

No evidence for WU polyomavirus infection in acute exacerbations of chronic obstructive pulmonary disease

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Abstract

Human polyomaviruses are known to cause persistent or latent infections, which are reactivated under immunosuppression. They are capable of immortalizing cell lines and possess oncogenic properties. Recently a novel human polyomavirus, WU polyomavirus (WUPyV), has been identified in respiratory specimens from patients with acute respiratory tract infections (ARTI). WUPyV has been proposed to be a pathogen in ARTI in early life and immunocompromised individuals, but so far its role as a causative agent of respiratory disease remains controversial.

The objective of our study was to determine the prevalence of WUPyV infection in adult hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease (AE-COPD) and to establish its clinical relevance by comparison to patients with stable COPD hospitalized for other reasons than acute exacerbation.

A total of 174 respiratory specimens, 61 induced sputum and 113 nasal lavage samples from 154 patients, who had been recruited in a prospective 2:1 ratio case-control set-up between 1999 and 2003, were evaluated for the presence of WUPyV DNA by real-time PCR.

In the present study we could not detect WUPyV DNA in 174 respiratory specimens from 154 adult hospitalized patients with acute exacerbation and stable COPD in four consecutive years.

Persistence of viral replication or reactivation of latent WUPyV infection did not occur. WUPyV may not play a major role in adult patients with AE-COPD.

Findings:

Polyomaviruses are small, non-enveloped viruses with a circular double-stranded DNA genome of approximately 5,000 base pairs. Human polyomaviruses are known to be capable of immortalizing animal and human cell lines. Their oncogenic potential has been demonstrated in vitro and in various cancer animal models and is effected by the integration of viral DNA into the host cell genome. Expression of the viral T-antigen is mandatory for cell transformation [1]. In the last year there has been a re-emergence of interest in polyomaviruses as possible human carcinogens as three novel polyomaviruses have been described in humans. While KI and WU polyomavirus have been detected in respiratory specimens [2, 3], the Merkel cell polyomavirus (MCPyV) was observed in Merkel cell carcinomas, a rare but aggressive human skin cancer of neuroendocrine origin [4]. However, the detection in the respiratory tract is not a unique feature of KI- and WUPyV as transmission by the respiratory route has already been suggested for the first two human polyomaviruses, BK and JC virus [5-7]. In 2007, WU polyomavirus (WUPyV) was identified in respiratory specimens from patients with acute respiratory tract infections (ARTI) [2]. It has been proposed to be a relevant pathogen in ARTI in early life and immunocompromised individuals, but so far its role as a causative agent of respiratory disease remains controversial as it was also found in healthy asymptomatic individuals [8, 9]. WUPyV infections appear endemic worldwide [2], detection frequencies vary from 0.4% [10] to 7% [11] and coinfections with other respiratory viruses are common [12].

The aim of the present study was to determine the prevalence of WUPyV infections in adult hospitalized patients with acute exacerbations of chronic obstructive pulmonary disease (AE-COPD) and to establish its clinical relevance by comparison to patients with stable COPD hospitalized for other reasons than acute exacerbation.

A total of 174 respiratory specimens, 61 induced sputum and 113 nasal lavage samples from 154 patients were retrospectively evaluated for the presence of WUPyV DNA. Subjects with acute exacerbations and stable COPD had been recruited in a prospective case-control

study in a 2:1 ratio between October 1999 and April 2003. Underlying criteria, definitions and procedures have been the same as described previously [13]. Notably, patients with known thoracic malignancies were excluded from the study. DNA was extracted from the samples using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) and stored at -70°C for further testing. Primers and probe for the WUPyV real-time PCR assay were selected from the highly conserved C-terminal region of the large T-antigen that has been used for a qualitative WUPyV PCR assay previously [14]. Blasting of primer and probe sequences against GenBank excluded significant homologies with other organisms. The real-time PCR was performed 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 1x Quantitect probe master mix (Qiagen, Hilden, 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 [2] cloned into the vector pCR2.1-TOPO (Invitrogen, Karlsruhe, Germany) was used as positive control and for the standard curve. Strict laboratory procedures were implemented to prevent PCR contamination. One negative control was amplified for every five samples. Data analysis was performed using SPSS, version 11.5 (SPSS Inc., Chicago, Illinois). Categorical data were compared by Pearson's chi-squared or Fisher's exact test, where appropriate. Normal distribution in continuous variables was determined with the Kolmogorov-Smirnov test and differences were subsequently determined either with student's t-test or Mann-Whitney-U test. All p values were calculated two-sided with statistical significance set to $p < 0.05$. The study was approved by the ethics committee of the Ruhr University, Bochum, Germany. All study participants gave their written informed consent prior to study inclusion.

