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CASE REPORT

Arm Paralysis After Routine Childhood Vaccinations: Application of Advanced Molecular Methods to the Causality Assessment of an Adverse Event After Immunization

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Post-licensure surveillance for adverse events following immunizations (AEFI) can identify rare complications of vaccinations and rigorous vaccine adverse event causality assessments can help to identify possible causal relationships. We report the development of arm paralysis after varicella vaccination in a 1-year-old child. Paralysis was initially presumed to be due to vOka because of the temporal relationship between vaccination and onset of arm weakness; however, molecular studies identified wild-type varicella zoster virus VZV (WT-VZV) in the CSF, leading the authors to conclude that WT-VZV was the probable cause. This case illustrates the complexity of assessing AEFI causality, and the importance of careful and complete evaluations when determining the most likely cause of an AEFI.

Keywords. adverse event after vaccination; child; paralysis; vaccine; varicella-zoster virus.

CASE REPORT

Clinical Scenario

A 1-year-old healthy child received hepatitis A (right thigh) and varicella (VAR) (right arm) vaccines in the late summer of 2014. Eight days later, he developed a febrile upper respiratory infection and bilateral otitis media, and he was treated with a third-generation oral cephalosporin. Ten days after vaccination, acute proximal weakness of the left upper arm (arm contralateral to vaccinated arm) developed, followed 2 days later by a mild maculopapular rash, vomiting, and diarrhea; the child was hospitalized for further evaluation. Physical examination was normal except for decreased muscle tone and left biceps reflex, normal range of motion, good movement of the left hand, but with a slightly decreased grip.

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The child was cared for at home with his healthy older children and adults who were healthy, asymptomatic, and vaccinated appropriately for age. The child was up-to-date with vaccines, had no recent travel or visitors to the home, and had no known exposure to VAR or zoster.

Diagnostic Evaluations

Examination of cerebrospinal fluid (CSF) revealed no organisms on Gram stain, red blood cell count 4188 per mm^3, white blood cell count 46 per mm^3 (95% mononuclear cells, 5% neutrophils), protein 39 mg/dL, and glucose 57 mg/dL. Magnetic resonance images (MRIs) of the brain and spine with contrast 3 days after the onset of arm paralysis were normal. Cerebrospinal fluid and serum were sent to the Wadsworth Center for encephalitis polymerase chain reaction (PCR) panel and serology. The laboratory is a federally funded Viral Vaccine Preventable Diseases Reference Center. Varicella-zoster virus (VZV) deoxyribonucleic acid (DNA) was detected in the CSF by real-time PCR [1], and differentiation of wild-type (WT) VZV from vaccine strain (vOka) was determined by 3 biallelic real-time PCR assays [2–4]. Results consistently showed all 3 single-nucleotide polymorphisms associated with WT VZV (Table 1). Attempts

Table 1. Primer and Probe Sequences and Sample Test Results, for VZV Wild-Type/Vaccine Distinguishing Real-Time PCR Assays

Target SNP	PCR Oligo Sequences	Sequence (5′-3′)[d]	Average C_T[a]	Reference
106262	Forward primer	CGTACACGTGATACTGAGACAAAGC		1
	Reverse primer	GCCGGTTGCTGGTGTTG		
	Wild-type standard probe	HEX-ATCCCTGGGCCACC- BHQ1	37	
	Vaccine standard probe	FAM- ATCCCCGGGCCAC- BHQ1	Neg	
107252	Forward primer	ACT GGA GCC CGT TGC CTC		2
	Reverse primer	TCC TAC AGA GTC TCC GCA GAG C		
	Wild-type MGB[b] probe	FAM-TTG CCA GCA TGG C- BHQ1	38	
	Vaccine MGB[b] probe	HEX-TTG CCG GCA TGG C- BHQ1	Neg	
108111	Forward primer	AAGACGAAACAAACTCACGACTCT		3
	Reverse primer	GGAAGATCCCACGCACCAGA		
	Wild-type LNA[c] probe	HEX- TTC[+T]CCAC[+T]GGGC[+T]GTC- BHQ1	35	
	Vaccine LNA[c] probe	FAM-TTCTCCAC[+C]GGGCTGTCA- BHQ1	Neg	

Abbreviations: C_T, cycle threshold; LNA, locked nucleic acid; MGB, minor groove binder; Neg, negative; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism; VZV, varicella-zoster virus.
[a]C_T values are the average across all tests run on the sample.
[b]Minor groove binding probe.
[c]Locked nucleic acid probe: square parenthesis indicate locked bases for LNA probes.
[d]Bases underlined in bold indicate the distinguishing nucleotide (vaccine vs wild type) at each SNP.

