Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Clinical Virology 44 (2009) 115–118

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Detection of WU polyomavirus DNA by real-time PCR in nasopharyngeal aspirates, serum, and stool samples

Florian Neske[a], Kerstin Blessing[b], Anika Pröttel[a,b], Franziska Ullrich[a,b], Hans Wolfgang Kreth[b], Benedikt Weissbrich[a,*]

[a] Institute of Virology and Immunobiology, University of Würzburg, Versbacher Str. 7, 97078 Würzburg, Germany
[b] Children's Hospital, University of Würzburg, Josef-Schneider-Str. 2, 97080 Würzburg, Germany

ARTICLE INFO

Article history:
Received 9 March 2008
Received in revised form
28 November 2008
Accepted 2 December 2008

Keywords:
WU polyomavirus
Respiratory tract infection
Epidemiology
Viral coinfections
Real-time PCR

ABSTRACT

Background: The human WU polyomavirus (WUPyV) has been recently described as a novel virus in respiratory tract samples.
Objective: To investigate the viral load of WUPyV in nasopharyngeal aspirates (NPAs), stool, and serum samples of pediatric patients with acute respiratory tract diseases.
Study design: We established a real-time PCR for WUPyV DNA and tested NPA obtained between 2002 and 2007 from pediatric in-patients with acute respiratory tract diseases. In addition, 14 stool and 14 serum samples of children with WUPyV DNA positive NPA were analysed.
Results: WUPyV DNA was found in 5.2% of 1232 NPA. The median viral load in the NPA was 950 copies/ml (maximum 3.4E10 copies/ml). The WUPyV load in NPA was neither associated with the coinfection status nor with the clinical diagnoses. WUPyV DNA was found in 3 of 14 serum samples and in 2 of 14 stool samples. The WUPyV load in NPA tended to be higher in viremic children.
Conclusion: WUPyV DNA was found in NPA, serum, and stool of hospitalised children with acute respiratory tract diseases. Further studies are necessary to determine whether WUPyV is a human pathogen.

1. Introduction

Respiratory tract infections are a leading cause of human morbidity and are mainly caused by viruses. Molecular methods have enabled the detection of a number of unknown viruses in respiratory tract samples such as several coronaviruses as well as the human bocavirus (hBoV).[1–3] Most recently, two novel viruses of the family polyomaviridae were detected in respiratory samples and named KI polyomavirus[4] and WU polyomavirus (WUPyV).[5] Two human polyomaviruses were known before, the JC and BK polyomavirus. These viruses cause persisting infections, which are usually asymptomatic in immunocompetent individuals but may lead to severe disease under immunosuppression.[6]

In the first description of WUPyV, DNA was detected in 37 (3.0%) of 1245 samples from Australian children with acute respiratory tract infection and in 6 of 890 respiratory samples obtained from adults and children in the United States.[5] Most of the WUPyV DNA positive patients (86%) were younger than 4 years of age. Strikingly, coinfections were detected in 72% of the patients. Meanwhile, similar findings of WUPyV detection have been reported from South Korea, Canada, Scotland, the United States, and Germany with prevalence rates ranging from 1.6% to 7%.[7–11] Because WUPyV DNA was also detected in samples of a control group without respiratory symptoms, the clinical relevance of positive WUPyV DNA results in respiratory samples has been questioned.[11] Rather, positive WUPyV DNA results in the respiratory tract may constitute an incidental detection of a persistent virus. However, further investigations are necessary to clarify this issue.

The initial studies on WUPyV infections have mostly used conventional single-round PCR for WUPyV detection. In order to facilitate WUPyV DNA analysis and to quantify WUPyV DNA in clinical samples, we designed a real-time PCR assay. Quantification of WUPyV DNA may be important with respect to the pathogenic relevance of positive WUPyV DNA results, as has been suggested for hBoV.[12] In the present study, we describe the evaluation of the WUPyV real-time PCR and its application for the detection of WUPyV DNA in nasopharyngeal aspirates (NPAs). In addition, we studied stool and serum samples of children with WUPyV DNA positive NPA.

