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WU and KI polyomavirus infections in Filipino children with lower respiratory tract disease☆

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ABSTRACT

Background: WU and KI are human polyomaviruses initially detected in the respiratory tract, whose clinical significance remains uncertain.
Objectives: To determine the epidemiology, viral load and clinical characteristics of WU and KI polyomaviruses.
Study design: We tested respiratory specimens collected during a randomized, placebo-controlled pneumococcal conjugate vaccine trial and related epidemiological study in the Philippines. We analyzed 1077 nasal washes from patients aged 6 weeks to 5 years who developed lower respiratory tract illness using quantitative real-time PCR for WU and KI. We collected data regarding presenting symptoms, signs, radiographic findings, laboratory data and coinfection.
Results: The prevalence and co-infection rates for WU were 5.3% and 74% respectively and 4.2% and 84% respectively for KI. Higher KI viral loads were observed in patients with severe or very severe pneumonia, those presenting with chest indrawing, hypoxia without wheeze, convulsions, and with KI monoinfection compared with co-infection. There was no significant association between viral load and clinical presentation for WU.
Conclusions: These findings suggest a potential pathogenic role for KI, and that there is an association between KI viral load and illness severity.

1. Background

WU and KI are small, icosahedral, non-enveloped double-stranded deoxyribonucleic acid (DNA) viruses [1] in the family *Polyomaviridae*. They were discovered in 2007 in the respiratory tract of children and adults, begging the question of their contribution to respiratory tract infections (RTI). Since their initial discovery, they have been detected in respiratory specimens worldwide [2–7]. Children appear to have higher detection rates in the respiratory tract than adults, with the highest rates (up to 13%) in 1–2 year olds [8–10]. The clinical presentation in persons with specimens positive for either KI or WU appears to be similar to other respiratory viruses, and includes fever, cough, and other upper and lower respiratory symptoms [5,8,10]. However, complete clinical characteristics including radiographic and laboratory findings have not been well defined for these viruses.

Abbreviations: PCR, polymerase chain reaction; LRTI, lower respiratory infection; GMVL, geometric mean viral load; CSF, cerebrospinal fluid; RSV, respiratory syncytial virus; CRP, C-reactive protein; hMPV, human metapneumovirus; PCT, procalcitonin.

☆ The polyomaviruses WU and KI were detected in the respiratory tract specimens of children with lower respiratory tract illness; our findings suggest that KI may be implicated in causing respiratory illness, and that viral load may correlate with illness severity.

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Controversy persists as to whether WU and KI are true respiratory pathogens. KI and WU have been identified as the sole pathogens in respiratory tract specimens that have undergone wide screening for other viruses, bacteria and fungi. There is, however, a high co-detection rate with other respiratory viruses; 31–79% for WU, and 33–74% for KI [10–12]. Furthermore, WU has been found in the respiratory tract of asymptomatic patients. Studies have detected viral sequences at similar or higher frequencies in asymptomatic patients [3,4,13], but few studies exist for KI.

Several real-time PCR methods have been developed for detecting both viruses [14–16], which offer higher sensitivity and specificity than traditional PCR. Quantitative real-time PCRs have recently been used to define the role of respiratory viruses such as RSV and bocavirus [17,18], and may contribute to further understanding the epidemiological and clinical impact of WU and KI. Quantitative PCR has been developed for WU, and studies report low viral loads in subjects with RTIs [19], with no correlation between viral load and clinical severity [6]. Their implementation for the study of KI, however, has been more limited, and the significance of viral load for KI, and correlation with illness severity, remain unknown.

2. Objectives

In order to further characterize the clinical presentation of these viruses, we sought to

1) determine the presence of WU and KI among children with lower respiratory tract infection,
2) describe their clinical features in more detail than previously reported, and
3) determine whether viral load correlates with illness severity.