REFERENCES TABLE
1.Quinlivan ML, Jensen NJ, Radford KW, Schmid DS (2012): Novel genetic variation identified at fixed loci in ORF 62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. J Clin Microbiol. 50: 1533–8.
2.Parker SP, Quinlivan M, Taha Y, Breuer J (2006): Genotyping of varicella-zoster virus and the discrimination of Oka vaccine strain by TaqMan real-time PCR. J Clin Microbiol. 44: 3911–4.
3.Campsall PA, Au NH, Prendiville JS, Speert DP, Tan R, Thomas EE (2004): Detection and genotyping of varicella-zoster virus by TaqMan allelic discrimination real-time PCR. J Clin Microbiol. 42: 1409–13.

at sequencing for viral genotyping were unsuccessful due to the low viral load. Duplicate CSF PCR testing at the Columbia University laboratory in New York City confirmed the presence of VZV DNA. This laboratory participates in the VZV Identification Program and is part of the Worldwide Adverse Experience System of Merck and Co., Inc [5, 6].

Tests for West Nile virus (WNV) antibody in CSF and serum were negative, as were serum antibody tests against *Borrelia burgdorferi*, *Bartonela henselae*, Powassan, Eastern equine encephalitis (EEE), Western equine encephalitis, St. Louis encephalitis (SLE), and California serogroup viruses. Polymerase chain reaction on CSF for *B burgdorferi*, herpes simplex virus (HSV) 1 and 2, adenovirus, cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6, enterovirus, WNV, EEE, SLE, Cache Valley virus, and California serogroup virus were also negative [1].

A nasopharyngeal (NP) specimen collected 2 days after onset of paralysis tested negative by PCR for multiple respiratory viral and bacterial pathogens including rhinovirus/enterovirus (FilmArray® Biofire Diagnostics, Salt Lake City, UT). Rectal and oral swab viral cultures were also negative. Sera collected at 4 days and 1 and 4 months after the onset of paralysis were positive (>2.5 $\log_2$) for neutralizing antibodies against the prototype EV-D68 virus and 3 enteroviruses isolated during the 2014 outbreak [7].

Extensive immune evaluation revealed age-appropriate levels or functions of immunoglobulin (Ig)G (391 mg/dL), IgA (19 mg/dL), IgM (50 mg/dL), lymphocyte subsets, T-cell subsets (naive/memory, CD45RA/RO distribution), cytotoxic lymphocyte function, natural killer cell function, CD107a mobilization (measure of lymphocyte degranulation), interleukin (IL)-1 receptor-associated kinase 4 sequence, and Toll-like receptor functions. Varicella-zoster virus IgG adjusted optical density levels were all positive at 1.222, 0.932, 1.164, 2.949, and 1.736 on 4 days, 1, 4, 9, and 16 months, respectively, after the onset of paralysis. The avidity indices were 0 at 4 days, and 1 month post-onset; and increased to 37, 49, and 73 at 4, 9, and 16 months post-onset paralysis, respectively [8], consistent with a "primary" VZV antibody response. T-cell stimulation responses to candida and tetanus antigens were normal, as were specific antibodies to diphtheria and Streptococcus *pneumoniae* antigens. However, tetanus antibody level was low at <0.1 g/mL (normal range, >0.15 IU/mL). Cell-mediated immunity was evaluated by interferon-gamma (IFNγ) enzyme-linked immunosorbent spot (ELISPOT) [9]. The VZV-specific response at 6, 9, and 16 months after onset of paralysis was below the limit of 2 spot-forming cells/10^5 for a positive result, whereas a robust response to phytohemagglutinin validated the assay and cell conditions. Cytokine production in VZV-stimulated peripheral blood mononuclear cells was further tested using Luminex MAP and showed the absence of IL-2 production in response to VZV stimulation, consistent with the impaired VZV Th1 responses detected by the IFNγ ELISPOT assay. Nerve conduction and electromyography studies at 3 days, 7 weeks, 4 and 9 months after presentation were consistent with injury of the motor neurons and/or axons at C5-7 level on the left and excluded brachial plexopathy.