Abbreviations: Ct, threshold cycle; hBoV, human bocavirus; NPA, nasopharyngeal aspirate; RSV, respiratory syncytial virus; WUPyV, WU polyomavirus.

* Corresponding author. Tel.: +49 931 201 49962; fax: +49 931 201 49561.
E-mail address: weissbrich@vim.uni-wuerzburg.de (B. Weissbrich).

1386-6532/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.jcv.2008.12.004

2. Materials and methods

2.1. Samples

The specimens tested for WUPyV infection consisted of unselected stored NPA that were received from January 2002 through September 2005 and from January 2007 through July 2007 from the Children's Hospital of the University of Würzburg for screening of respiratory viruses. The aspirates were collected using a catheter tube and an aspiration trap. After suction the tube was rinsed with sterile saline. All samples had been tested for the presence of antigens of adenovirus, influenza A and B virus, parainfluenza viruses 1–3, and respiratory syncytial virus (RSV) by immunofluorescence assay and for the presence of hBoV DNA as described previously.[13] In addition, archived stool and serum samples of children with positive WUPyV DNA in NPA were tested if they were obtained no longer than 14 days before or after the NPA. All samples had been stored at −20 °C or below until DNA extraction. The study was approved by the ethics committee of the medical faculty of the University of Würzburg.

2.2. Real-time WUPyV PCR

DNA was extracted from 200 μl of the NPA and serum samples using the High Pure Viral Nucleic Acid Kit (Roche, Mannheim, Germany) and from 140 μl of stool suspensions using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), which is known to purify both RNA and DNA.[14] Both kits were used according to the instructions of the manufacturers. The elution volume of the extractions was 50 μl in case of the NPA and serum samples and 60 μl for the stool samples. Less than the stated starting material was used for the extraction procedures in exceptional cases, if the volume of the archived samples was otherwise insufficient. One negative control was extracted and amplified for every five patient samples. Extracted nucleic acids were stored at −70 °C until PCR testing.

Primers and probe for the real-time PCR were selected by Primer Express 2.0 software (Applied Biosystems, Darmstadt, Germany) from the C-terminal region of the large T-antigen that was used for a qualitative WUPyV PCR previously.[10] Sequencing of 62 WUPyV positive samples had revealed high sequence conservation in this region. Blasting of primers and probe against GenBank to exclude unspecific binding did not reveal any significant homologies with other organisms. The real-time PCR was carried out in a final volume of 20 μl consisting of 5 μl of extracted DNA, primers WU2958s (CCTGTTAGTGATTTTCACCCATGTA) and WU2865a (TGTCAGCAAATTCAGTAAGGCCTATATAT) at a final concentration of 400 nM, the probe WU2925s-TM (6FAM-AAAGTTGTGTATTGGAAAGAACTGTTAGACA-TAMRA) at a final concentration of 100 nM, and 1× Quantitect probe master mix (Qiagen). Amplification was performed on an ABI7500 real-time PCR system (Applied Biosystems, Darmstadt, Germany). The cycling conditions were 50 cycles with 30 s at 95 °C and 60 s at 60 °C after a preheating step of 15 min at 95 °C.

A plasmid containing the PCR product obtained with the primers AG0048 and AG0049[5] cloned into the vector pCR2.1-TOPO (Invitrogen, Karlsruhe, Germany) was used as positive control and for the standard curve. Viral loads were calculated from the threshold cycle (Ct) values of the individual samples with respect to the standard curve. General laboratory procedures to prevent PCR contamination were strictly adhered to.

2.3. Statistical analysis

Statistical analysis was carried out by using GraphPad Prism Version 3.0c for Mac (GraphPad Software, San Diego, USA) and SPSS Version 15 for windows (SPSS, Chicago, USA).