The standard curve was linear over the range from 1×10^1 to at least 1×10^8 copies per reaction. The lower detection limit of the applied real-time PCR assay was determined to ten copies per reaction. A total of 174 respiratory specimens, 61 induced sputum and 113 nasal lavage samples, from 154 adult hospitalized COPD patients were investigated for the presence of WUPyV DNA by real-time PCR. Of those, 102 patients (66.2%) had AE-COPD and 52 (33.8%) had stable COPD. The distribution of specimens with regard to the COPD status is shown in Table 1. PCR results were available both for induced sputum and nasal lavage specimens in 20 patients. The two groups were comparable in terms of age, sex, body mass index, smoking behavior, pack years and medication (Table 1). Spirometric data before discharge were available for 59 of 102 patients (57.8%) with AE-COPD, had significantly improved after treatment for acute exacerbation and were comparable to the baseline airflow in the control group. Significant differences were apparent for increased airflow limitation on admission and higher C-reactive protein levels in the AE-COPD group (Table 1). WUPyV DNA was not detected in any of the tested samples when using a sensitive real-time PCR assay.

Our findings are in agreement with two recent studies from China [15] and UK [16] which failed to detect WUPyV DNA in adults. The initial investigation by Gaynor et al. found four adults with altered immune status or multiple comorbidities to be positive for WUPyV [2]. None of the mentioned studies explicitly included patients with (AE-)COPD. The present population consisted of predominantly elderly COPD patients with severely impaired lung function and steroid medication. In a previous study performed on a comparable population we demonstrated that AE-COPD was significantly associated with the detection of respiratory viruses, foremost human rhinovirus, influenza virus A and respiratory syncytial virus, and that induced sputum had a higher viral yield than upper respiratory tract specimens in patients with AE-COPD [13]. However, in the present study we could not detect WUPyV DNA in 174 respiratory specimens from 154 adult hospitalized patients with acute exacerbation and

stable COPD in four consecutive years between 1999 and 2003, whereas a recent report found WUPyV circulating in a German pediatric population within our study period [14].

Our findings support the hypothesis that primary WUPyV infection is acquired in early life rather than adulthood and suggest that persistence of viral replication or reactivation of latent WUPyV infection is not a common phenomenon in the adult COPD population. Hence, WUPyV may not play a major role in patients with AE-COPD. A clear linkage between WUPyV and human disease still remains to be determined.

Abbreviations

AE-COPD (acute exacerbation of chronic obstructive pulmonary disease); ARTI (acute respiratory tract infection); DNA (deoxyribonucleic acid); PCR (polymerase chain reaction); WUPyV (WU polyomavirus);

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

FCR conceived and designed the study, performed the statistical analysis, interpreted the data, supervised the study and drafted the manuscript. MH conducted the PCR experiments and revised the manuscript critically for important intellectual content. IB was involved in the processing of the specimens, UA, JK and BMH recruited the patients and all revised the manuscript critically for important intellectual content. GSW contributed to the study design and supervised the study. GR contributed to the study design, analysis and interpretation of data, supervised the study and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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References

1. zur Hausen H: **Novel human polyomaviruses--re-emergence of a well known virus family as possible human carcinogens.** *Int J Cancer* 2008, **123**:247-250.
2. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP, Wang D: **Identification of a novel polyomavirus from patients with acute respiratory tract infections.** *PLoS Pathog* 2007, **3**:e64.
3. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T, Andersson B: **Identification of a third human polyomavirus.** *J Virol* 2007, **81**:4130-4136.
4. Feng H, Shuda M, Chang Y, Moore PS: **Clonal integration of a polyomavirus in human Merkel cell carcinoma.** *Science* 2008, **319**:1096-1100.
5. Goudsmit J, Wertheim-van Dillen P, van Strien A, van der Noordaa J: **The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils.** *J Med Virol* 1982, **10**:91-99.
6. Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T: **Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients.** *J Clin Microbiol* 1994, **32**:1390-1394.
7. Monaco MC, Jensen PN, Hou J, Durham LC, Major EO: **Detection of JC virus DNA in human tonsil tissue: evidence for site of initial viral infection.** *J Virol* 1998, **72**:9918-9923.
8. Abed Y, Wang D, Boivin G: **WU polyomavirus in children, Canada.** *Emerg Infect Dis* 2007, **13**:1939-1941.
9. 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**:307-311.

