Trichodysplasia Spinulosa Polyomavirus in Respiratory Tract of Immunocompromised Child

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Trichodysplasia spinulosa polyomavirus causes trichodysplasia spinulosa, a skin infection, in immunocompromised persons, but the virus is rarely detected in respiratory samples. Using PCR, we detected persistent virus in respiratory and skin samples from an immunocompromised boy with respiratory signs but no characteristic skin spicules. This virus may play a role in respiratory illness.

Trichodysplasia spinulosa is a rare skin disease that occurs exclusively in immunocompromised persons. It is characterized by facial keratotic spicules formed by trichohyalin accumulation in the inner root sheath cells of affected hair follicles. In 1999, electron microscopy identified a novel polyomavirus, subsequently named trichodysplasia spinulosa polyomavirus (TSPyV) or human polyomavirus 8, in sections of skin spicules of a solid organ transplant patient (1); in 2010, the virus was more completely characterized (2). TSPyV is 1 of 5 polyomaviruses associated with human diseases, particularly those that affect immunocompromised persons (3). Although worldwide seroprevalence of TSPyV antibodies among the general population is estimated at 70% (4) and a respiratory route of infection has been hypothesized (5,6), as of 2015, only 32 cases of trichodysplasia spinulosa had been reported (7), suggesting that other pathology caused by TSPyV may have gone undiagnosed. We describe PCR detection of TSPyV in an immunocompromised boy with respiratory signs and symptoms.

To elucidate potential causes of undiagnosed viral respiratory infection, during January 2015–February 2016, we used a panpolyomavirus degenerate primer PCR to screen archived samples for polyomavirus. The archived samples were nucleic acid of respiratory specimens from 218 children 6 months to 5 years of age, previously negative for typical respiratory viruses in a panel used for routine diagnosis (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/9/18-0829-Techapp1.pdf). Of the 218 samples screened in 22 pools, we obtained positive results for polyomavirus in 1 pool and, subsequently, 1 sample (from the patient reported here). Subsequent Sanger sequencing and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis of the 274-bp degenerate primer PCR product indicated that the sample contained TSPyV. The complete genome of this TSPyV strain was amplified in 4 overlapping PCR fragments and Sanger sequenced (online Technical Appendix). Phylogenetic analysis of the assembled complete 5,232-nt genome with all available 23 reference sequences revealed that the TSPyV strain was most closely related to TSPyV 1312, which had been isolated in 2012 in Dallas, Texas, USA (online Technical Appendix), but bootstrap support was limited because of the highly conserved nature of TSPyV genomes.

The patient from whom this TSPyV-positive sample was collected was a 4-year-old boy in Nottinghamshire, United Kingdom, who had common acute lymphoblastic leukemia and was receiving maintenance chemotherapy during the study period. Retrospective clinical analysis for March 2014–February 2016 revealed that the child had had frequent cough with fever and coryzal symptoms of varying severity (Table). Concurrently collected nasopharyngeal aspirate and throat swab specimens were negative for bacterial and viral pathogens routinely tested for, except at the start of the study period, when rhinovirus and adenovirus were detected, and the end of the period, when rhinovirus and respiratory syncytial virus were detected (Table). No bacteria were cultured from paired specimens. On this basis, in conjunction with unremarkable physical examination and radiologic findings and stable neutrophil and leukocyte counts (data not shown), the patient’s respiratory signs were treated conservatively on an outpatient basis. However, on 2 occasions (August and November 2015), the child required hospital admission, without and with co-infection, respectively.

Further retrospective laboratory investigation found that all 11 additional samples collected from this patient during November 2014–2015 were positive for TSPyV, with co-infection at the 4 time points (November and December 2014, September and November 2015); testing showed fluctuating cycle threshold (C_0) levels on quantitative PCR (Table; online Technical Appendix). Of note, various forms of rashes appeared in different anatomic regions of the patient but did not resemble the characteristic appearance of trichodysplasia spinulosa and, thus, did not raise any clinical suspicion for this condition. Indeed, retrospective testing found that a single skin swab sample taken from a suspected viral rash (site undocumented) that looked like blisters and
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Table. Clinical and laboratory data from TSPyV-positive patient, Nottinghamshire, United Kingdom, November 2014–2015