3. Results

3.1. Validation of the quantitative real-time PCR

In order to validate the quantitative real-time PCR, the linearity of the assay, the lower limit of detection, the intra-assay and interassay variation, and the specificity were investigated. The amplification curve was linear over the range from 5 to at least 5×10^8 copies/reaction. For standard curves of 28 independent runs, the mean value of the slope was −3.53 with a standard deviation of 0.1. The mean of the coefficient of correlation was 0.992 with a standard deviation of 0.014. The lower limit of detection was determined by probit analysis based on a total of 24 replicates in three independent runs of a twofold dilution series. A concentration of 7.1 copies/reaction (95% confidence interval: 4.8–20.4 copies/reaction) was detectable with 95% probability (data not shown). This corresponds to 360 copies/ml of starting material (serum or NPA). The reliability of the real-time PCR was assessed by analyzing the intra-assay and interassay variation. The intra-assay coefficient of variation of the Ct values for three replicates was 1.32% or lower for standard concentrations of 5–5×10^5 copies/reaction (data not shown). The interassay coefficient of variation of a run control (nominal concentration, 5×10^5), which was regularly included in each run, was 15.1% (data not shown). To test the primers and probe for unspecific binding, samples known to be positive for JC, BK, and KI polyomavirus, hBoV DNA and, adenovirus DNA were tested in the WUPyV real-time PCR assay. All samples were negative in the WUPyV assay.

3.2. Quantitative detection of WUPyV DNA in NPA

For clinical evaluation of the WUPyV real-time PCR, 1232 samples from 1134 children that had previously been tested by conventional qualitative WUPyV PCR[10] were retested with the real-time assay. After analysis of discrepant results between the qualitative and the real-time PCR assay by repeat-testing and by additional PCR assays in different genome regions, two samples remained that were positive only in the real-time assay (110 and 140 copies/ml) and one sample that was positive only in the qualitative assay. Overall, the frequency of WUPyV DNA detection in our study population was 5.2% with the real-time PCR assay. The median viral load of all WUPyV positive NPA was 9.5×10^2 copies/ml. The maximum value was 3.4×10^{10} copies/ml. Twenty-two (34.4%) of the positive samples had viral loads below the 95% limit of detection (360 copies/ml). A comparison of WUPyV loads with respect to the clinical diagnosis did not reveal any significant differences ($p = 0.98$ by Kruskal–Wallis test; Fig. 1). In 32 (50%) of the WUPyV positive NPA, evidence of infection with other respiratory viruses was detected. The median WUPyV loads of the

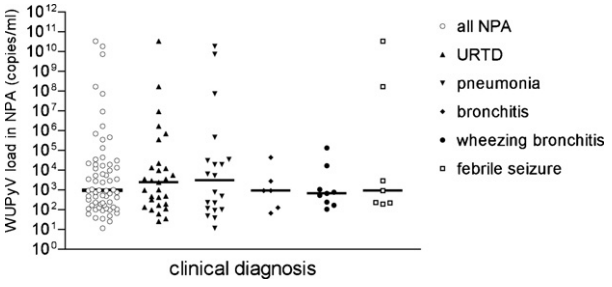

Fig. 1. Viral load of WUPyV in NPA according to the clinical diagnosis. The median values (horizontal bars) were 9.3×10^2 copies/ml for all NPA, 2.5×10^3 copies/ml for upper respiratory tract disease (URTD), 3.0×10^3 copies/ml for pneumonia, 9.3×10^2 copies/ml for bronchitis, 6.8×10^2 copies/ml for wheezing bronchitis, and 9.3×10^2 copies/ml for febrile seizure.

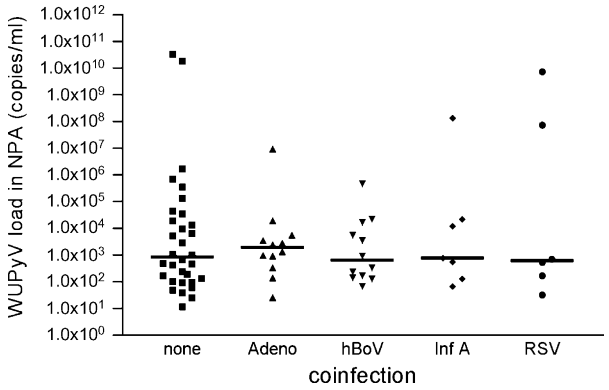

Fig. 2. WUPyV load in NPA of children with and without respiratory coinfections. In addition to WUPyV DNA, all samples were tested for antigen of RSV, influenza A/B virus, adenovirus, and parainfluenza viruses 1–3 by immunofluorescence assay and for hBoV DNA by PCR. Coinfecting agents found more than five times are displayed. Horizontal bars indicate median values.

