sudan virus or Bundibugyo virus are usually low because of outbreak dynamics and societal structure. For example, nosocomial Ebola virus disease infections mostly occur in adults working on hospital wards, and children are not usually caregivers for Ebola virus disease patients. However, the 2000–2001 Sudan virus outbreak in the Gulu district of Uganda, the largest recorded Ebola virus disease outbreak to that point, resulted in 425 cases; 145 cases were in patients around 21 years of age, and 55 of these cases were laboratory confirmed. The case-fatality rate for pediatric patients in this outbreak was lower than for adults, but the reasons for this increased survival were unknown. The relatively large number of pediatric cases in this outbreak enabled closer investigation of factors associated with increased survival of pediatric patients with Ebola virus disease.

Samples collected during the Gulu outbreak have been invaluable for advancing understanding of Ebola virus disease pathophysiology. Studies using these samples found associations between fatal outcomes and elevated liver enzyme levels, renal dysfunction, cytokine dysregulation, and genetic factors. Recently, we analyzed serum biomarkers by using samples from the Gulu outbreak and identified associations between cytokines/chemokines, acute-phase reactants, markers of coagulopathy, and markers of endothelial function and patient death, hemorrhage, and viremia. In this study, we used a series of multiplex assays to measure the concentrations of 55 serum analytes in specimens from patients from the Gulu outbreak to identify biomarkers that had age-specific associations with survival, hemorrhagic manifestations, or both.

During the 2000–2001 Gulu Ebola virus disease outbreak, an international response team, including representatives from the US Centers for Disease Control and Prevention, or CDC, provided clinical and technical assistance. Serum samples were obtained as part of the management of these patients and were stored in liquid nitrogen. In addition to the samples from the 55 pediatric patients, people less than 21 years of age, who had laboratory-confirmed Ebola virus disease, we selected samples from 50 adult patients, more than 21 years of age, who had laboratory-confirmed infection. This selection was designed to be representative of overall sex ratios, hemorrhagic manifestations, and death rates observed during the outbreak. A total of 45 of the 55 pediatric patients and 49 of the 50 selected adult patients had sufficient serum available for the proposed studies.

Specimens were prioritized for novel analyses first; if a sufficient sample amount was available, serum chemistry analyses were also performed.

Patients were considered to have hemorrhagic manifestations if they exhibited any of the following signs: vomiting blood; blood in the stool; or bleeding from the gums, skin, or eyes. We found that a higher percentage of pediatric than adult patients exhibited hemorrhage, but overall case-fatality rates remained lower for children than for adults.

To determine whether pediatric patients were more likely to survive as viral replication decreased, we measured viremia levels in each sample by using real-time reverse transcription PCR and compared the results with a standard curve generated from stock virus of known titer. No statistically significant differences were found between viral loads in adults and pediatric patients. Viral loads were higher for patients who died, as previously demonstrated; however, in the pediatric population, this difference did not reach statistical significance, likely because of the small sample size and the wide range of observed values in the pediatric patients with nonfatal cases.

Serum chemistry tests were performed on all samples that had sufficient available volume after initial testing. Blood urea nitrogen, creatinine, and albumin levels varied by age, as expected, given the normal physiological differences between adults and children. No age-specific associations were found between any analyte in the serum chemistry results and death or hemorrhage. More labile analytes, such as carbon dioxide and electrolytes, were excluded from analysis.

Differences in disease severity for patients of different ages are not uncommon in infectious diseases. For example, tuberculosis is associated with disseminated disease in children under 5 years of age and focal pulmonary disease in adults but causes infrequent and mild disease in school-aged children and adolescents. A similar pattern was observed in this evaluation of patients infected with Sudan virus. Two possible explanations for the increased rate of death among children under 5 years of age are the contributions of co-occurring conditions or the immature immune systems in children of this age.