10. Lin F, Zheng M, Li H, Zheng C, Li X, Rao G, Wu F, Zeng A: **WU polyomavirus in children with acute lower respiratory tract infections, China.** *J Clin Virol* 2008, **42**:94-102.
11. 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**:1766-1768.
12. Le BM, Demertzis LM, Wu G, Tibbets RJ, Buller R, Arens MQ, Gaynor AM, Storch GA, Wang D: **Clinical and epidemiologic characterization of WU polyomavirus infection, St. Louis, Missouri.** *Emerg Infect Dis* 2007, **13**:1936-1938.
13. Rohde G, Wiethage A, Borg I, Kauth M, Bauer TT, Gillissen A, Bufe A, Schultze-Werninghaus G: **Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study.** *Thorax* 2003, **58**:37-42.
14. Neske F, Blessing K, Ullrich F, Prottel A, Wolfgang Kreth H, Weissbrich B: **WU polyomavirus infection in children, Germany.** *Emerg Infect Dis* 2008, **14**:680-681.
15. Ren L, Gonzalez R, Xie Z, Zhang J, Liu C, Li J, Li Y, Wang Z, Kong X, Yao Y, et al: **WU and KI polyomavirus present in the respiratory tract of children, but not in immunocompetent adults.** *J Clin Virol* 2008. **43**:330-333
16. Abedi Kiasari B, Vallely PJ, Corless CE, Al-Hammadi M, Klapper PE: **Age-related pattern of KI and WU polyomavirus infection.** *J Clin Virol* 2008, **43**:123-125.

Table. Demographic and clinical characteristics of the study population.

Variables	AE-COPD		Stable COPD		P value
	n	%*	n	%*	
Patients (total n=154)	102	66.2	52	33.8	
	n	% [¶]	n	% [¶]	
Specimens (total=174)					
Induced sputum (n=61)	38	37.3	23	44.2	0.49
Nasal lavage (n=113)	75	73.5	38	73.1	1.0
	n	% [#]	n	% [#]	
Sex					
Female	26	25.5	7	13.5	0.10
Male	76	74.5	45	86.5	
Smoking behavior					
Active smokers	25	24.5	14	26.9	0.10
Non-smoker	19	18.6	3	5.8	
Ex-smoker	58	56.9	35	67.3	
Oral steroid medication					
Yes	69	67.6	34	65.4	0.86
No	33	32.4	18	34.6	
Inhaled corticosteroids					
Yes	65	63.7	37	71.2	0.38
No	37	36.3	15	28.8	
	Mean	SD	Mean	SD	
Age in years [range]	68 [41-83]	9.4	66 [43-81]	10.9	0.13
Body mass index (kg/m ²)	27.2	5.7	27.2	5.7	0.98
Leukocytes/nl	11.2	3.5	10.5	3.8	0.28
	Median	Range	Median	Range	
Pack years	30	4 - 120	30	2 - 120	0.73
FEV1 ^a (l)	1.0	0.5 – 2.2	1.2	0.5 – 2.6	<0.001
FEV1 ^a (% predicted)	38.0	18.7 – 79.0	42.5	19.4 – 77.3	0.046
FEV1 ^b (l)	1.2	0.5 – 2.9	1.2	0.5 – 2.6	0.49
FEV1 ^b (% predicted)	44.0	19.8 – 78.9	42.5	19.4 – 77.3	0.64
C-reactive Protein (mg/dl)	0.9	0.0 – 35.0	0.6	0.0 – 3.8	0.002
Oral steroid dose (mg) [§]	20	2 – 150	14	5 – 150	0.12

* Percent in line. [¶] Percent of all patients in the respective group. [#] Percent in column. [§] Oral steroid dose in prednisone equivalent in those patients receiving oral steroid medication before admission. (AE-)COPD, (acute exacerbation of) chronic obstructive pulmonary disease. FEV1^a, forced expiratory volume in one second on admission. FEV1^b, baseline FEV1 for the control group and before discharge after recovery from exacerbation for the AE-COPD group.