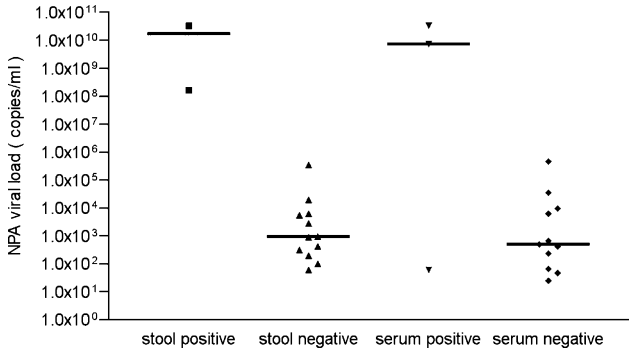

Fig. 3. Comparison of WUPyV loads in NPA according to WUPyV status of stool and serum samples of the same patient. Median values are indicated by horizontal bars.

samples with and without evidence of coinfection were almost identical for any coinfecting agent that was found more than five times ($p = 0.99$ by Kruskal–Wallis test; Fig. 2).

3.3. Detection of WUPyV DNA in serum and stool samples

For some of the children with WUPyV positive NPA, archived stool and serum samples from the same hospitalisation period were available. These samples were also tested by WUPyV real-time PCR. Three (21.4%) of 14 serum samples and two (14.3%) of 14 stool samples were positive for WUPyV DNA. Inhibition of the WUPyV-negative stool samples was excluded by spiking experiments with WUPyV plasmid DNA (data not shown). The median WUPyV load in NPA tended to be higher in children with positive serum samples (7.5×10^9 copies/ml versus 5.0×10^2 copies/ml; $p = 0.09$ by Mann–Whitney test) or positive stool samples (1.7×10^{10} copies/ml versus 9.4×10^2 copies/ml; numbers too small for calculation of p-value; Fig. 3). Interestingly, the patient with the highest WUPyV viral load (3.4×10^{10} copies/ml) of all tested NPA was positive for WUPyV DNA in stool and serum.

4. Discussion

In order to improve the diagnostic tools for WUPyV detection, we have established a real-time PCR method. In our validation experiments, this assay proved to be sensitive, specific, and reliable for WUPyV DNA amplification. Quantification was possible over a broad linear range. We found WUPyV DNA in 5.2% of NPA obtained from infants and children with respiratory tract diseases during the years 2002–2005 and 2007 in the region of northern Bavaria in Germany. PCR inhibition by NPA was not assessed as part of this study, but data from our routine diagnostic lab demonstrate that inhibition occurs in less than 1% of NPA (unpublished observation). Two other studies using real-time PCR and probe detection for WUPyV amplification have been published thus far.[9,15] However, detailed descriptions of analytical assay characteristics and quantitative data of clinical samples were not provided.

There are some striking features of the quantitative WUPyV DNA results in our study. While very high viral loads of greater than 10^9 copies/ml were found in a small proportion (4.7%) of the WUPyV DNA positive NPA, the majority of these samples (51.6%) contained only low viral loads of less than 1000 copies/ml. Although the significance of viral load results in NPA is limited to some extent because of technical variations in the acquisition procedure (e.g. the volume of the NPA on arrival in the lab ranged approximately from 1 to 10 ml), the observed viral loads in patient samples differed by a factor of 10^8, a range much larger than could be accounted for by variation in NPA volumes obtained. In analogy to hBoV,[16] the observed WUPyV load distribution is compatible with primary WUPyV infection associated with high viral loads in few of the children and incidental detection of low copy numbers of persistent virus in most of the children.