Differential Diagnosis

The differential diagnosis includes stroke, radiculopathy, myelitis, meningitis, and brachial plexopathy. In children, stroke may be associated with a recent attack of VAR [10]. Cerebral infarct was excluded by the normal diffusion-weighted cranial MRI. Spinal infarct was excluded by the absence of upper motor

neuron findings on examination and a normal spinal MRI. The spinal MRI may have been falsely negative for myelitis because it was performed soon after onset of symptoms, and spinal infarct could have been missed because the MRI did not include a diffusion-weighted sequence. Normal sensory conduction studies excluded brachial plexus involvement.

Treatment and Clinical Course

The patient had not recovered left arm function at 22 months after onset of paralysis. He was not treated with antiviral medications because the molecular information implicating VZV was not available during hospitalization. The child received supportive care, including physical therapy, shoulder kinesiotaping to correct scapular subluxation, and electrical stimulation to the left upper extremity.

DISCUSSION

When unusual illnesses occur after vaccination, vaccines are often presumed to be the cause of the adverse event. In this case, molecular studies helped clarify the etiology of the paralysis. In this patient, there was no scientific evidence that vaccine-type VZV caused the paralysis.

Wild-type VZV DNA was detected in the CSF, and despite extensive testing, no other pathogens were identified in CSF, NP, oral, or rectal specimens. The presence of WT VZV DNA strongly implicates this virus as the likely cause of arm paralysis. Laboratory error or contamination is unlikely because the virus was found in 2 independent laboratories and in multiple CSF aliquots. Reversion from vaccine strain (vOka) to WT VZV is highly unlikely given the inherent stability of the VZV genome [11]. Three genetic mutations, separated by 990 and 859 base pairs, respectively, would have all needed to independently revert to WT within the short period between vaccination and acquisition of the test sample (Figure 1). Although a small subset of vaccine viruses may contain 1 of the WT markers, there have been no isolates identified that contain all of them [12, 13]. Although recombination between WT VZV and vOKA has been described in vitro [14], it has never been demonstrated in vivo.

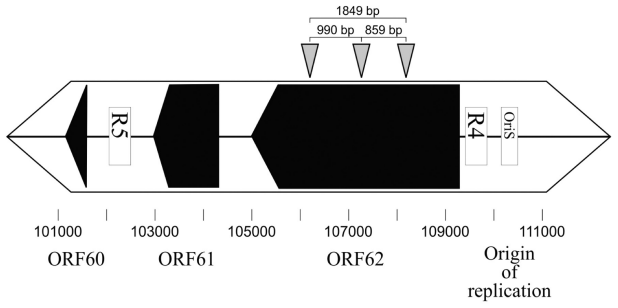

Figure 1. Location of the ORF62 single-nucleotide polymorphism used to discriminate vaccine from wild type. Display of varicella-zoster virus ORF62 together with other features found in the sequence immediately flanking the gene. The gray triangles mark the location of the fixed markers used to discriminate vaccine virus from wild type at positions 106262, 107262, and 108111 (left to right) and the distances in base pairs between them. The positions are based on the standard reference genome sequence, Dumas strain (GenBank accession number: NC_001348.1).

Varicella-zoster virus is a neurotropic virus that can infect neurons and cause them to die. Neurons may also harbor latent VZV for years and are the only cells that have the ability do so. During latency, only a few of the 71 genes of VZV are expressed. When VZV switches from latent infection to lytic infection in a neuron, most or all VZV genes are expressed, and neurologic disease associated with VZV is presumed to be a consequence of damage to neurons and satellite cells. Normal cellular mechanisms and structures would be expected to be disrupted in this context [15]. Central nervous system complications from VAR have become less common since VZV vaccination was instituted, but it remain serious and a leading cause of VZV-associated hospitalization [16–19]. Limb paralysis due to VAR has been previously described, but this is the first report of limb paralysis due to proximal axonopathy [19]. The absence of vesicular rash is rare but has been well documented among children with neurological manifestations of VZV infection [16, 20].