| Collection date | Sample type   | Signs and symptoms at time of sample collection | Documented skin lesion | Hospital admission | Viral/bacterial co-infection | TSPyV Cv value |
|-----------------|---------------|-----------------------------------------------|------------------------|-------------------|-----------------------------|----------------|
| 2014 Nov        | Throat swab   | Cough, sore throat, fever                      | Tiny skin colored pustules on hand | Not required      | Rhinovirus                  | 31.83          |
|                 | NPA           | Cough, fever                                  | None                   | Not required      | Adenovirus                  | 31.17          |
| 2015 Jan        | Skin swab     | None recorded                                 | Suspected varicella zoster virus rash | Not required      | None                        | 24.97          |
| Feb             | NPA           | Dry cough, fever                              | None                   | Not required      | None                        | 31.23          |
| Mar             | Throat swab   | Dry cough, fever                              | None                   | Not required      | None                        | 21.43          |
| Jul†            | Throat swab   | Cough with runny nose                         | None                   | Not required      | None                        | 23.90          |
| Jul‡            | NPA           | Cough with runny nose                         | None                   | Not required      | None                        | 22.70          |
| Jul‡            | Throat swab   | Dry cough, fever                              | Few blisters on fingers | Not required      | None                        | 22.30          |
| Aug             | Throat swab   | Cough, fever (high)                           | Erythematous rash with tiny white center on face | Hospitalized 4 d | None                        | 21.47          |
| Sep Nov         | NPA           | Cough, wheeze, fever (high), corylzial symptoms | None                   | Not required      | Respiratory syncytical virus | 26.87          |

*Cv., cycle threshold; NPA, nasopharyngeal aspirate; TSPyV, trichodysplasia spinulosa polyomavirus; VZV, varicella zoster virus.
†Collected on the same date.
‡Collected on the same date.

About the Author
Ms. Bagasi is a PhD student at the University of Nottingham. Her research interests are epidemiology and cellular entry of viral infections.

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Wohlfahrtiimonas chitiniclastica Bacteremia in Hospitalized Homeless Man with Squamous Cell Carcinoma

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We report a case of Wohlfahrtiimonas chitiniclastica bacteremia in an elderly man in Japan who had squamous cell carcinoma. Blood cultures were initially negative for W. chitiniclastica but were positive on day 20. Careful attention needs to be paid to this organism in patients who have chronic wounds with maggots.

We report Wohlfahrtiimonas chitiniclastica bacteremia in a 75-year-old man in Japan who had squamous cell carcinoma on his shoulder. In September 2016, an unidentified patient was found unconscious on the ground by a passerby and admitted to the emergency department of the National Center for Global Health and Medicine (Tokyo, Japan). He had a necrotic lesion on his left shoulder with maggots. Blood analysis showed leukocytosis (26.61 × 10^9 cells/L [reference range 3.30–8.60 × 10^9 cells/L]), thrombocytosis (626 × 10^9/L [reference range 158–348 × 10^9/L]), anemia (hemoglobin, 9.6 g/dL [reference range 13.7–6.8 g/dL]), and elevated C-reactive protein (87.9 mg/L [reference range 0.00–1.40 mg/L]). Albumin was 2.4 g/dL (reference range 4.1–5.1 g/dL) and calcium was 12.6 mg/dL (reference range 8.8–10.1 mg/dL). He was diagnosed with disturbance of consciousness caused by hypercalcemia and was hospitalized.

After saline infusion and intravenous cefazolin (3 g/d) were initiated, the patient’s condition improved. A blood culture taken at the time of admission grew Peptoniphilus harei. A swab culture of the ulcer site grew Proteus mirabilis, Morganella morganii, and Kerstersia gyiorum. A biopsy was performed on day 3, and the patient was diagnosed with squamous cell carcinoma. Enhanced computed tomography scanning revealed an ulcer and ring-enhancing lesion on his left shoulder (which was suspected of being a tumor or abscess) and multiple enlarged lymph nodes and 10-mm pulmonary nodules in the right lung.

On day 20, the patient had fever and disturbance of consciousness; therefore, he was transferred to the Infectious Disease department of the hospital. Intravenous therapy with vancomycin (1.5 g/d), cefepime (3 g/d), and metronidazole (1,500 mg/d) was initiated, and the patient’s fever and consciousness improved. Two cultures of blood taken on day 20 grew P. mirabilis, M. morganii, Streptococcus anginosus, Streptococcus agalactiae, Bacteroides fragilis, and gram-negative rods. After we obtained the culture results, vancomycin was stopped in accordance with the susceptibility test results. We identified the gram-negative rods as W. chitiniclastica by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Billerica, MA, USA), which showed scores of 2.239. We further confirmed the isolate to be W. chitiniclastica by using 16S rRNA sequencing; the isolate was 99.08% identical to strain S5 (GenBank accession no. AM397063). We assessed the isolate’s antimicrobial susceptibility profile (Table). The patient improved and was later discharged to another hospital.