3. Study design

3.1. Study population

From 2000 to 2004, a cohort of 12,194 children less than 2 years of age were enrolled in a randomized, placebo-controlled, double-blinded trial testing the efficacy of an 11-valent pneumococcal conjugate vaccine (11 PCV) in 6 barangays (villages) in Bohol, Philippines [20]. Concurrently, all children from the 6 barangays who were 5 years of age or less, who were not in the trial, and who were admitted to the hospital or who visited the outpatient department of the Bohol Regional Hospital were enrolled in a separate prospective epidemiologic study (the EpiGreyStudy). Hospitalized and outpatient subjects in the EpiGreyStudy were examined, treated and investigated exactly the same as those in the vaccine trial. Nasal wash specimens and sera were collected from all patients hospitalized with lower respiratory infection (LRI), and one in 5 outpatients with LRI, within two years of enrolment or by the end of study follow-up at the end of December 2004. The protocol included a blood culture and chest x-ray for all episodes, and CSF culture when indicated.

In these 2 groups of subjects, 5570 clinical episodes of LRI were observed, and 2066 nasal washes were obtained as described previously [21]. Data regarding hospital admissions and other important medical events were collected until the end of the study or study follow-up.

3.2. Definitions and clinical characteristics

An LRI was defined as the presence of lower respiratory tract signs (crackles, wheezing or bronchial breathing) in children with cough or difficulty breathing, or the presence of WHO-defined pneumonia [22] or radiographic pneumonia. Clinical pneumonia was classified using World Health Organization (WHO) severity grades as non-severe, severe and very severe in infants and children with cough and/or difficulty breathing. Fast breathing (respiratory rate $\geq$ 60/min if age <2 months, $\geq$ 50/min if age 2–11 months and $\geq$40/min if age 12–59 months) identified non-severe pneumonia; lower chest-wall indrawing defined severe pneumonia, and cyanosis, the inability to feed or drink and convulsions were the features of very severe pneumonia [23]. Other detailed information collected for the study included presenting symptoms, vital signs, physical examination findings, radiographic findings and laboratory data.

3.3. Pathogen detection

Samples were screened by Quantitative Real-Time PCR for the presence of WU and KI. The method for Quantitative Real-Time PCR, including the primer and probe sequences, has been described previously [24]. Briefly, viral nucleic acids were extracted from the respiratory specimens using the TRIzol® extraction method (Invitrogen, Camarillo, CA), following the manufacturer's instructions. The DNA primers and probes for the VP1 region of WU and KI were designed using Primer Express software 3.0 (Applied Biosystems, Foster City, CA). Basic Local Alignment Search Tool (BLAST) searching of the primer and probe sequences found the sequences to be specific to WU and KI, and control PCR reactions with other polyomavirus VP1 sequences (BK Virus, JC Virus, Simian Virus 40 (SV40), lymphotrophic polyomavirus (LPV), Merkel cell virus (MCV)) confirmed this analysis. Quantitative real-time PCR was performed on the ABI Prism® 7750 Sequence Detector. All real-time assays used previously described PCR buffer and cycling conditions [24]. Results initially expressed as cycle threshold (Ct), were converted to copy number by creating a standard curve using serial dilutions of a control plasmid of known concentration. Viral copy numbers were normalized to human cells in the specimen using the C-reactive protein (CRP) copy number, and were expressed as copies per mL of sample and copies per cell.

Specimens were previously tested for the presence of other respiratory viruses including respiratory syncytial virus (RSV), influenza, parainfluenza, human metapneumovirus (hMPV) and rhinovirus using routine respiratory tissue culture, conventional respiratory shell vial culture, and the PLx Multi-Code Respiratory Virus Panel (PLx-RVP), manufactured by Eragen Biosciences, Inc. (Madison, WI). The methods for testing followed the standard protocols and have been described previously [21].

3.4. Statistical analysis

We estimated that with a sample of 1200 individuals, we would be able to construct a 95% confidence interval around the proportion of WU and KI positive specimens with a precision of 0.03, assuming a baseline rate for both KI and WU to be 0.50. Demographic and clinical features were compared between WU or KI-infected and non-infected children. In addition, for those who tested positive, comparisons were made between WU or KI as the sole virus detected and WU or KI co-detected with other respiratory viral and invasive bacterial pathogens. Categorical variables were compared using the chi-square test or Fisher's exact test for association. Continuous variables with non-normal distributions were compared using the Mann Whitney *U* test. All comparisons were two-sided and a P value of <0.05 was considered significant. All statistical analyses were performed using SPSS (SPSS, Inc., v.21, IBM) and SAS version 9.0 (SAS Institute Inc., Cary, NC).