Severe VZV infection most commonly develops in patients with cell-mediated immunity defects [21–23]. This child had abnormal VZV-IFNγ responses. A full characterization of cell-mediated immune responses against *Herpesviridae* was impossible due to lack of exposure demonstrated by absence of HSV, CMV, or EBV antibodies. Lacunar defects in the immune repertoire have been invoked in cases of severe infections limited to a single agent [24].

This child presented with a neurological illness at the time there was an epidemiologic cluster of acute flaccid myelitis (AFM) and at the peak of a state- and community-wide outbreak of EV-D68 [25, 26]. Similarly to our patient, 53 of 56 children in the outbreak with presumed EV-D68 paralysis have not fully recovered [25]. Nevertheless, it remains unclear what role, if any, EV-D68 played in our patient's illness because he had "serological" evidence of prior (not acute) EV-D68 infection and no "virological" evidence of acute illness.Widespread seropositivity to EV-D68 in the US population limits its specificity. Although our patient could clinically fit into the syndromic epidemiological cluster, VZV clearly does not appear to be part of the large EV-D68 outbreak [25, 27]. This finding is inconsistent with a prior report indicating an association between EV-D68 in a cluster of 7 of 11 patients with AFM with an abscence of an alternative diagnosis [28]. We cannot exclude the possibility that the VZV DNA detected in CSF was due to blood contamination from a traumatic spinal tap, but we believe that this is unlikely to have occurred; in addition, blood PCR was not performed.

Clinical Immunization Safety and Assessment Project

Given the severity and unique nature of the patient's illness shortly after vaccination, the case was referred to the Centers for Disease Control and Prevention (CDC)-Clinical Immunization

Safety and Assessment (CISA) Project for consultation. Advice from CDC and CISA is meant to assist in decision making, rather than provide direct patient management.

CONCLUSIONS

The CISA experts agreed that the prepoderance of epidemiological and laboratory evidence indicated that the acute flaccid monoplegia was not caused by VAR and that paralysis was most consistent with WT VZV infection. A minority of CISA experts commented that it was not possible to completely exclude EV-D68 as contributing to the child's arm paralysis [25, 26]. Because the child had laboratory evidence of WT VZV infection, a second dose of VAR vaccine should not be needed. However, due to the VZV-specific cell-mediated immune defect, some experts recommended checking VAR titers at 4 years of age, to evaluate a need for second dose of VAR vaccine.