RANTES is an acronym for regulated on activation normal T cell expressed and secreted. Now RANTES was the only factor we studied that demonstrated an association with higher survival rates in children. Decreased levels of RANTES in adult patients infected with chikungunya virus and in children infected with respiratory syncytial virus have been associated with more severe disease, and lower RANTES levels in children have been associated with death from cerebral malaria. The data from all of these studies suggest that, during Sudan virus infection, RANTES could recruit and activate T cells, leading to a stronger Sudan virus-specific T cell–mediated response and thus to improved survival. The occurrence of this phenomenon only in children is notable, but in models of familial hypercholesterolemia, the monoctytes of children, but not of adults, have increased RANTES expression, which suggests that children might have a greater capacity for RANTES production than do adults.

Interleukin 10, or IL-10 levels, were significantly elevated at the earliest times of infection in pediatric patients who died. The role of IL-10 in inhibiting antigen-stimulated T cell proliferation supports the assumption that a T cell–mediated response is *critical* for survival during Ebola virus disease. Levels of Intercellular Adhesion Molecules and Vascular Cell Adhesion Molecules for children are normally higher than for adults, and the levels that we detected in surviving

pediatric patients were consistent with these normal levels. Pediatric patients who died had sICAM and sVCAM levels 2 to 3 times above the reference range 0 to 10 days after symptom onset, but these levels dropped to within the reference range at 11 to 15 days. This pattern may reflect early, excessive, and ultimately detrimental endothelial activation in these patients. Consistent with this theory are the increased Plasminogen Activation Inhibitor, or PAI-1, levels also seen in pediatric patients who died; PAI-1 is released by endothelial cells in response to activating cytokines.

IgG levels were higher in samples from pediatric patients than in samples from adult patients; this difference is notable because children usually have slightly *lower* levels of total IgG than do adults. The higher IgG levels might suggest a higher degree of immune activation, perhaps secondary to other infectious co-existing conditions, which are likely to be present in children living in a rural area of Africa.

We also observed association between age and hemorrhagic manifestations for PAI-1 and Serum Amyloid A, or SAA, levels. Elevated PAI-1 levels in pediatric patients with hemorrhagic manifestations likely represent the overactive endothelium and not a functional inhibition of fibrinolysis, since PAI-1 activity is likely to be low, as it rapidly converts to the inactive form under physiologic conditions. In our study, higher SAA levels were seen in pediatric patients without hemorrhagic manifestations than in those with hemorrhagic manifestations. These levels were also higher than those seen in adults, regardless of the presence or absence of hemorrhagic manifestations. SAA is known to induce TF production by monocytes; we found that levels of TF were higher in pediatric patients, but these levels were not outside the normal range for adults or children and were not associated with hemorrhage or its absence. Alternatively, another, as yet undefined, function of SAA could be responsible for its association with pediatric patients who did not experience hemorrhagic manifestations. Finally, despite the association of PAI-1 with both hemorrhage and death, no statistically significant differences in survival rate were observed between patients with or without hemorrhagic manifestations. This finding suggests that these physiologic observations about hemorrhage are not causally related to survival.

An overactive endothelial response, as evidenced by elevated sICAM, sVCAM, and PAI-1 levels, was associated with death in children and adolescents. However, the adults in our study did not seem to be affected by this phenomenon. Co-existing conditions in the pediatric patients, such as malaria or other childhood illnesses, could have contributed to endothelium reactivity, or this finding could be secondary to the known physiologic differences that exist between the adult and juvenile endothelium.

In summary, our data suggest that different pathophysiologic mechanisms of disease may be at work in pediatric patients, and children may benefit from different treatment than their adult counterparts. Therapeutic interventions targeted at decreasing endothelial activation in pediatric patients early during the course of infection might include drugs that affect endothelial activation, such as statins. The clear association between survival and increased RANTES in pediatric patients also suggests that a better understanding of the mechanisms and molecular consequences of increased levels of this chemokine could be useful in future therapeutic design, especially in design of drugs that induce a stronger, earlier, antigen-specific T cell response.

I'm Dr. Mike Miller, for *Emerging Infectious Diseases*, and I've been reading an abridged version of the article Biomarker Correlates of Survival in Pediatric Patients with Ebola Virus Disease.You can read the entire October 2014 article online now at cdc.gov/eid.

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