W. chitiniclastica is a gram-negative, short, facultative anaerobic, straight-rod gammaproteobacterium that was first isolated from the parasitic fly Wohlfahrtia magnifica (1). This fly has not been reported in Japan. However, W. chitiniclastica has also been isolated from the Chrysomya megacephala fly, and this species has been reported in Japan (2), and from the Musca domestica housefly, which is widely distributed all over the world (3). Campisi et al. reported that the Lucilia sericata fly might be a vector for W. chitiniclastica (4); this fly is common and widely distributed throughout Japan, and a
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Technical Appendix

Supplementary Information

Nucleic acids (NA) were extracted from respiratory specimens of symptomatic individuals referred to Nottingham University Hospitals Trust, Nottingham, UK by the Nuclisens EasyMAG system (Biomerieux). Routine diagnostic PCR / RT-PCR assays for Human Adenovirus, Influenza (A and B), Human Metapneumovirus, Parainfluenza (1–4), Rhinovirus and Human Respiratory Syncytial Virus (RSV, A and B) were undertaken and surplus NA archived at −72°C. A total of 198 NPA and 20 throat swabs negative for the aforementioned viruses were identified from patients aged 6 months to 5 years old between January 2015 and February 2016 and subjected to extended diagnostic tests. All subsequently analyzed longitudinal samples from the sole TSPyV positive individual identified had been subject to the same diagnostic pathway, with the exception of January 2015 skin swab. This sample was screened by PCR / RT-PCR for Herpes Simplex Virus 1 and 2, Varicella Zoster Virus, Mumps virus, Enterovirus and Parechovirus and found to be negative for all targets.

Five µl NA from 8 –10 samples were combined to make a total of 22 pools and 1 µl of each screened for Polyomaviruses using a previously described degenerate pan-Polyomavirus assay (1) modified by using only the VP1–2 primer pair (see table below) and HotStarTaq (QIAGEN) in 15 µl reactions with the following PCR cycle conditions: 95°C for 15 minutes then 55 cycles of 95°C for 20s, 57°C for 20s and 72°C for 20s. Constituents of any positive pools were then re-screened individually to isolate the positive sample component(s). All putative positive samples were confirmed by Sanger sequencing (Source Bioscience, Nottingham UK) and BLAST analysis of the resulting nucleotide sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
TSPyV-positive samples were quantitatively investigated by SYBR green Realtime PCR (qPCR) using novel in-house primers TSPyV326f and TSPyV510r (see table below, PCR conditions as above, Ct values represent an average of triplicate values) designed in Primer3 (http://primer3.ut.ee/) using an alignment created in MEGA7 (http://www.megasoftware.net/) of all 23 complete reference sequences available on Genbank in March 2018 (https://www.ncbi.nlm.nih.gov/nuccore/?term = txid862909[Organism:noexp]) to target a highly conserved genomic region. The entire circular 5232 nt genome of a single TSPyV-positive sample was amplified in 4 overlapping PCR products using additional primers designed as above (see table below, PCR conditions as above except with a 2 minute extension step and 45 cycles only). Amplicons were Sanger sequenced using both forward and reverse primers and a contiguous sequence representing the complete genome (Genbank ID MH351677) was assembled using Seqman Pro (DNASTAR, Madison WI) and compared to the reference genomes in MEGA7 (see figure below).

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| Technical Appendix Table. Primers for TSPyV PCR amplification |
|----------------------------------------------------------------|
| **Assay**               | **Primer** | **Genome position** | **Sequence**                                   | **Product size** | **Source**                  |
|-------------------------|------------|---------------------|------------------------------------------------|------------------|----------------------------|
| Pan-polyoma | VP1-2f     | 2068–2097           | ATGAAAATGGCCGTGGCCGCCCCTNTGYAARG               | 274bp            | Johne et al 2005 (1)       |
| screen                  | VP1-2r     | 2313–2341           | CCCTCATAAACACCCTCATCCTGCTACAC                 |                  |                            |
| TSPyV specific PCR      | TSPyV326   | 326–345             | TAACACACATCATCTCCCCTGCT                     | 185bp            | This study                 |
| Full genome amplification | TSPyVr510  | 491–510             | GCTGCTTCTCTCTGACTACAC                  | as above         | 1577bp                     | This study                 |
|                         | TSPyV326   | as above            | as above                                    |                  |                            |
| Full genome amplification | TSPyVr1902 | 1883–1902           | GATTGAGGGCTTGTGTGCTC                       | 1403bp           | This study                 |
|                         | TSPyV1777  | 1774–1793           | ATATGGTTCGATGGGTGGGG                      | 1404bp           | This study                 |
|                         | TSPyV3176  | 3156–3176           | TGGCTAGTGAATTTCCCTGG                     | 1404bp           | This study                 |
| Full genome amplification | TSPyV3045  | 3045–3065           | AGTGCTTTTGACCCCTTGAACA                  | 1404bp           | This study                 |
|                         | TSPyVr4448 | 4428–4448           | GGGGTAGAATTAMAGGTGGGGQA                  | 1488bp           | This study                 |
| Full genome amplification | TSPyV4255  | 4255–4274           | AGAGRTCTGGGCTTGCATG                  | as above         | This study                 |
|                         | TSPyVr510  | as above            | as above                                    |                  |                            |
Technical Appendix Figure. Phylogenetic tree of the complete 5232 bp Trichodysplasia Spinulosa-associated Polyomavirus genome. The tree was constructed using the maximum likelihood method based on Tamura-Nei model and 1000 bootstrapping replications. Twenty-three available complete TSPyV genomes (open circles) were retrieved from Genbank in March 2018 and compared with the strain identified in this study (filled circle).