Notes

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Disclaimer. The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the Centers for Disease Control and Prevention or the US Department of Health and Human Services.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Dupuis M, Hull R, Wang H, et al. Molecular detection of viral causes of encephalitis and meningitis in New York State. J Med Virol **2011**; 83:2172–81.
2. Quinlivan ML, Jensen NJ, Radford KW, Schmid DS. Novel genetic variation identified at fixed loci in ORF62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. J Clin Microbiol **2012**; 50:1533–8.
3. Parker SP, Quinlivan M, Taha Y, Breuer J. Genotyping of varicella-zoster virus and the discrimination of Oka vaccine strains by TaqMan real-time PCR. J Clin Microbiol **2006**; 44:3911–4.
4. Campsall PA, Au NH, Prendiville JS, et al. Detection and genotyping of varicella-zoster virus by TaqMan allelic discrimination real-time PCR. J Clin Microbiol **2004**; 42:1409–13.
5. LaRussa P, Lungu O, Hardy I, et al. Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. J Virol **1992**; 66:1016–20.
6. Sharrar RG, LaRussa P, Galea SA, et al. The postmarketing safety profile of varicella vaccine. Vaccine **2000**; 19:916–23.
7. Zhang Y, Moore DD, Nix WA, et al. Neutralization of enterovirus D68 isolated from the 2014 US outbreak by commercial intravenous immune globulin products. J Clin Virol **2015**; 69:172–5.
8. Behrman A, Lopez AS, Chaves SS, et al. Varicella immunity in vaccinated healthcare workers. J Clin Virol **2013**; 57:109–14.
9. Smith JG, Liu X, Kaufhold RM, et al. Development and validation of a gamma interferon ELISPOT assay for quantitation of cellular immune responses to varicella-zoster virus. Clin Diagn Lab Immunol **2001**; 8:871–9.
10. Donahue JG, Kieke BA, Yih WK, et al.Varicella vaccination and ischemic stroke in children: is there an association? Pediatrics **2009**; 123:e228–34.
11. Loparev VN, Rubtcova EN, Bostik V, et al. Identification of five major and two minor genotypes of varicella-zoster virus strains: a practical two-amplicon approach used to genotype clinical isolates in Australia and New Zealand. J Virol **2007**; 81:12758–65.
12. Quinlivan ML, Jensen NJ, Radford KW, Schmid DS. Novel genetic variation identified at fixed loci in ORF62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. J Clin Microbiol **2012**; 50:1533–8.
13. Thiele S, Borschewski A, Küchler J, et al. Molecular analysis of varicella vaccines and varicella-zoster virus from vaccine-related skin lesions. Clin Vaccine Immunol **2011**; 18:1058–66.
14. Dohner DE, Adams SG, Gelb LD. Recombination in tissue culture between varicella-zoster virus strains. J Med Virol **1988**; 24:329–41.
15. Gershon AA, Breuer J, Cohen JI, et al. Varicella zoster virus infection. Nat Rev Dis Primers **2015**; 1:15016.
16. Science MD, MacGregor SE, Richardson SM, et al. Central nervous system complications of varicella-zoster virus. J Pediatr **2014**; 165:779–85.
17. Rosenfeld J, Taylor CL, Atlas SW. Myelitis following chickenpox: a case report. Neurology **1993**; 43:1834–6.
18. Gilden D, Nagel MA, Cohrs RJ, Mahalingam R. The variegate neurological manifestations of varicella zoster virus infection. Curr Neurol Neurosci Rep **2013**; 13:374.
19. Ziebold C, von Kries R, Lang R, et al. Severe complications of varicella in previously healthy children in Germany: a 1-year survey. Pediatrics **2001**; 108:E79.
20. Ibraheem M, Marin M, Leung J, et al. Fatal wild-type varicella-zoster virus encephalitis without a rash in a vaccinated child. Pediatr Infect Dis J **2013**; 32:183–5.
21. Gershon AA, Mervish N, LaRussa P, et al. Varicella-zoster virus infection in children with underlying human immunodeficiency virus infection. J Infect Dis **1997**; 176:1496–500.
22. Arvin AM, Pollard RB, Rasmussen LE, Merigan TC. Selective impairment of lymphocyte reactivity to varicella-zoster virus antigen among untreated patients with lymphoma. J Infect Dis **1978**; 137:531–40.
23. Levy O, Orange JS, Hibberd P, et al. Disseminated varicella infection due to the vaccine strain of varicella-zoster virus, in a patient with a novel deficiency in natural killer T cells. J Infect Dis **2003**; 188:948–53.
24. Wu UI, Holland SM. Host susceptibility to non-tuberculous mycobacterial infections. Lancet Infect Dis **2015**; 15:968–80.
25. Sejvar JJ, Lopez AS, Cortese MM, et al. Acute flaccid myelitis in the United States, August–December 2014: results of nationwide surveillance. Clin Infect Dis **2016**; 63:737–45.
26. Shaw J, Welch TR, Milstone AM. The role of syndromic surveillance in directing the public health response to the enterovirus D68 epidemic. JAMA Pediatr **2014**; 168:981–2.
27. Greninger AL, Naccache SN, Messacar K, et al. A novel outbreak enterovirus D68 strain associated with acute flaccid myelitis cases in the USA (2012–14): a retrospective cohort study. Lancet Infect Dis **2015**; 15:671–82.
28. Aliabadi N, Messacar K, Pastula DM, et al. Enterovirus D68 infection in children with acute flaccid myelitis, Colorado, USA, 2014. Emerg Infect Dis **2016**; 22:1387–94.