For two of the three children with very high viral load in NPA, serum samples from the same disease episode were available for retrospective testing. WUPyV viremia was found in both of these children. Again in analogy to the findings described for hBoV,[16] this may be an indication of the clinical relevance of WUPyV in a subset of the children. Serological studies to determine the time point of primary WUPyV infection will be necessary to further elucidate this issue.

Based on the hypothesis that high viral loads may have pathogenic relevance, we compared the WUPyV load in NPA according to the clinical diagnosis. Significant differences of the median viral load were not observed between the different disease groups. However, this analysis is limited by the retrospective nature of our study, by the high number of coinfections, and by the fact that clinical information was analyzed only for WUPyV positive patients.

Stool samples from 2 of 14 children were positive for WUPyV DNA. This finding may be explained by replication of WUPyV in gastrointestinal epithelium. However, both of the children had high WUPyV loads in NPA, which points to swallowing of WUPyV as an alternative explanation for the presence of WUPyV DNA in stool samples. Whether the WUPyV DNA in stool samples is derived from infectious or from degraded virus particles and if WUPyV may be a cause of gastroenteritis remains to be determined.

In conclusion, we have successfully established and used a real-time PCR assay for the detection of WUPyV DNA. Application of this assay for quantitative analysis has shown that WUPyV DNA can be found in NPA samples at very high titers. In addition to the respiratory tract, WUPyV was also found in serum and stool samples. However, the clinical relevance of WUPyV infections remains uncertain both for the respiratory and for the gastrointestinal tract.

Acknowledgements

We thank the technicians of the viral diagnostic lab for skilful and dedicated assistance.

References

1. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci USA* 2005;**102**:12891–6.
2. Fouchier RA, Rimmelzwaan GF, Kuiken T, Osterhaus AD. Newer respiratory virus infections: human metapneumovirus, avian influenza virus, and human coronaviruses. *Curr Opin Infect Dis* 2005;**18**:141–6.

3. Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;**79**:884–95.
4. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, et al. Identification of a third human polyomavirus. *J Virol* 2007;**81**:4130–6.
5. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* 2007;**3**:e64.
6. Ahsan N, Shah KV. Polyomaviruses and human diseases. *Adv Exp Med Biol* 2006;**577**:1–18.
7. Abed Y, Wang D, Boivin G. WU polyomavirus in children, Canada. *Emerg Infect Dis* 2007;**13**:1939–41.
8. Han TH, Chung JY, Koo JW, Kim SW, Hwang ES. WU polyomavirus in children with acute lower respiratory tract infections, South Korea. *Emerg Infect Dis* 2007;**13**:1766–8.
9. Le BM, Demertzis LM, Wu G, Tibbets RJ, Buller R, Arens MQ, et al. Clinical and epidemiologic characterization of WU polyomavirus infection, St. Louis, Missouri. *Emerg Infect Dis* 2007;**13**:1936–8.
10. Neske F, Blessing K, Ullrich F, Pröttel A, Kreth HW, Weissbrich B. WU polyomavirus infection in children. *Germany Emerg Infect Dis;* 2008;**14**: 680–1.
11. Norja P, Ubillos I, Templeton K, Simmonds P. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. *J Clin Virol* 2007;**40**:307–11.
12. Allander T. Human bocavirus. *J Clin Virol* 2008;**41**:29–33.
13. Weissbrich B, Neske F, Schubert J, Tollmann F, Blath K, Blessing K, et al. Frequent detection of Bocavirus DNA in German children with respiratory tract infections. *BMC Infect Dis* 2006;**6**:109.
14. Weissbrich B, Harms F, ter Meulen V. Detection of enterovirus RNA and herpes virus DNA in CSF by multiplex PCR. *Qiagen News* 1995;2/95.
15. Bialasiewicz S, Whiley DM, Lambert SB, Gould A, Nissen MD, Sloots TP. Development and evaluation of real-time PCR assays for the detection of the newly identified KI and WU polyomaviruses. *J Clin Virol* 2007;**40**:9–14.
16. Allander T, Jartti T, Gupta S, Niesters HG, Lehtinen P, Osterback R, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007;**44**: 904–10.